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Invited review: Advances in nisin use for preservation of dairy products

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ABSTRACT

Dairy product safety is a global public health issue that demands new approaches and technologies to control foodborne pathogenic microorganisms. Natural antimicrobial agents such as nisin can be added to control the growth of pathogens of concern in dairy foods, namely *Listeria monocytogenes* and *Staphylococcus aureus*. However, several factors affect the antimicrobial efficacy of nisin when directly added into the food matrix such as lack of stability at neutral pH, interaction with fat globules, casein, and divalent cations. To overcome these limitations, new and advanced strategies are discussed including nisin encapsulation technology, addition to active packaging, bioengineering, and combination with other antimicrobials. This review highlights advanced technologies with potential to expand and improve the use of nisin as a dairy preservative.

Key words: encapsulation, nisin, antimicrobial packaging, dairy preservation, bioengineering

INTRODUCTION

Microbial contamination of dairy products is a worldwide food safety and quality concern as contamination can occur at any part along the food chain. Pathogens of concern in dairy products include both gram-positive bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* and gram-negative bacteria such as *Salmonella* spp. and *Cronobacter sakazakii* (Jack et al., 1995; Deegan et al., 2006; Gandhi and Chikindas, 2007). Preservation using natural antimicrobials is desirable due to increased consumer demand for clean label foods (Meira et al., 2017). Nisin A is a 34 AA, heat-stable bacteriocin produced by certain *Lactococcus lactis* ssp. *lactis* strains, with a proven track record as an effective antimicrobial for selected dairy foods. It is suitable for clean label applications as it is abundant in the fermentates of nisin-producing *L. lactis* strains and is primarily active against gram-positive bacteria such as *L. monocytogenes* and *S. aureus* (Al-Holy et al., 2012; Karam et al., 2013a). In addition, nisin inhibits the outgrowth of spores from several *Bacillus* and *Clostridium* species in dairy products but has little, if any, effect against gram-negative bacteria, yeast, and molds (de Arauz et al., 2009; Cao-Hoang et al., 2010). Nisin antimicrobial activity is based on the inhibition of cell wall biosynthesis, which is coordinated via binding to the cell wall precursor lipid II. This interaction leads to pore formation and subsequent bacterial death (Cui et al., 2016; Ahmad et al., 2017).

Nisin has been approved for use in over 50 countries (Favaro et al., 2015) and was granted generally recognized as safe (GRAS) status by the FDA in 1988 (Santos et al., 2018). The FAO/WHO Codex Committee on milk and milk products allows nisin as a food additive for processed cheese at a concentration of 12.5 mg/kg product, whereas up to 250 mg/kg is permitted by the US FDA (Sobrino-Lopez and Martin-Belloso, 2008). Nisin, either added directly in purified form or produced in situ by live bacteria, is used in several dairy food applications to ensure safety, extend shelf-life, and preserve quality (Mitra et al., 2010; Mills et al., 2011; Dal Bello et al., 2012; Cui et al., 2017; Santos et al., 2018; Silva et al., 2018; Kondrotiene et al., 2018). However, several factors can reduce nisin efficacy when used directly in dairy foods (Kim et al., 2002; Sobrino-Lopez and Martín-Belloso, 2008; Khan and Oh, 2016; Silva et al., 2018).

The purpose of this review is to address the limitations related to nisin applications for dairy preservation. Strategies to improve nisin antimicrobial activity in dairy products are described, emphasizing nisin encapsulation and nanoencapsulation, active antimicrobial packaging with nisin, nisin bioengineering, and the combination of nisin with other antimicrobials.

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LIMITATIONS OF NISIN USE IN DAIRY PRESERVATION

It is well established that nisin is less effective in certain dairy products (Gharsallaoui et al., 2016). In particular, dairy foods with a neutral pH made with whole milk are known to be poor candidates for nisin application (Sobrino-Lopez and Martín-Belloso, 2008; Gharsallaoui et al., 2016). For example, the addition of 250 μg/g nisin to Queso Fresco, a Hispanic-style fresh cheese that has a neutral pH and is made with whole milk, provides *L. monocytogenes* control for just a few days (Van Tassell et al., 2015; Ibarra-Sánchez et al., 2018; Feng et al., 2019). Several factors have been proposed to explain nisin limitations in certain dairy foods including (1) lack of nisin stability at neutral pH, (2) potential for nisin to partition to fat globules, (3) cationic nisin binding to anionic casein, (4) presence of divalent cations blocking access to cell membrane phospholipids, and (5) potential for nisin resistance to develop.

