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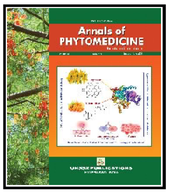

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A comprehensive review of potent antimicrobial agent

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Abstract

Nisin, a bacteriocin produced by *Lactococcus* lactis, offers a multifaceted approach to food safety and gut health. This review thoroughly examines Nisin's role as a preservative for food, its structural and biological properties, and its unique amino acid composition. An important area of study is Nisin's biological activity, which demonstrates its effectiveness against Gram-positive bacteria, particularly spoilage and pathogenic strains. Isolation and extraction methods for Nisin production are addressed. The document also explores reliable methods for determining Nisin concentration. Applications of Nisin across various foods, including processed cheese, meats, and drinks. Emphasizing its role in extending shelf-life is the main goal. The concept of combining Nisin with additional preservation techniques is explored to enhance efficacy. Recent research on Nisin as a regulator of the gut microbiota is presented, shedding light on its potential to alter gut microbiome composition safely. Food safety and legal aspects of Nisin use, including regulations and approvals, are addressed. The review finally examines the established Nisin safety profile and addresses its low toxicity. The development of Nisin-based delivery systems for targeted antimicrobial effects and their use in smart packaging are possible future advancements. Further investigation into *L. lactis* genetic engineering could potentially broaden the range of Nisin uses in the food and pharmaceutical sectors.

1. Introduction

Food can be preserved by a naturally occurring substance called Nisin. It originates from a particular kind of bacteria known as *Lactococcus lactis*. Nisin is a naturally occurring defense mechanism produced by this bacterium to eliminate rival bacteria. It excels in removing Gram-positive bacteria, a common culprit behind food spoilage. By inhibiting their growth, Nisin helps these products stay fresh for longer. Highly potent, it requires minimal amounts (1-25 ppm) to be effective, thus minimally impacting food's taste and texture. This benefit paved the way for its commercialization as an antibacterial agent in England by 1953 (Jozala *et al*., 2015). Nisin, first discovered in fermented milk around 1928, has a well-established safety profile supported by decades of use (Jozala *et al*., 2015). Recognized as safe for human consumption due to its digestibility, non-toxicity, and biodegradability, it received formal approval from the Joint FAO/WHO Expert Committee in 1969 and the US FDA for use in processed cheeses in 1988 (Favaro *et al*., 2015; Santos *et al*., 2018; Gharsallaoui *et al*., 2016; Todorov *et al.,* 2022). Today, it is approved in over 50 countries, making it a widely trusted natural preservative. Nisin's versatility as a natural preservative across a wide range of foods has been a game-changer for the food industry, significantly enhancing food safety and shelf-life. The Nisin market was valued at an estimated $443 million in 2020 and is projected to grow to $553 million by 2025, indicating a promising future for this natural preservative.

Nisin's mode of action involves binding to lipid II, a key molecule in bacterial cell wall synthesis, thereby halting cell growth and triggering cell death (Lubelski *et al.,* 2008). Its heat stability and pH tolerance make it especially suitable for use in dairy products, canned foods, and meat items. Moreover, new Nisin variants like Nisin Q, F, and J have shown potential for enhanced activity and broader spectrum applications. These naturally occurring variants arise through minor genetic changes in producer strains, leading to differences in structure and bioactivity. Efforts are ongoing to engineer Nisin derivatives through bioengineering, aiming to overcome resistance or broaden

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antimicrobial coverage (Field *et al.,* 2012). In addition to safety, its biodegradability and non-toxicity make it an environmentally friendly option compared to synthetic preservatives. Researchers are investigating synergistic effects when Nisin is combined with other hurdles like essential oils or mild heat treatment. Recent advances in fermentation and purification have improved yields and cost efficiency, making industrial-scale use more viable.

2. Classification of Nisin

Since the initial discovery of Nisin A found in fermented milk, scientists have uncovered a treasure trove of natural and bio-designed Nisin variants, expanding the potential applications of this powerful preservative (Sullivan *et al.,* 2020). Nisin A, the first discovered variant, paved the way for identifying a fascinating array of naturally occurring Nisin analogs. These eleven variants, including Nisin Z, F, Q, H, O (A1-A3 and A4), U, U2, P, and J, exhibit unique solubility, chemical reactivity, and the spectrum of bacteria they target.

Nisin Z and A, both located in dairy products and produced by *L. lactis*, vary by a single amino acid swap at position 27. This single change (histidine to asparagine) significantly impacts solubility. Nisin Z is more soluble due to asparagine's additional polar side chains. Interestingly, this difference has little effect on other properties like resistance to enzymes, bacteria, pH variations, and heat (Gharsallaoui *et al.,* 2016). Nisin F deviates further from Nisin A, containing different amino acids at positions 27 (aspartic acid) and 30 (valine). Other Nisin variations include Nisin Q (aspartic acid at 27), and shorter versions like Nisin U, O, U2, and P (with 31-33 amino acids). Nisin J, consisting of 35 amino acids, is currently the longest naturally occurring form of Nisin identified (Sullivan *et al.,* 2020).

