Nisin Production from Waste: A Review

Bhavana NR[1], Pooja Muralidas [1], PR Shivadarshini [1], Dr. S Narendra Kumar[2]

Department of Biotechnology, RV College of Engineering, Bangalore, India

Abstract

Nisin is a bacteriocin produced by microbial fermentation of the well-known starter culture for dairy products, *Lactococcus lactis*. A major challenge in food safety and security is the prevention of the emergence of unwanted microbes. Artificial preservatives added to prolong the shelf life of foods may have negative effects on consumer health. An alternative approach is to use bio-preservatives. However, the cost of production of nisin is relatively higher than other chemical-based alternatives, because of which it has low marketability. Traditional production techniques are expensive and harmful to the environment. This review investigates sustainable alternatives employing waste products as fermentation substrates, including whey, corn stover, cane molasses, soya-bean cake, bean pulp and mussel processing waste. These eco-friendly strategies minimize negative environmental effects while providing resource and cost-efficient solutions. This review demonstrates the possibility for India to become self-sufficient in nisin production and reduce reliance on imports by emphasizing waste-to-value techniques.

Keywords: Nisin, Bio-preservatives, Waste-to-value, Sustainable, Eco-friendly

1. Introduction

Nisin, a natural antimicrobial peptide has attracted interest as a food preservative due to its significant antibacterial activities against Gram-positive bacteria and spores. It is made through microbial fermentation, usually using lactic acid bacterial strains like *Streptococcus lactis* or *Lactococcus lactis*. Nisin has several benefits over chemical preservatives, including a natural source, dietary safety, and effectiveness at low doses. However, issues with conventional nisin production include high production costs, negative environmental effects from unsustainable raw resources and dependency on pricey carbon and nitrogen sources such sugar, yeast powder, and peptone. There is an increasing need for sustainable solutions that use waste products as fermentation substrates to address these problems which can replace synthetic preservatives and lower the negative effects of their manufacture on the environment. It will not only lower prices but also enhance the general sustainability of the food sector. Adopting sustainable practises can pave the way for more resourceful and environmentally conscious methods of food production

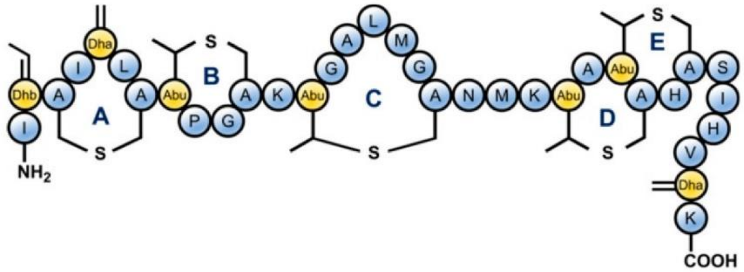

Fig 1: Structure of Nisin

1.1 Mechanism of action

Nisin has a clearly known mode of action that allows it to produce strong antibacterial activities. It begins by identifying and precisely attaching to the Lipid II molecule, a vital part of the bacterial cell membrane involved in the formation of cell walls. After binding, nisin functions as a membrane-depolarizing substance, generating a nisin-phospholipid complex that causes momentary membrane holes. By altering its conformation, nisin can breach the lipid bilayer and enter the cytoplasmic membrane. As a result, the membrane becomes more permeable, allowing ions or adenosine triphosphate (ATP) to flow through and inactivating sulphydryl groups in the cytoplasmic membrane. The several effects of nisin prevent spore and vegetative cell proliferation as well as hamper the germination procedure. Notably, the antibacterial properties of nisin are particularly sensitive to spores destroyed by heat. Therefore, nisin is an efficient and adaptable natural preservative with broad-spectrum antibacterial characteristics because of its method of action, which targets the bacterial cell membrane.

1.2 Properties

Nisin demonstrates remarkable safety for human consumption, as it is inactivated by enzymes in the stomach and cannot be detected in saliva within 10 minutes after ingestion. Its usage in food goods to increase their shelf life is justified by this safety profile. However, at temperatures below 4°C, modifications in lipid-hydrocarbon chains prevent its function. Nisin needs to be kept out of direct sunlight and at temperatures under 25°C to be stable. Also, it continues to function even at pH values that are acidic and, when necessary, at high temperatures.

When fatty acids are present in non-uniform compositions, nisin's hydrophobic nature can be impacted, which can hamper its function. It can therefore exercise its antibacterial characteristics without interference in liquid and homogeneous food products, where they are most effective.

Overall, nisin is a helpful and justifiable addition to food products to improve their preservation and shelf life due to its safety, stability, and effectiveness under specific circumstances.

1.3 Applications

In the food industry, nisin is used to prevent cheese from producing clostridial gas and to remove beer deterioration brought on by specific Gram-positive bacteria, enabling room-temperature storage. Shelf life of milk and dairy desserts are extended while being transported, and canned food deterioration is reduced.