Nisin is greatly affected by pH and has maximum solubility and stability at pH 3 (Liu and Hansen, 1990; Davies et al., 1998) possibly due to its structure, which is pH dependent. At pH 3, nisin secondary structure contains random coiling, whereas at pH 6 and above, β-turns are favored, suggesting that pH-induced structure variations may be associated with solubility and stability of nisin (Dykes et al., 1998; Modugno et al., 2018). Nonfermented dairy products such as milk and fresh cheeses are characterized by having a near neutral pH, which favor the growth of pathogenic and spoilage bacteria; however, the reduced nisin solubility and stability in these products is expected to reduce nisin effectiveness.

Nisin is a hydrophobic peptide, and the concentration and integrity of the fat globules in the food matrix may affect nisin partitioning and thus nisin activity. It has been shown that nisin is more effective at inhibiting *L. monocytogenes* in skim milk compared with whole milk (Jung et al., 1992). Bhatti et al. (2004) observed that nisin was more effective at reducing *L. monocytogenes* cell numbers in nonhomogenized whole milk compared with homogenized whole milk, suggesting that the surface area increase of fat globules, caused by homogenization, increases nisin adsorption to fat globules, resulting in decreased nisin effectiveness. Other studies have shown that the residual activity of nisin in milk was decreased by more than 88% in milk with 12.5% fat (Jung et al., 1992), and a similar effect was observed when nisin was added to a water-salmon oil mixture, where only 30 to 40% of the initial amount of nisin added was recovered in the water phase (Aasen et al., 2003). More work is needed to determine how the fat content of other dairy products such as fresh cheeses affects nisin activity.

It has been suggested that nisin can interact with proteins present in foods. At neutral pH, nisin is a cationic peptide, whereas caseins and other milk proteins are anionic, suggesting that there may be ionic interactions between nisin and milk proteins (Wirjantoro et al., 2001). Analysis of nisin absorption to proteins in homogenates of chicken and salmon determined that up to 90% of the added nisin was absorbed to proteins, and when the pH of the homogenates was adjusted from 6.2 to 2.5 (neutralizing the negative charges of muscle proteins), nisin absorption was reduced to 40%, suggesting that ionic interactions are important in adsorption of nisin to proteins (Aasen et al., 2003). The fact that nisin can interact with milk proteins may be desirable in cheese-making, ensuring a high retention of nisin in the cheese matrix and minimizing loss in cheese whey; however, the absorption of nisin to caseins could also reduce the amount of available nisin to exert antimicrobial activity.

The presence of divalent cations in dairy foods, such as calcium and magnesium, may also affect the activity of nisin. Houlihan and Russell (2006) found that calcium and magnesium significantly reduced the potassium loss from nisin-sensitive *Streptococcus bovis* cells when nisin was added. It has been suggested that the ionic interaction between the positive charges of nisin and the negatively charged phospholipids of the cell is necessary to initiate the formation of transient pores (O'Brian et al., 2018). However, divalent cations can interact with the phospholipid head groups of the cells, which can inhibit nisin insertion (Kaur et al., 2011). It is not clear whether the concentrations and availability of calcium and magnesium found in different dairy products affect the antimicrobial activity of nisin; however, these cations may be a contributing factor to the limited effectiveness of nisin in dairy products.

The naturally occurring variability in tolerance to nisin within a given species, and the acquisition of nisin resistance derived from repeated exposure to nisin may also limit nisin use. For example, nisin at concentrations ranging from 0.125 to 37.5 mg/L in liquid or solid media can inhibit *L. monocytogenes* depending on the strain (Rasch and Knøchel, 1998; Ennahar et al., 2000; Szendy et al., 2019). Although some authors have defined *L. monocytogenes* nisin resistance or high tolerance for strains able to grow at 12.5 (Rasch and Knøchel, 1998) or 37.5 (Szendy et al., 2019) mg/L, the legal limit of 250 mg/kg nisin in US dairy products can inhibit those strains. Nisin resistance is a complex phenotype and the natural high tolerance to nisin in certain *L. monocytogenes* strains may be associated

with mutations in the glutamate decarboxylase system (Szendy et al., 2019). On the other hand, prior exposure to nisin may select for nisin-resistant mutants, and examples of microorganisms capable of acquiring nisin resistance include *L. monocytogenes*, *Listeria innocua*, *Clostridium botulinum*, *Streptococcus thermophilus*, *S. aureus*, and *S. bovis* (Kaur et al., 2011; Zhou et al., 2014). Bacterial nisin resistance development seems to be restricted to laboratory media studies. For example, *L. monocytogenes* strains isolated from Queso Fresco treated with nisin have the same nisin sensitivity as the parental strains (Ibarra-Sánchez et al., 2018). Similarly, nisin-adapted *S. aureus* cells gradually lost the resistant phenotype in the absence of nisin (Martínez et al., 2008). The occurrence of bacteria developing nisin resistance in food might be limited in part due to stability and antimicrobial activity loss of nisin in dairy foods as discussed above. The FAO/WHO agrees that bacterial resistance against nisin in foods is not of concern (EFSA, 2006).