2.1 Composition and biological characteristics

Nisin boasts a unique and complex structure that contributes to its biological and physical properties. The physical properties of Nisinare shown in Table 1**.** Molecular composition:Nisin, a tiny molecule with just 34 amino acids, is a heat-stable class IA lantibiotic bacteriocin. These building blocks link together in a specific order, forming a complex 3D structure with loops and rings (Al-holy *et al.,* 2012). Non-standard Amino Acids: Nisin stands out from most peptides because it incorporates non-standard AA, including lanthionine (Lan) and methyllanthionine (MeLan). These molecules are formed by linking cysteine units within the protein chain through a unique ether bond. In doing so, a ring is formed. The way Nisin is put together helps it stay strong and function properly. Additionally, Nisin contains dehydrated amino acid derivatives such as didehydroalanine (Dha) and didehydrobutyrine (Dhb), which arise from the dehydration of serine and threonine residues, respectively. These dehydrated forms of alanine and butyrate play a crucial role in Nisin's antibacterial action (Karam *et al.,* 2013). Thus, they are essential to Nisin's effectiveness in inhibiting bacterial growth.

Naturally occurring antimicrobial fighters called antibacterial peptides come in various shapes and sizes. Antibacterial peptides are built from short chains of AA, ranging from just a few to hundreds long(Jenson *et al.,* 2006). These chains can be straight (linear) or circular (cyclic). What makes them special is their chemical makeup. Antibacterial peptides have a mix of properties: Cationic, positively charged, attracting them to negatively charged targets like bacteria. Hydrophobic: repelled by water but attracted to fats and oils, which are abundant in bacterial membranes. Amphipathic: having both cationic and hydrophobic regions. The unique properties of antibacterial peptides enable them to latch onto and dismantle microbes (Thapa *et al.,* 2020; Caballero *et al.,* 2003). Their lengths can range dramatically, from just an individual amino acid to a chain of up to one hundred (Jenson *et al*., 2006).

The strong antibacterial properties displayed by antimicrobials against a vast collection of microbes and their many ways of acting, low propensity for bacterial resistance, rapid eradication of target cells, and minimal cytotoxic effects, have sparked a heightened interest in research about antimicrobial resistance (Tong *et al.,* 2014; Makhlynets *et al.,* 2021; Hafeez *et al.,* 2021; Reiners *et al.,* 2020).

Another AMP is Nisin, which is a colorless, tasteless, and odorless substance (Tong *et al.,* 2014). Modifications made after translation: The bacteria do not naturally produce the unique amino acids that occur in Nisin. They are created by enzymatic modifications after the initial peptide is built. The ultimate structure and the purpose of Nisin depend on these alterations (Dos Santos*et al.,* 2018; Cooper *et al.,* 2010). This molecule features a unique structure with five thioether rings and four constituent amino acids. The N and C terminals are capped by two hydrophobic residues (Caballero *et al.,* 2003).

Nisin's unique power comes from its structure. The occurrence of special rings called thioethers grants Nisin several advantages: resistance to breakdown by enzymes (proteolytic degradation), stability at high temperatures (heat stability), and the ability to kill bacteria even at incredibly small scales (nanoscale). Each thioether ring has a distinct function. The first two rings bind specifically to Lipid II, a molecule essential for bacterial cell wall formation. Meanwhile, the flexible hinge region cooperates with the last two thioether rings to insert themselves into the bacterial membrane, ultimately forming a pore. Interestingly, only 34 of Nisin's amino acids are essential for its antibacterial activity. This means Nisin is initially produced with an extra 23-amino acid leader peptide that gets cleaved off to activate Nisin (Lie *et al.,* 2018).

2.2 The physical attributes of Nisin

2.2.1 Nisin's bioactivity

Nisin is a powerful weapon against Gram-positive bacteria, including those that develop out of spores and take the vegetative form, such as *Bacillus* and *Clostridium* species. *Lactococcus, Enterococcus, Streptococcus, Staphylococcus, and Micrococcus* are some of these genus (Punyauppa-path *et al.,* 2015; Aveyard *et al.,* 2017).

2.3 Mechanism of action

Disrupts cell membranes: The peptidoglycan precursor is attached to Nisin, which is necessary for Gram-positive bacteria to produce their cell walls. This binding breaks down the bacterial cell wall, leading to internal leakage and eventual cell death. Prevents the formation of cell walls**:**Nisin throws a double punch at bacteria. By targeting Lipid II and interfering with other cell wall components, it significantly weakens the bacteria's protective barrier. As shown in Figure 1. Because of their outer lipopolysaccharide membranes, which function as a barrier or shield to prevent Nisin from penetrating the cytoplasmic membrane, Gram-negative bacteria like *E. coli* are typically resistant to it (Wang *et al.,* 2019; Vukomanovic *et al.,* 2017). Furthermore, Nisin does not inhibit the growth of viruses, filamentous fungi, or yeast cells. Various research investigations (Pandey *et al.,* 2020a; Shin *et al.,* 2012; Naghmouchi *et al.,* 2010; Wang *et al.,* 2019; Punyauppapath *et al.,* 2015; Field *et al.,* 2012;

Pinile *et al.,* 2016) indicated that further methods such as heat treatment or freezing, chelating agents like EDTA, Nisin-chelating high purity, Nisin-bioengineered variants, Nisin-antibiotics, Nisin-inorganic nanoparticles like magnesium oxide, gold, or silver, or other methods of destabilizing the outer membrane may also be useful against Gram-negative bacteria.