As part of the hurdle technology, Nisin is essential for achieving product safety and a longer shelf life. In bakery goods, it prevents food-poisoning bacteria from growing, and in liquid egg products, it works with lysozyme. Nisin also has potential in dental care by obstructing biofilms, suppressing *Streptococcus mutans*, and preventing the development of plaque. Nisin suppresses *Propionibacterium acnes* and lessens inflammation in the treatment of acne. Additionally, by causing melanoma cells to undergo apoptosis, limiting tumour growth, and acting as a therapeutic agent, nisin has demonstrated potential in the study of cancer. It has also shown potential in lowering intestinal colonization of Vancomycin-resistant Enterococci (VRE) in mouse infection models and can aid in preventing the development of biofilm in bacteria that are resistant to antibiotics. Due to many applications in various fields, the production of nisin is very profitable and beneficial.

Fig 2: Cheese Whey

2. Production of nisin

2.1 From whey

Whey is collected from the cheese-making industry. To get rid of big debris, the whey is filtered. Evaporation or ultrafiltration are two techniques used to boost the nisin concentration in the whey solution. Nisin is separated from other substances found in the whey using chromatographic techniques such ion-exchange chromatography, size-exclusion chromatography, or affinity chromatography. Nisin and other compound-containing fractions are collected separately. To get a larger concentration of nisin, the nisin-containing fraction is further concentrated. The concentration of nisin and its antibacterial activity against target microorganisms are measured as part of the characterization of the isolated nisin fraction.

Using stabilizing agents or encapsulating methods, nisin is shielded from thermal deterioration. Nisin is incorporated into polymers to confer antimicrobial properties through methods such as blending, coating, or grafting.

The modified polymers are tested for their antibacterial qualities using standardized procedures to ascertain their inhibitory efficacy against diseases or spoilage microorganisms.

Fig 3: Corn stover

2.2 Using corn stover

Using fermentable sugars created from maize stover, lactic acid and nisin can be produced. Using Lactococcus lactis strains, the procedure comprises pre-treating maize stover to weaken its lignocellulosic structure, enzymatic hydrolysis to break down complex polysaccharides into simple sugars, and microbial fermentation. To make the best use of the sugars produced during enzymatic hydrolysis, simultaneous saccharification and fermentation (SSF) is used. During the SSF process, nisin production is measured using analytical techniques like HPLC or spectrophotometry. The production of nisin is increased through the optimization of process variables. The goal is to increase the production and yield of the targeted products.

2.3 Using cane molasses, soya-bean cake or bean pulp

It was carried out in two phases. In Phase 1, seeds are grown in a mixture of nutrients for six hours at 30 °C and 50 rpm in a shaking flask. A 10L fermentor tank is then used for batch fermentation utilising an 8% inoculum and 20% liquid caustic soda to maintain a pH of 6.5. The final nisin concentration reaches 5263 IU/mL after 24 hours. Phase 2 entails shake-flask culture, cane molasses fermentation in a 10L fermentor, and 100L fermentor culture. The final nisin content in the fermented liquid is 5915 IU/mL after 20 hours of fermentation.

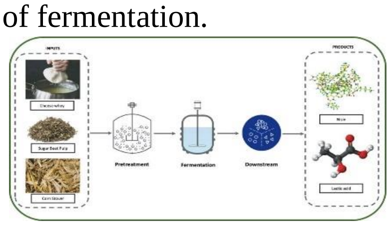

Fig 4: Cheese whey, Sugar beet Pulp and Corn Stover waste for production of Nisin

The use of industrial waste as fermentation raw material can significantly reduce production costs and turn waste into wealth. The bacterial strain used is *Streptococcus acidi lactici*.

2.4 Using mussel processing waste

The waste from the processing of mussels can be used as a substrate, together with glucose and five other nitrogen sources. Here, the chemical is produced sustainably using waste from the mussel-processing sector. The strain of *Lactococcus lactis subsp. lactis* CECT 539 that produces nisin is cultivated. After the mixture settled and the precipitates were removed, the pH was adjusted to 4-5. Using commercial amylase, glucose was produced from glycogen. The media was sterilised, and its impact on the formation of bacteriocin and biomass was investigated.

The results show that glycine inhibits the formation of bacteriocin. Glutamic acid boosted biomass production while having a somewhat beneficial impact on nisin synthesis. Although high quantities of yeast extract led to a decrease in nisin production with rising glucose concentration, the greatest positive output for nisin production was seen with yeast extract. It was discovered that adding glucose was essential for increasing the creation of antimicrobial peptides, and that the type of nitrogen source had a big impact on both peptide synthesis and bacterial growth.

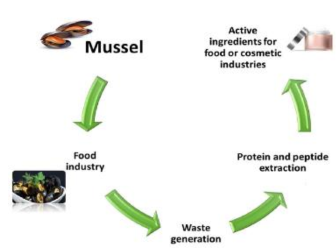

Fig 5: Use of Mussel processing waste in Bacteriocin production

3. Conclusion

There is a growing demand for dairy and agricultural products as India is primarily a dairy and plant-rich diet. Due to the huge demand, there is also an enormous increase in the waste associated with it. This increases the pressure on the environment and leads to various types of pollution. The generated waste is so high that we are pushed to find out new ways to extract any benefit from the unusable surplus. Applying biotechnological inventions and advancements can help us utilise waste to its fullest potential and thereby reducing the environmental effects. The usage of waste products to produce nisin can offer an affordable and sustainable replacement for conventional production techniques and can also make India self- sustained and reduce the dependence on import of nisin.

4. References

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