STRATEGIES TO IMPROVE NISIN ANTIMICROBIAL EFFICACY FOR DAIRY APPLICATIONS

Encapsulation and Nanoencapsulation of Nisin

Whereas the legal limit for nisin in US dairy products is 250 mg/kg, the nisin MIC for *L. monocytogenes* is approximately 12.5 mg/L (Van Tassell et al., 2015; Ibarra-Sánchez et al., 2018). Several studies have encapsulated nisin with a view to extending its antimicrobial activity by allowing a controlled and sustained release of nisin into the food matrix, thereby maintaining a minimum antimicrobial concentration during product storage. Unfortunately, most of the studies have shown very limited effectiveness using this encapsulation strategy in milk and fresh cheese (da Silva Malheiros et al., 2010, 2012; Xiao et al., 2011a; Ruiz Martinez et al., 2016). Presumably, the release rate of nisin is not maintaining the required antilisterial concentration in the food matrix. However, Feng et al. (2019) recently demonstrated enhanced efficacy of nisin-loaded zein microcapsules against *L. monocytogenes* in Queso Fresco, resulting in up to 2 log cfu/g lower pathogen counts compared with free nisin cheeses. There is potential for even further improvements in nisin encapsulation technology.

Another strategy has been to co-encapsulate nisin with other antimicrobial substances to potentially elicit synergistic effects (Xiao et al., 2011b; Pinilla and Brandelli, 2016; Lopes et al., 2019). However, at least in the 3 studies we identified, no benefit to co-encapsulation has been demonstrated in milk. Generally, the co-encapsulated nisin and other antimicrobial have similar effectiveness as adding these antimicrobials without encapsulation.

In some cases, encapsulation of nisin using other GRAS materials including chitosan, pectin, alginate/pectin, among others, have shown potential to extend the antimicrobial activity of nisin when tested in vitro (McClements, 2018). However, they need to be evaluated in a food matrix to demonstrate that the encapsulation system could actually increase the antimicrobial activity of nisin. Moreover, the minimal antimicrobial enhancement of nisin after encapsulation observed in most studies when tested in milk and cheese, described in the aforementioned studies, suggest that optimization of the encapsulation system is required to achieve the appropriate release rate of nisin. Additionally, most nisin encapsulation studies lack sensory evaluations of the capsules added into the food; thus, it is unknown whether these capsules negatively affect the characteristic mouthfeel and flavor of dairy products.

Active Antimicrobial Packaging Using Nisin

The development of antimicrobial packaging by incorporating nisin into the packaging material is one possible strategy to overcome nisin limitations. This approach can reduce the interaction of antimicrobials with food components (mainly protein and fat), dilution into bulk foods, neutralization, and rapid diffusion in the food mass (Quintavalla and Vicini, 2002; Guiga et al., 2010; Limbo and Khaneghah, 2015; Cui et al., 2016). Antimicrobial packaging is a promising type of active packaging that offers better stability and controlled release of antimicrobial agents to improve the quality and safety of food products (Balasubramanian et al., 2009; Guiga et al., 2010; Karam et al., 2016; Mlalila et al., 2018).

Nisin is a highly surface-active molecule that can bind to different compounds, making it suitable for adsorption on solid surfaces and killing bacterial cells that subsequently adhere (Sobrino-Lopez and Martín-Belloso, 2008). Therefore, nisin-activated surfaces showed potential for applications as antimicrobial packaging (Sobrino-Lopez and Martín-Belloso, 2008; Karam et al., 2013a,b,c,d; Meira et al., 2017). However, to date, only a few antimicrobial packaging systems have been applied in real food matrices/dairy food applications (Table 1).