Table 1: Physical properties

Property	Comment	References
Acidity	In acidic solutions, Nisin is soluble and very stable. At a basic Ph, Nisin's solubility drastically drops and loses is ability to function properly in the body, possibly due to changes caused by chemicals. The cationic surface-active detergent quality and the inhibitor of murein formation, which is an essential part of the cell wall of gram-positive bacteria, are additional features.	Muller-Affermann *et al.,* 2015
Temperature	Nisin activity is less negatively impacted by mild temperatures, specifically those used during pasteurization, and remains at least 80% effective. Even after autoclaving, Nisin maintains its biological activity at Ph 2.8. At high temperatures, it drastically drops, losing twice as much activity and becomes less stable as the pH rises. Nisin offers excellent storage stability at cold temperatures.	Muller-Affermann *et al.,* 2015
Solubility	In acidic solutions, Nisin is soluble and very stable. At a basic Ph, Nisin's solubility drastically drops and it becomes physiologically inactive, most likely due to chemical reactions.	Muller-Affermann *et al.,* 2015
Enzymes	Proteases can break down bacteriocins since they are hydrophobic peptides. Proteases, like proteinases A, are released by yeast cells during fermentation, which is an important step because they can break down the hydrophobic polypeptides that stabilize beer foam. Alpha chrymotrypsin inactivates it but remains resistant to protease, trypsin, and heat treatments in acidic environments.	Suganthi *et al.,* 2012

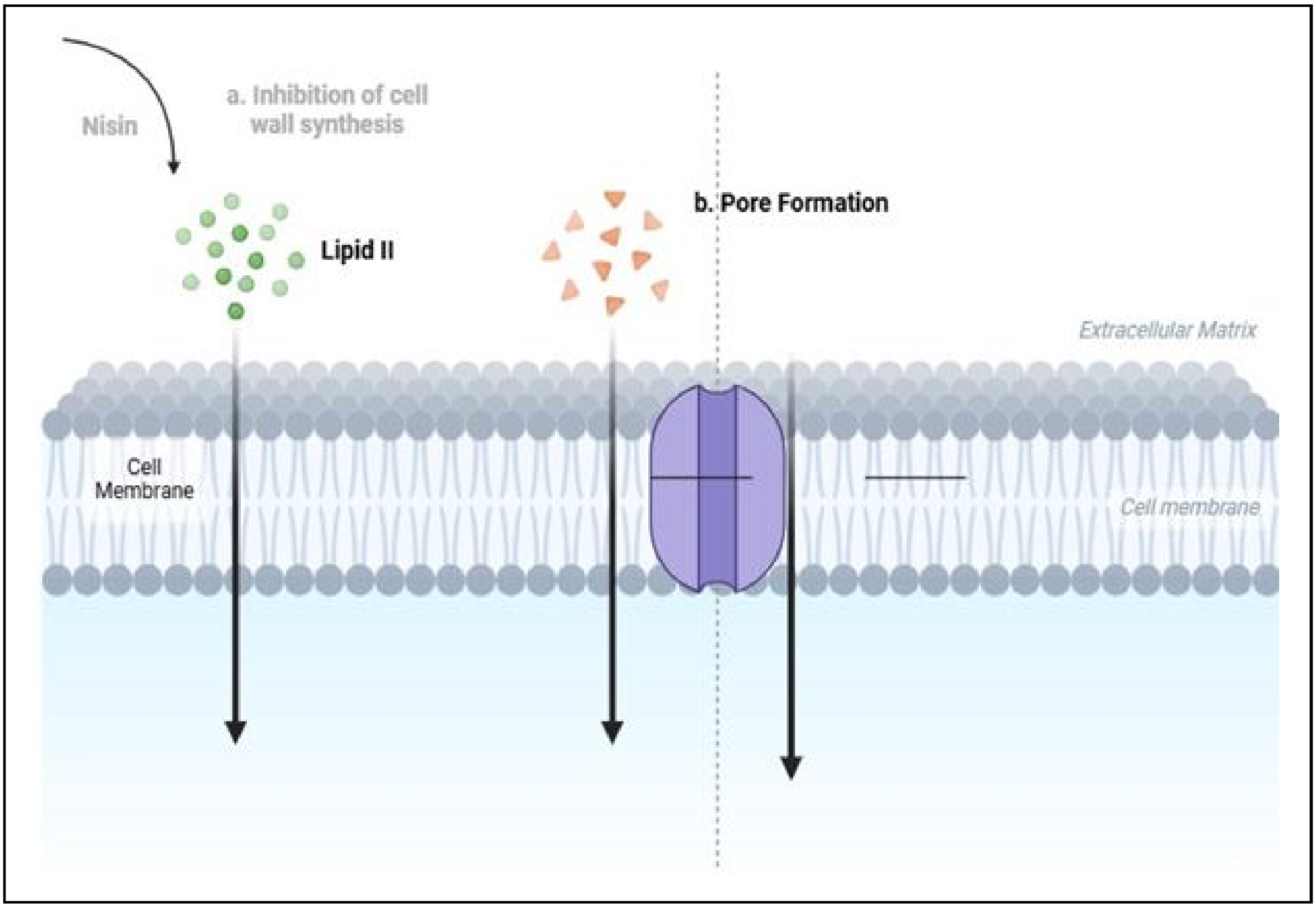

Figure 1: Mechanism of action: a. inhibition of cell wall synthesis, b. pore formation (The illustration was prepared using BioRender software).

2.4 Nisin's production aspects

2.4.1 Isolation of Nisin

Isolation refers to separating a specific strain of microorganisms from a mixed population. The initial process requires isolating Nisin-producing bacterial taxa, including *Lactococcus, Streptococcus, Staphylococcus, and Blautia* (Garcia Gutierrez *et al.,* 2020), and cultivating them on a particular growth medium that makes it possible to identify colonies that produce Nisin. These colonies typically exhibit clear zones around them, indicating inhibition of surrounding bacteria. Once a Nisin-producing strain is identified, it is grown in a large-scale fermentation process. Centrifugation is then used to extract the bacterial cells from the culture broth, producing a cell-free supernatant containing the Nisin the bacteria have produced. The Nisin isolation process is shown in Figure 2.

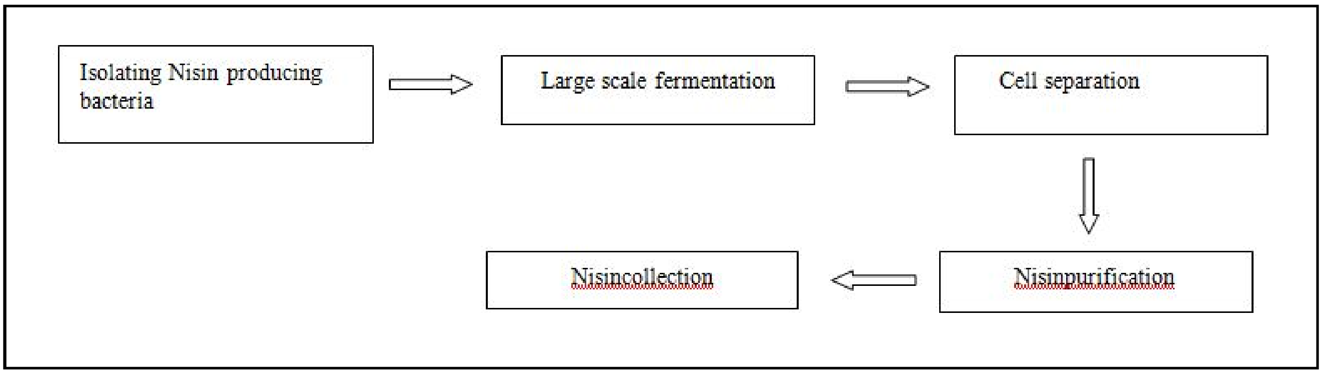

Figure 2: Isolation process.

Nisin production has gotten a big boost lately. This is owing to innovative fermentation techniques and gene manipulation of Nisin-producing strains**.** Recently, lactis cells have produced 15,400 IU ml^{-1} of Nisin by eliminating genes after genetic alterations. substances that block the yield of Nisin or that raise the cell density of the strains that produce it in the fermentation medium (Burcuozel *et al.,* 2018).

2.4.2 Nisin extraction

The main purpose of extraction is to obtain concentrated and purified Nisin for various applications. Various methods are employed for purifying Nisin from the culture medium, each with distinct mechanisms. Direct chromatography of the culture medium involves passing the culture liquid through a chromatographic column, where Nisin is separated based on its affinity for the stationary phase. This technique effectively isolates Nisin, leveraging its interaction with the column material. Solvent extraction, on the other hand, uses solvents such as chloroform to extract Nisin at different temperatures, namely 20°C, 4°C, and -10°C. The varying temperatures influence the solubility and extraction efficiency of Nisin, helping to refine the purification process. Lastly, salting out by ammonium sulfate is a method where ammonium sulfate is added to the culture medium at 40% saturation. This induces Nisin to precipitate out of the solution, allowing for its collection. These techniques offer complementary approaches for isolating Nisin, each with its specific advantages depending on the desired yield and purification level.

Among various purification methods are shown in Table 2, ammonium sulfate precipitation at 40% saturation proved to be the most efficient for concentrating Nisin. (fold of purification= 168.80, yield = 90.04%). This method achieved significantly higher yield and fold of purification compared to solvent extraction with chloroform and direct cation exchange chromatography of culture media. For optimal Nisin purification, a low pH of 3.0 and an acidic environment are necessary (Tafreshi *et al.,* 2020).