Several antimicrobial packaging systems using nisin were able to reduce or eliminate *L. innocua*, *S. aureus*, *Bacillus cereus*, and *Micrococcus luteus* in packaged cheeses or milk products (Scannell et al., 2000; Mauriello et al., 2005; Cao-Hoang et al., 2010; Hanušová et al., 2010). The efficiency of antimicrobial packaging

Table 1. Active antimicrobial packaging incorporating nisin (NI), alone or in combination with other antimicrobials, for preservation of dairy products

Packaging material type	Antimicrobials	Antimicrobial concentration	Active antimicrobial packaging	Target microorganisms	Dairy food application	Reference
Cellulose-based packaging (bioactive inserts) used with modified atmosphere packaging	NI	7,650 AU[1]/cm^2	Adsorption of bacteriocins to the packaging material	*Listeria innocua*, *Staphylococcus aureus*	Young, white, Cheddar cheese	Scannell et al., 2000
Polyethylene (PE)/polyamide (PA) pouches (70:30, PE:PA) used with vacuum packaging	NI	7,860 AU/cm^2	Adsorption of bacteriocins to the packaging material	*L. innocua*, *S. aureus*	Young, white, Cheddar cheese	Scannell et al., 2000
Sodium caseinate film	NI	1,000 IU/cm^2 or 0.04 g/cm^2	NI incorporated into sodium caseinate and film formation by casting method	*L. innocua*	Mini red Babybel cheese	Cao-Hoang et al., 2010
Starch-halloysite nanocomposites films	NI	2 and 6 g/100 g of NI in nanocomposite films	NI incorporated in nanocomposite films by direct melt-extrusion	*Listeria monocytogenes*	Minas Frescal cheese	Meira et al., 2016
Polyamide/polyethylene films coated by polyvinyl dichloride (PVdC) lacquer	Natamycin (NA) NI	16.7% wt/wt of each antimicrobial in lacquer	Coextruded polyamide/ polyethylene film coated with the PVdC lacquer containing NI and NA (INVOS film[2])	*B. cereus*, total bacterial count, yeast and molds	Blatácké cheese, Olomoucké tvaruzky cheese	Hanušová et al., 2010
Chitosan coating	NI	5 mg/mL NI in NI-silica liposomes	Chitosan coating embedded with NI encapsulated in silica liposome	*L. monocytogenes*	Grang'or Cheddar cheese	Cui et al., 2016
Aluminum foil	NI	5 mg/mL NI in NI-loaded poly-y-glutamic acid/chitosan (NGC) nanoparticles	Aluminum foil coated with polyethylene oxide nanofibers containing NGC nanoparticles	*L. monocytogenes*	Kerrygold Cheddar cheese	Cui et al., 2017
Cellulose films	NA NI	8% NA + 50% NI in films	Antimicrobial agents incorporated into cellulose polymer and film formation by casting method	Yeast and molds, *Staphylococcus* sp., psychrotrophic bacteria	Mozzarella cheese	dos Santos Pires et al., 2008
Tapioca starch edible films	NA NI	9.25 mg of NA/dm^2, 2.31 mg of NI/dm^2	Antimicrobial agents incorporated into starch films and film formation by casting method	*Saccharomyces cerevisiae*, *L. innocua*	Port salut cheese[3]	Resa et al., 2014
Cellulose-chitosan film	NI	500 and 1,000 ppm of NI in chitosan-zinc oxide nanocomposite	Bilayer film: chitosan solution containing NI prepared by sol-gel method and then coated on cellulose paper by dip coating method	*L. monocytogenes*	UF white cheese	Divsalar et al., 2018
Low-density polyethylene (LDPE) film	NI	6,400 AU/mL	LDPE film coated with NI	*Micrococcus luteus*	Milk	Mauriello et al., 2005

[1]AU = activity units.

[2]INVOS, Svárov, Czech Republic.

[3]La Serenisima, Longchamps, Buenos Aires, Argentina.

is affected by several factors such as the method of incorporation of antimicrobials into the polymer, the packaged food/dairy product, and the packaging.

Previous studies presented the advantages and disadvantages of different types of methods used to set up antimicrobial packaging (Karam et al., 2013a). One of the most promising methods is to add encapsulated nisin to the packaging to enhance its stability and bioactivity in a food environment and to ensure its controlled release to the dairy product. Successful studies were reported to inhibit *L. monocytogenes* on Cheddar cheese without affecting its sensory properties (Cui et al., 2016, 2017), on the Minas Frescal cheese surface (Meira et al., 2016), and on commercial UF milk cheese (Divsalar et al., 2018). Another innovative approach to avoid the progressive depletion of antimicrobials from the packaging is to include live microorganisms able to produce bacteriocins in the film. For example, live-*Enterococcus*-doped films had a significantly higher antimicrobial activity than films with nisin or enterocin 416K1, showing full inactivation of *L. monocytogenes* in precooked chicken fillets and were very effective in cold chain break conditions when *Listeria* growth is rapid (Degli Esposti et al., 2018). The positive results suggest it would be interesting to assess the antimicrobial effect of incorporating live microorganisms producing nisin rather than purified nisin peptide into cheese packaging. However, this novel approach requires additional studies.