Table 2: Comparison of Nisin extraction techniques

Techniques	Foldof purification	Yield (%)
Direct chromatography of culture medium	1.80	20.00%
Solvent extraction at 20°C,4°Cand -10°C	37.43	24.23%
Salting out with ammonium sulfate at 40%	168.80	90.04%

3. Determination methods for Nisin concentration

3.1 Chemical methods

A colorimetric assay could be developed for Nisin concentration determination. Nisin may have some mild oxidizing properties. In theory, it could potentially oxidize a suitable colorless indicator dye to a colored form. Depending on how quickly a particular strain of microbe may change the color of an indicator substance, such as methylene blue (Reunanen *et al.,* 2007).

3.2 Methods based on growth inhibition

3.2.1 Techniques utilizing liquid media

Varying amounts of Nisin are introduced into tubes containing the indicator strain. These tubes are incubated at specific times and a specific temperature. The culture's optical density at 600 nm wavelength is used to gauge the growth. Plotting the optical densities versus the Nisin concentration logarithms yields a standard curve. The quantity of Nisin present is expressed as the reciprocal of the least dilution that triggers the development of the indicator strain (Reunanen *et al.,* 2007).

3.2.2 Techniques utilizing solid media

A round (clear) zone of inhibition is created when a solution containing a specific amount of Nisin is added to the circular cup, a cut in the agar gel medium that holds a culture of a test microorganism. Nisin diffuses swiftly through the agar layer in these zones, preventing the strain's ability to proliferate in these areas. To improve the Nisin's poor diffusion properties, Tween 20 or 80 is added to the agar. The diameter of the inhibitory zone has a direct relationship with the concentration of Nisin (Reunanen *et al.,* 2007).

3.2.3 Immunological methods

Concentrations of Nisin can also be ascertained quantitatively using the enzyme-linked immunosorbent test (ELISA). Polyclonal antibodies and a color shift are employed to identify Nisin (Reunanen *et al.,* 2007).

3.2.4 Harnessing Nisin for food preservation

The main objective of Nisin application in food goods is to extend shelf life and enhance food safety by inhibiting the growth of undesirable microorganisms. It is a good substitute for artificial preservatives, which some customers might want to stay away from.

3.3 Use of Nisinin dairy

Most FDA-reported listeriosis outbreaks are dependent on dairy products. For instance, the centre for disease control and prevention (CDC) and FDA reported a multi-state listeriosis outbreak linked to queso fresco cheese in New York, Maryland, and Virginia, which resulted in 12 infections and 1 death (CDC, 2021) Maryland, and Virginia, causing 12 infections and 1 death (CDC, 2021) It is rare for dairy products, such as fresh cheese and whole milk, to have Nisin with a pH of neutral. Since Nisin's antibacterial efficacy is significantly diminished when the pH exceeds 6 (Gharsallaui *et al.,* 2016). Nisin treatment alone was unable to successfully stop *L. monocytogenes* from growing in Queso Fresco (pH greater than 6) (Ibarra-Sanchez *et al.,* 2018).

In cheese and pasteurized cheese spreads, Nisin is frequently used in place of nitrate to obviate the formation of *Clostridium* spores. It has been demonstrated that Nisin lowers *L. monocytogenes* and *S. aureus* in chilled milk. Additionally, Nisin has been added to several varieties of cheese (cottage cheese, cheddar, and ricotta-type cheeses) in several trials. Nisin treatment significantly reduced the growth of *L. monocytogenes*, although the effect was limited to a 1-3 log cycle reduction. The study demonstrated that the presence of Nisin resulted in a reduction in the levels of bacteria that contaminated the product after processing, such as *L. monocytogenes,* in five different pasteurized dairy products, such as clotted cream, flavored milk, cooled desserts, butter, and evaporated milk in a can (Silva *et al.,* 2018).

When Nisin is combined with antimicrobials obtained from plants (such as organic compounds, essential oils, and extracts), the manifestation of synergy has been observed in the reduction of populations of *S. aureus, C. sakazakii,* and *L. monocytogenes*in both milk and chocolate milk products (Yoon *et al.,*2011; Bajpai *et al.,*2014; Alveset *al.,*2016; Zhaoet *al.,* 2016; Campion *et al.,* 2017; Chen and Zhong, 2017).Certain studies have indicated that the quantity of fat present in milk plays a role in influencing the efficacy of Nisin when used in combination with plant-derived antimicrobial agents. For example, the same combination of antibiotics may work synergistically in skim milk, but it may also show antimicrobial enhancement, meaning that when treated independently, the antibacterial activity is a little bit more than it would be in whole milk (Yoon *et al.,* 2011; Bajpai *et al.,* 2014). Nisin and magnesium oxide nanoparticles demonstrated a potent synergistic antibacterial effect against *S. aureus* and *E. coli* in unpasteurized cow milk. Employing bacteriophages along with their other potential bio-preservation method was the combination of endolysins with Nisin. Studies have shown that the staphylococcal phage cocktail and Nisin's endolysin (Garcia *et al.,* 2010) increase antibacterial activity to reduce *S. aureus* from pasteurized milk.

3.4 Use of Nisin in the brewery

3.4.1 Malting

When added to the steeping water during malting, Nisin may inhibit the growth of the naturally occurring molds, bacteria, and yeast in the barley. One possible treatment for mycotoxin generation is Nisin.

3.4.2 Wort

Unboiled wort is protected from spoilage with the addition of Nisin. Research has shown that over 30% of the original Nisin activity remains intact after boiling wort for 60 min (Muller-Affermann *et al.,* 2015). This suggests that Nisin plays a role in reducing the probability of thermophilic Gram-positive bacterial proliferation. Furthermore, it is plausible that Nisin could offer a degree of protection against potential microbiological contaminations at subsequent stages of the brewing process. In particular, studies have demonstrated that Nisin retains significant stability and antimicrobial activity even after exposure to high temperatures during brewing (Moretti-Almeida *et al.,* 2020).

3.5 Canned vegetables

The first to document Nisin's efficacy in canned veggies was Gillepsy (1953). She described how Nisin at a dose of 5 micrograms did not cause any degradation and how effective Nisin studies were conducted utilizing *C. Thermosaccharolyticum*-inoculated canned beans in tomato sauce. To guarantee that *C. botulinum* is destroyed, low-acid canned goods with a pH of more than 4.5 should typically undergo a minor heat treatment. Even at temperatures higher than this, heat-resistant spores of the thermophilic bacteria *B. stearothermophilus* or *C. thermosaccharolyticum* may endure. This spoiling, which happens especially when cans are kept at high ambient temperatures for extended periods, can be managed using Nisin. Nisin can also be used to lessen the amount of heat treatment, which will conserve energy and enhance the food's texture, look, and nutritional content, according to Thomas *et al.* (2005).

3.6 Meat products

Nisin demonstrated synergistic antibacterial activity in raw pork loin at 4°C for 12 h, reducing *L. monocytogenes* levels by 3 $\log_{10}$ CFU/g when combined with grapefruit seed extract and cinnamon aldehyde. When mixed with naturally occurring antibacterial compounds found in pork, Nisin's concentration against *L. monocytogenes* may be lowered to 5-7 ppm, which would still demonstrate a 10-fold inhibitory concentration against the bacteria (Yu *et al.,* 2019). The blend includes tea polyphenols, chitosan, and Nisin (TPs) to preserve fresh meat in the fridge. The mutton samples had a sensory score of six, meaning they were regarded as fit for human eating. The best possible treatment might increase the duration of the shelf life of 18 days (5.47 $\log_{10}$ CFU/g) of fresh mutton at 4°C (He *et al.,* 2016).

Nisin rapidly reduced the *L. monocytogenes* contamination in RTE meat products with little effect on the meat products' sensory quality. The concentration of *L. innocua* (FH1836lux) decreased by 1.3 $\log_{10}$ CFU/g after a 1h treatment with Nisin (12.5 µg/ml) (Nyhan *et al.,* 2021). The combination of isothiocyanate (AITC), Nisin, and garlic extract lowered the *E. coli* O157: H7 levels to 3.88 $\log_{10}$ CFU/g at 6°C by day 20, maintaining the fresh sausage's physical and chemical properties without sacrificing its sensory appeal (Araujo *et al.,* 2018).

Nisin and avocado peel extract microcapsules were added to ground beef to increase its shelf-life. Samples containing Nisin and avocado peel extract microcapsules demonstrated reduced oxidation and bacterial growth. Vacuum-packed microcapsules helped to significantly slow down both chemical and microbial spoilage compared to the control group (Calderon-Oliver *et al.,* 2020).

3.7 Aquatics

Safe and fresh aquatic goods have gained increased attention in recent decades because of their health benefits. Because aquatic products are more likely to be contaminated by foodborne pathogenic bacteria like *L. monocytogenes*, which can cause infections associated with food, they become a major food category (Jasour *et al.,* 2015). According to Li *et al.* (2019), 2.6% of freshwater fish in China had *L. monocytogenes* prevalence overall in 2019. The European Food Safety Authority (EFSA) and the European CDC combined data from 18 European countries to determine that the total incidence of *L. monocytogenes* in RTE fish products in 2017 was 7.0% (EFSA, 2018). Nisin, a natural preservative, is sometimes detected in aquatic foods like frozen vacuum-packed tuna and gilthead sea bream (Sofra *et al.,* 2018). Its presence helps control the growth of harmful bacteria like *L. monocytogenes.*