Antimicrobial packaging releases the antimicrobial on the food surface to control surface-contaminated cheeses but may affect the aging process of surface-ripened cheeses. Cao-Hoang et al. (2010) reported that sodium caseinate-nisin films reduced 90% of *L. innocua* counts on surface-inoculated Mini red Babybel cheese. In contrast, the antimicrobial effectiveness of nisin decreased as the distance between the surface in contact with the active films increased. Similarly, Hanušová et al. (2010) developed films containing both natamycin and nisin that inhibited the culture microorganisms on the Olomoucké tvaruzky cheese surface, which affected its ripening process. The food matrix characteristics and storage temperature can also affect the release of antimicrobials from the films as, for example, nisin release from low-density polyethylene film was higher with low pH and high temperature (Mauriello et al., 2005).

The antimicrobial activity of the active films is defined by the activity of added antimicrobials. To expand the limited spectrum of nisin activity, some authors have developed packaging containing a combination of nisin and natamycin to inhibit psychrotrophic bacteria, *L. innocua*, yeasts, and molds in cheeses (dos Santos Pires et al., 2008; Hanušová et al., 2010; Resa et al., 2014). In addition, the films developed by dos Santos Pires et al. (2008) were not able to inhibit *Staphylococcus* spp. growth due to the lack of nisin diffusion from the films to the cheese.

To advance the field of antimicrobial packaging, future studies should (1) focus more on full-fat dairy products with a near-neutral pH that are at higher risk for pathogen contamination, (2) develop innovative methods that retain the activity of the antimicrobials and improve their release to the food, (3) characterize the interactions between the active packaging and the food matrix, (4) study the kinetics of release of antimicrobials from the film for controlled shelf-life extension of products, (5) assess the potential of incorporating different antimicrobials with nisin into the packaging material, (6) evaluate the stability of the developed antimicrobial systems during storage time, (7) assess the effect of adding the antimicrobials on the mechanical, physico-chemical, and barrier properties of the active films in order not to compromise the basic protective functions of the food packaging, and (8) address legislative requirements related to antimicrobial packaging especially when nano-encapsulated antimicrobials are used.

Nisin Bioengineering

Nisin A, produced by *L. lactis* ssp. *lactis*, is a gene-encoded, ribosomally synthetized peptide that is suitable for bioengineering strategies. Amino acid modifications in the primary structure of nisin A change the physicochemical and antimicrobial properties of nisin A, as observed in various natural variants (Z, F, Q, H, U, and U2; Field et al., 2015a; O'Connor et al., 2015). For instance, nisin Z has shown better solubility at neutral pH, and only differs from nisin A at a single substitution of histidine to asparagine at position 27 (H27N) (Rollema et al., 1995).

The intentional introduction of mutations in the nisin gene sequence, targeting single residues such as in the hinge region (residues 20–22), has focused mainly on identifying nisin derivatives with enhanced antimicrobial activity such as improved potency and expanded antimicrobial spectrum (Field et al., 2015a), and several studies have reported nisin derivatives with enhanced antimicrobial activity in solid or liquid media against *S. aureus*, *L. monocytogenes*, *B. cereus*, *Enterococcus* spp., and *Streptococcus* spp. (Field et al., 2012; Rouse et al., 2012; Healy et al., 2013; Molloy et al., 2013; Field et al., 2015c; Hayes et al., 2019). In some cases, nisin derivatives (Field et al.,2012) and fusion mutants of nisin with anti-gram-negative peptides (Zhou et al., 2016;

Li et al., 2018) have displayed antimicrobial activity against gram-negative pathogens such as *C. sakazakii*, *Escherichia coli, E. coli* O157:H7, *Salmonella*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacter aerogenes*.