Table 3: Multi-hurdle approach with Nisin

Multi-hurdle technology	Notable antibacterial action against	Mode of action	References
Chelating agents (EDTA) + Nisin	Gram negative-bacteria	The Gram-negative cell wall's permeability is increased when Mg^{2+} and Ca^{2+} ions are removed by ethylene diamine tetraacetic acid and other chelating agents, which also permits the release of phospholipids.	Suganthi *et al.,* 2012
Thermal Sterilization + Nisin	*Listeria monocy to showgenes*	The growth of *L. monocytogenes* might be successfully inhibited by thermal sterilization. But it also kills some heat-sensitive nutrients, like vitamin C, and taints food's flavor and color.	De Souza *et al.,* 2020
Sonication + Nisin	*Listeria monocytogenes* *Escherichia coli* *Salmonella typhimurium*	It may lessen the quantity of pathogenic microorganisms (yeasts, molds, and bacteria) that contaminate kiwifruit juice. However, the treated kiwifruit juice showed a noticeable color shift and a decrease in ascorbic acid content.	Ordonez-Santos *etal.*, 2017; Yýldýz *et al.,* 2022
Phenolic compound + Nisin	*Listeria monocytogenes* *Escherichia coli* *Salmonella typhimurium*	Enhance the synergistic effect of antilisteria activity.	Hossain *et al.,* 2022
Encapsulating Nisin + chitosan	*Listeria monocytogenes*	The formation of silica liposomes by encasing Nisin with chitosan has the potential to dramatically suppress *L. monocytogenes* in cheddar cheese (down to 1.16 log10 CFU/g) while maintaining the cheese's organoleptic qualities (taste, texture, color, and off-color).	Cui *et al.,* 2016.
Nisin + Sodium citrate	*Listeria monocytogenes*	Reduced the concentration of *L. monocytogenes* by 2.56-3.48 log10 CFU/ml on fresh-cut tomatoes, improved color saturation, and vitamin C contents.	Ola-dunjoye *et al.*, 2016
Nisin + lacto-peroxidase	*Listeria monocytogenes*	Displays synergistic effects and increases Nisin's effectiveness.	Chen and Hoover, D.G., 2003

3.8 Beverages

3.8.1 Fruit juice

Fruit juice and other low-pH products can become spoiled and turned sour by the new acid-tolerant, endospore-forming Gram-positive bacteria *Alicyclobacillus acidoterrestris*. These heat-resistant bacteria are particularly sensitive to Nisin, which lowers the D-value by around 40% when it is present during pasteurization (Yamazaki *et al.,* 2000).

3.8.2 Alcoholic beverages

Nisin was utilized to "wash" pitching yeast polluted by lactic acid bacteria, in addition to being used during fermentation and bottling. Commercial brewing yeast strains maintained their activity and functionality even in the presence of Nisin. Nisin at 25µg/ml decreased the viability of the contaminated deteriorating organisms by 92% after a 4h therapeutic session. The lactic acid bacterial inactivation method employed here was superior to conventional acid-washing techniques since the *Saccharomyces* cultures remained flocculative and fermentative despite the Nisin treatment enhancing their viability and vigor. Additionally, Nisin has been tested in fruit brandy and wines (Thomas *et al.,* 2005).

3.8.3 Fruits and vegetables

Nisin prevented *Bacillus acidoterrestris* from growing vegetatively and from sporulating in orange juice, apples, and grapes (Barbosa *al.,*2017). At all temperatures, storage times, and circumstances, the growth of *Alicyclobacillus acidoterrestris* was suppressed by Nisin-containing fruit juices. Nisin is administered at 5ºC to decrease the quantity of *L. monocytogenes* and *S. aureus* in mangos (Barbosa *et al.,* 2017). To prevent listeria, tomatoes are combined with Nisin at 4, 10, and 25°C.

3.9 Multi-hurdle technology

A "multi-hurdle" approach, as opposed to relying exclusively on one technique, has become more popular because of worries about emerging pathogenic bacteria being resistant to traditional methods for preserving food and customers' reluctance to consume large amounts of preservatives made of chemicals. This tactic has demonstrated more efficacy when applied to Nisin in particular combinations areshown in Table 3.

4. Nisin as a modulator of the intestinal microbiota

The term "microbiota" describes the population of microscopic organisms that reside on or within a particular surface, such as the human body. It has a significant impact on digestion, boosts immunity, and may have an impact on general health and well-being. Nisin affects the makeup and activity of gut bacteria, which modulates the intestinal microbiota (Prabha, 2024).

Nisin's potential as a therapeutic agent is hampered by its breakdown in the gut. Studies have shown that digestive enzymes like chymotrypsin, trypsin, and pepsin readily degrade Nisin A (Slootweg *et al.,* 2013). Similar results were observed in recent tests simulating digestion in the mouth, stomach, and small intestine, where both Nisin Z and Nisin A were broken down (Soltani *et al.,* 2021b; Gough *et al.,* 2017).