A limited number of studies have investigated the application of nisin derivatives in food matrices, in particular dairy foods. Rouse et al. (2012) reported that the nisin hinge variant, SVA (substitution of amino acids NMK to SVA at positions 20–22), was better able to reduce *L. monocytogenes* cells to nondetectable levels in commercial chocolate milk at 4°C than nisin A. In a similar study conducted at 37°C in commercial chocolate milk, nisin V (M21V) was more effective than nisin A at reducing *L. monocytogenes* counts, and in addition, the antilisterial synergy (antimicrobial activity is greater compared with individual treatments) with cinnamaldehyde was stronger with nisin V than with nisin A (Field et al., 2015b). Finally, nisin V and nisin S29A did not exhibit enhanced antimicrobial activity relative to nisin A against *C. sakazakii* when tested in reconstituted powdered infant milk formula despite these variants being more potent against *C. sakazakii* in media (Campion et al., 2017).

As stated previously, bioengineering of nisin has focused mainly on identifying nisin variants with enhanced antimicrobial activity or extended-antimicrobial spectrum, without testing for relevant biochemical properties such as improved solubility at neutral pH and lower absorption to milk fat, which are desirable characteristics for nisin applications in nonfermented and whole-fat dairy products. Additionally, the antimicrobial assessment of nisin derivatives has been mainly performed in laboratory media, which cannot be extrapolated to food matrices as shown by Campion et al. (2017). Consequently, it is currently unknown which nisin modifications would be most beneficial for application in dairy foods. The availability of nisin variant libraries (Field et al., 2015a; Zhou et al., 2016; Li et al., 2018) opens the possibility to screen these mutants for improved biochemical characteristics that will improve nisin performance in problematic food matrices such as low-acid, whole-fat dairy products.

Combining Nisin with Other Antimicrobials (Hurdle Technology)

Strategies for controlling spoilage and pathogenic microorganisms are currently oriented toward hurdle technology such as combining different antimicrobials to inhibit microbial growth and improve food safety (David et al., 2013). Additionally, effective antimicrobial mixtures can be optimized, allowing food processors to identify treatments with lower costs, minimal sensory effect, or both, while maintaining the desired level of safety in the food product (David et al., 2013). Examples of recent studies applying combinations of nisin with other antimicrobials in milk and cheese are listed in Tables 2 and 3.

Nisin has shown synergy when combined with plant-derived antimicrobials (extracts, essential oils, and organic compounds) to reduce the populations of *S. aureus*, *C. sakazakii*, and *L. monocytogenes*, in some cases to non-enumerable levels of pathogen, in milk and chocolate milk products (Yoon et al., 2011; Bajpai et al., 2014; Field et al., 2015c; Alves et al., 2016; Zhao et al., 2016; Campion et al., 2017; Chen and Zhong, 2017; Shi et al., 2017a,b). Some reports have shown that the effectiveness of combinations of nisin with plant-derived antimicrobials is affected by the amount of fat in milk. For example, the same antimicrobial combination can be synergistic in skim milk but display antimicrobial enhancement, where antimicrobial activity is to some degree elevated compared with individual treatments in whole milk (Kim et al., 2008; Yoon et al., 2011; Bajpai et al., 2014). However, in contrast to studies conducted in milk, combinations of nisin with plant-derived antimicrobials were less effective in Queso Fresco. For example, Gadotti et al. (2014) and Lourenço et al. (2017) reported initial *L. monocytogenes* reduction caused by nisin followed by a bacteriostatic effect associated with the plant-derived compound, whereas similar treatments were only bacteriostatic against *Salmonella* in Queso Fresco (Gadotti et al., 2020). Despite the synergistic potential of nisin with plant-derived antimicrobials, the strong odor and flavor of plant-derived compounds could limit their use in some food products such as milk and fresh cheeses (Calo et al., 2015).

The combination of nisin with other bacteriocins, inorganic antimicrobials, and fermentation products has been assessed for milk and cheese preservation. Combinations of nisin with reuterin (Arqués et al., 2011), bovicin HC5 (Pimentel-Filho et al., 2013), and enterocin AS-48 (Sobrino-Lopez et al., 2009) have been effective at reducing the populations of *L. monocytogenes* and *S. aureus* in skim and whole milk. *Listeria monocytogenes* counts were reduced to non-enumerable levels in curdled milk when nisin was combined with the lactoperoxidase system (Arqués et al., 2008). Nisin combined with magnesium oxide nanoparticles showed significant synergistic antibacterial effect against *E. coli* and *S. aureus* in unpasteurized cow milk (Mirhosseini and Afzali, 2016). Additionally, combining antimicrobial mixtures with other treatments could result in an enhanced antimicrobial effect. For example, the

Table 2. Applications of nisin in combination with other antimicrobials for preservation of milk products