As evidenced by several studies (Mousa *et al.*2017; Garcia-Gutierrez *et al.*2019a), the importance of the gut microbiota to human health is now expanding. Maintaining a balanced gut microbiome is emerging as a key therapeutic target. Research shows that imbalances in gut bacteria are linked to the development or worsening of conditions like diabetes, obesity, inflammatory bowel disease, and *C. difficile* infections (Duan *et al.,* 2022). Through *in situ* bacterial secretion or direct injection, an abundance of recent research has explored the promise of Nisin to regulate particular pathogens linked to chronic intestinal disorders and/or affect the gut microbiome. For example, in animal models, the inclusion of Nisin in the feed of hens has been demonstrated to promote growth rate and have a good impact on gastrointestinal ecology (Jozefiak *et al.,* 2013; Kieronczyk *et al.,* 2017). Comparable results were noted in models using rabbit models (Laukova *et al.,* 2014). Similarly, in a mouse model of bacterial diarrhea, Nisin treatments significantly altered the gut microbiome by diminishing disease-causing strains of *Enterococcus* spp. and *E. coli*while increasing helpful species like *Lactobacillus, Bacteroides*, and *Bifidobacterium* (Jia *et al.,* 2018). Studies using an emulated human colon system (mimicking the different sections of the large intestine) have shown that Nisin A is a powerful impediment to *C. difficile*. Nisin A demonstrated remarkable efficacy against *C. difficile*, reducing cell numbers by over 100-fold at a concentration of 76 μmol/l (20x the minimum inhibitory concentration) (Cinquin *et al.,* 2004; Le Lay *et al.,* 2015).

5. Food safety and legal aspects

A person's lifetime tolerable daily intake (TDI) is the maximum amount of a chemical they can ingest each day without significantly endangering their health. food safety and legal aspects of Nisin shown in Table 4.

Table 4: History of Nisin legislation

Year	Fact	References
1928	Nisin was discovered.	Jozala *et al.*, 2015
1951	First documented intentional use in a food process.	Rulis *et al.*, 2017
1969	Nisin and other bacteriocins are beneficial in providing antibiotic activity, as suggested by the UN Joint Expert Committee on Food Additives (JECFA), the Food and Agriculture Organization, and the World Health Organization. As a result, Nisin is recognized as a legitimate food preservative (Additive No. 234).	Jones *et al.*, 2005
1988	After reviewing Nisin, the USA Food and Drug Administration (FDA) determined that it should be granted GRAS certification, or "Generally Recognized as Safe." With a tolerable daily intake of 2.94 mg per day, the FDA declared Nisin safe for human consumption and authorized its use as a *Clostridium botulinum* inhibitor addition in canned goods.	Jones *et al.*, 2005
1995	The european food safety authority (EFSA) approved for some food products the use of Nisin (E234) as a preservative for the industry by Directive 95/2/EC.	EFSA *et al.*,2006

5.1 Toxicity

The FDA has approved this bacteriocin to be used as a safe preservative and categorized it as "Generally Recognized as Safe." It was principally employed in the dairy business. Nisin's usage of food additives prompted a response from European Food Safety Authority (EFSA).

The panel decided not to change the previous tolerable daily intake (TDI) of 0.13 $mgkg^{-1}$ body weight (bw), as established by the expert committee on food (ECF), after reviewing the applicable data. Taking into account that humans have been exposed to Nisin for numerous thousands ofyears,a safety panel has deemed it acceptable for use.

Maurício *et al.* (2017) reported that the oral lethal dose (LD_{50}) of the Nisin formulation in mice was 6950 $mgkg^{-1}$ body weight (bw), while the LD_{50} of the pure Nisin in rats was more than 2000 mg kg^{-1} body weight. Furthermore, Nisin has not been linked to any damage to the reproductive or developmental systems, nor is it known to have carcinogenic or mutagenic properties.

The Panel determined, based on the results of Hagiwara *et al.* (2010), that there was enough toxicological data to calculate a revised TDI using a no observed effect level (NOEL) of $mgkg^{-1}$ body weight (bw) per day. For the extrapolation of sub-chronic studies and inter- and intra-species variability, a TDI of 1 $mgkg^{-1}$ body weight (bw) per day for Nisin (E 234) was developed using a default unknown variable of 200 (EFSA, 2012). After considering all demographic categories, the Panel determined that the overall exposure estimate for Nisin A was less than the new TDI of 1 mg/kg bw per day. The proposed extension of use as a food additive (E 234) in heat-treated beef products (at an upper limit of 25 $mgkg^{-1}$) and young cheese (at an upper limit of 12 $mgkg^{-1}$) would not constitute a safety risk, according to the updated TDI of 1 $mgkg^{-1}$ body weight (bw) per day for Nisin A (EFSA, 2017).

6. Conclusion

Nisin is a potent bacteriocin in the field of food safety. This naturally occurring substance protects against harmful bacteria, especially Gram-positive ones that can cause spoilage or illness. Scientists have developed reliable methods to extract and measure Nisin, making it easy to incorporate into food production. Nisin's usefulness extends far and wide. It can be used in many different types of food, and when combined with other preservation techniques, it becomes even more effective. Exciting new research suggests that Nisin might even benefit our gut health by influencing the good bacteria that live there. Safety regulations allow the use of Nisin with confidence, and because it is minimally toxic, it is a natural choice for food preservation. As research continues, we may discover even more ways to utilize Nisin in new food systems and potentially even use it to manage the bacteria in our guts. Overall, Nisin is a promising tool for keeping our food safe.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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