	Combined antimicrobial					
Nisin concentration	Antimicrobial type	Antimicrobial concentration	Other treatment	Target microorganisms	Dairy food application	Reference
1/4 MIC[1]	Thymol, eugenol, carvacrol, and cinnamaldehyde	1/4 MIC[2]	NA[3]	*Staphylococcus aureus*, *Listeria monocytogenes*	Cow milk	Alves et al., 2016
0.008 mg/mL	Magnesium oxide nanoparticles	2 mg/mL without heat 0.5 mg/mL	NA Heat (60°C)	*Escherichia coli*, *S. aureus*	Unpasteurized cow milk	Mirhosseini and Afzali, 2016
25 μg/mL[4]	Thymol	100 μg/mL	NA	*L. monocytogenes*	Chocolate milk	Field et al., 2015c
	Carvacrol *trans*-cinnamaldehyde	304 μg/mL 327.6 μg/mL				
60 μ*M* and 250 μg/mL[5]	Carvacrol	300 μg/mL	NA	*Cronobacter sakazakii*	Reconstituted powdered infant milk formula	Campion et al., 2017
	Citric acid	30 m*M*				
16 μg/mL	Perilla oil	1 mg/mL	NA	*S. aureus* *L. monocytogenes*	Pasteurized milk	Zhao et al., 2016
8 mg/mL	Cinnamaldehyde	0.25 mg/mL	NA	*S. aureus*	Pasteurized milk	Shi et al., 2017a
0.37 and 0.75 μg/mL	Phage-encoded endolysin LysH5	7.5 and 15 U/mL	NA	*S. aureus*	Pasteurized milk	García et al., 2010
8 μg/mL	p-Anisaldehyde	1 mg/mL	NA	*S. aureus*	Pasteurized milk	Shi et al., 2017b
1.5 μg/mL	Bacteriophage Φ35 Bacteriophage Φ88	1:1 cocktail of phages Φ35 and Φ88 at 10^3 pfu/mL	NA	*S. aureus*	Pasteurized milk	Martínez et al., 2008
62.5, 125, 250, and 500 IU/mL	Garlic shoot juice	2.5 and 5%	NA	*L. monocytogenes*	Whole (3.5%), low (1%), and skim (no fat content) milk	Kim et al., 2008
250 and 500 IU/mL	Lactobionic acid	10 mg/mL	NA	*L. monocytogenes*	UHT processed 2% reduced-fat milk and whole milk	Chen and Zhong, 2017
	Thymol	1–2 mg/mL				
62.5, 125, 250, and 500 IU/mL	Cone essential oil of *Metasequoia glyptostroboides*	1 and 2%	NA	*L. monocytogenes*	Whole, low, and skim milk	Yoon et al., 2011
62.5, 125, 250, and 500 IU/mL	Leaf essential oil of *Metasequoia glyptostroboides*	1, 2, and 5%	NA	*L. monocytogenes*	Whole, low, and skim milks	Bajpai et al., 2014
100 IU/mL	Reuterin	8 AU[6]/mL	NA	*L. monocytogenes*, *S. aureus*, *E. coli* O157:H7, *Salmonella enterica*, *Yersinia enterocolitica*, *Aeromonas hydrophila*, *Campylobacter jejuni*	UHT skim milk with 0.04% fat	Arqués et al., 2011
1 to 20 IU/mL	Lysozyme	300 to 5,000 IU/mL	High-intensity pulsed-electric field: 120 to 1,200 μs	*S. aureus*	Homogenized UHT skim milk	Sobrino-Lopez et al., 2009
	Enterocin AS-48 (AS-48)	28 AU/mL				
400, 600, 800, and 1,200 AU/mL	Bovicin HC5	400, 600, 800, and 1,200 AU/mL	NA	*L. monocytogenes*, *Listeria innocua*, *S. aureus*	UHT whole milk	Pimentel-Filho et al., 2013

[1]Nisin MIC: 110 ± 14.14 and 125 ± 7.07 μg/mL for *S. aureus* and *L. monocytogenes*, respectively (means ± SD).

[2]Thymol MIC: 155 ± 7.07 and 150 ± 14.14 μg/mL; carvacrol: 60 ± 0 and 65 ± 7.07 μg/mL; cinnamaldehyde: 100 ± 0 and 65 ± 7.07 μg/mL; eugenol: 25 ± 7.07 and 90 ± 14.14 μg/mL, for *S. aureus* and *L. monocytogenes*, respectively (means ± SD).

[3]NA = not applicable.

[4]Nisin and nisin derivative M21V (nisin V).

[5]Nisin and nisin derivatives nisin V and nisin S29A.

[6]AU = activity units.

Table 3. Applications of nisin in combination with other antimicrobials for preservation of cheese products

	Combined antimicrobial				
Nisin concentration	Antimicrobial type	Antimicrobial concentration	Target microorganisms	Dairy food application	Reference
0.49 g/kg	Caprylic acid *trans*-cinnamaldehyde	0.36 and 0.72 g/kg 0.3 and 0.6 g/kg	*Listeria monocytogenes*	Queso Fresco	Gadotti et al., 2014
0.5 g/kg	Caprylic acid	0.73, 1.09, and 1.46 g/kg	*L. monocytogenes*	Queso Fresco	Lourenço et al., 2017
250 μg/g	Phage endolysin PlyP100	2.5 and 10 U/g PlyP100	*L. monocytogenes*	Queso Fresco	Ibarra-Sánchez et al., 2018
0.5 g/kg	Caprilic acid *trans*-cinnamaldehyde	0.4, 0.7, and 1.6 g/kg 0.3, 0.6, and 1.2 g/kg	*Salmonella*	Queso Fresco	Gadotti et al., 2020
100 IU/mL	Reuterin Lactoperoxidase system	2 AU[1]/mL 0.2 AB-TSU[2]/mL	*L. monocytogenes*, *Staphylococcus aureus*	Cuajada (curdled milk)	Arqués et al., 2008

[1]AU = arbitrary units.

[2]AB-TSU = 2,2′-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid units.

addition of mild heat (60°C) to nisin-magnesium oxide nanoparticles (Sobrino-Lopez et al., 2009) and combining nisin-enterocin AS-48 with a high-intensity pulsed-electric field (Mirhosseini and Afzali, 2016) increased the antimicrobial efficacy of the antimicrobial mixtures in milk.

Another promising biopreservation intervention involved the use of bacteriophages and their endolysins in combination with nisin. The combination of nisin with a staphylococcal phage cocktail (Martínez et al., 2008) or with its endolysin (García et al., 2010) has shown enhanced antimicrobial activity to reduce *S. aureus* from pasteurized milk, notably with the nisin-endolysin combination (synergy). Additionally, Martínez et al. (2008) reported that nisin-adapted *S. aureus* cells (generated in vitro) had lower susceptibility to staphylococcal phages, but phage-resistant *S. aureus* cells had the same nisin sensitivity as the parental strain. However, nisin-bacteriophage cross-resistance might not be of concern in dairy products due to instability issues as discussed above. Finally, the combination of nisin with phage endolysin PlyP100 showed a strong synergy achieving complete elimination of *L. monocytogenes* in most Queso Fresco samples after 4 wk of refrigerated storage. Moreover, *L. monocytogenes* isolates from Queso Fresco cheeses did not develop resistance to nisin or PlyP100 (Ibarra-Sánchez et al., 2018). To the best of our knowledge, the latter study represents the most effective intervention using antimicrobials to eliminate *L. monocytogenes* in Queso Fresco.

The combination of nisin with other antimicrobials has mainly focused on the preservation of fluid milk, and fewer studies have been oriented toward cheese safety, in particular Queso Fresco and other fresh cheeses, dairy products that are at high risk of *Listeria* contamination (Ibarra-Sánchez et al., 2017). Although several antimicrobials have been shown to act synergistically with nisin in dairy products, most studies in milk have evaluated antimicrobial combinations at room temperature or 37°C rather than refrigeration temperature, and the majority of the studies lack sensory evaluations, especially when plant-derived antimicrobials are used with nisin.

CONCLUSIONS

This review addresses both the limitations and the major trends and advances in the use of nisin for dairy product preservation. In several studies, nisin was effective in dairy products using antimicrobial packaging, bioengineering, encapsulation, and combined antimicrobials. Active antimicrobial packaging containing nisin can provide an efficient hurdle to control cheese contamination during processing or postprocessing activities, but its activity is limited to the cheese surface. Screening studies to identify bioengineered nisin variants with enhanced solubility and stability in a variety of food matrices, including low-acid and whole-fat dairy foods, are required to expand their potential use by the food industry. Nisin encapsulation techniques need to be optimized and should include sensory evaluation of the capsules added to food. Interestingly, the incorporation of encapsulated nisin into antimicrobial packaging presents a promising solution. Other hurdles and natural antimicrobials could be further investigated and added to the food matrix or food packaging to achieve greater stability and microbiological safety without affecting the nutritional and sensory properties of food. Overall, further studies on nisin applications in dairy products are required to understand its performance in complex food environments. In addition, the legislative requirements and challenges related to those advanced technologies need to be assessed.

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