Nisin as a food preservative

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Nisin is a natural, toxicologically safe, antibacterial food preservative. It is regarded as natural because it is a polypeptide produced by certain strains of the food-grade lactic acid bacterium *Lactococcus lactis* subsp. *lactis* (hereafter referred to as *L. lactis)*, during fermentation. Nisin exhibits antimicrobial activity towards a wide range of Gram positive bacteria, and is particularly effective against spores. It shows little or no activity against Gram negative bacteria, yeasts, and moulds.

In 1969, nisin was approved for use as an antimicrobial in food by the Joint FAO/WHO Expert Committee on Food Additives. Since then nisin has been given the food additive number 234 and is permitted currently for use in over 50 countries. In Australia and New Zealand it is allowed in cream products (flavoured, whipped, thickened, and sour cream) at a maximum of 10 mg/kg; in crumpets, flapjacks and pikelets (hot plate flour products) at a maximum of 250 mg/kg; and in cheese and cheese products, oil emulsions (<80% oil), tomato products pH 4.5, beer and related products, liquid egg products, dairy and fat based desserts, dips and snacks, sauces, toppings, mayonnaises and salad dressings at levels compliant with good manufacturing practice.

The suitability of nisin as a food preservative arises from the following characteristics: it is non-toxic, the producer strains of *L. lactis* are regarded as safe (food-grade); it is not used clinically; there is no apparent cross-resistance in bacteria that may effect antibiotic therapeutics; and it is quickly digested. Since 1953, nisin has been sold under the trade name of *Nisaplin®*. *Nisaplin®* contains approximately 2.5% nisin, the balance consisting of milk and milk solids derived from the fermentation of a modified milk medium by nisin producing strains of *L. lactis*. The product is standardised to an activity of one million international units per gram. Throughout this article all levels of nisin are expressed as mg pure nisin per kilogram or litre. The assay method in most common use involves measuring zones of inhibition in agar seeded with the test organism, *Micrococcus lactis*.

Mode of action

Nisin works in a concentration dependent fashion; thus the more bacteria present in a food the more nisin may be required. Nisin initially forms a complex with Lipid II, a precursor molecule in the formation of bacterial cell walls. The nisin–lipid II complex then inserts itself into the cytoplasmic membrane forming pores and allows the efflux of essential cellular components resulting in inhibition or death of the bacteria.

Gram negative bacteria are resistant to nisin because their cell walls are far less permeable than those of Gram positive bacteria. However any treatment of Gram negative bacteria to make their cell walls permeable to nisin makes them susceptible to nisin. Such treatments include exposure to chelating agents, sub-lethal heat, osmotic shock and freezing. Although chelating agents show good in vitro effects in buffer systems, results in food systems are far less pronounced owing to preferred interaction between chelating agents and divalent ions in foods. Nisin action against spores is far less understood. It is predominantly sporostatic rather than sporocidal, the more the spores are heat damaged the more they are susceptible to nisin, and nisin appears to bind to sulphhydryl groups on the spore surface.

Stability and solubility

Nisaplin® is an extremely stable product, showing no loss of activity over two years when stored under dry conditions in the dark, below 25°C. Nisin shows increased solubility in an acid environment and becomes less soluble as the pH increases. However, owing to the low level of nisin used in food preservation, solubility does not present a problem. Nisin solutions are most stable to autoclaving (121°C for 15 min) in the pH range 3.0–3.5 (<10% activity loss). At pH values below and above this range, there is marked decrease in activity (>90% loss at pH 1 or 7). Losses of activity at pasteurisation temperatures are significantly less (approximately 20% during standard processed cheese manufacture at pH 5.6–5.8). Food components can also protect nisin during heat processing as compared to a buffer system.

The stability of nisin in a food system during storage is dependent upon three factors: incubation temperature, length of storage and pH. Greater nisin retention occurs at lower temperatures. For instance, the manufacture of a pasteurised processed cheese spread (85–105°C for 5–10 min at pH 5.6–5.8) results in an initial 20–30% loss, nisin retention after 30 weeks storage being approximately 80% at 20°C, 60% at 25°C and 40% at 30°C. Thus a higher level of nisin addition will be required if storage at unusually high ambient temperature is intended.

In cold processed foods, proteolytic enzymes can affect nisin stability. The food additives, titanium dioxide and sodium metabisulphite can also adversely affect nisin stability.

Table 1 summarises the major categories of food in which nisin is used, and the typical spoilage or pathogenic bacteria in these products that are controlled by nisin.

Dairy products

Processed cheese products

Processed cheese products cover a wide range, including block cheese (44–46% moisture), slices (46–50% moisture), spreads (52–60% moisture) and sauces and dips (56–65% moisture). All are heat processed and contain emulsifying salts. Formulations can be of low-fat or reduced sodium chloride content and may contain various flavour additives such as herbs, fish, shellfish and

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meat. All these factors along with bacterial quality of the raw ingredients, severity of the melt process, filling temperature, and shelf-life requirement can affect the microbial stability of processed cheese products and hence the requirement for and level of nisin.

Ingredients used in the manufacture of these products are raw cheese, butter, skimmed milk powder, whey powder, phosphate or citrate emulsifying salts and water. Spores of anaerobic clostridial species are often present in these ingredients, particularly the cheese, and are able to survive the heat process of 85–105°C for 6–10 minutes. The composition of processed cheese, relatively high pH (5.6–6.0) and moisture content and low redox potential (anaerobic conditions), can result in spore germination and growth. *Clostridium* species often associated with the spoilage of processed cheese are *C. sporogenes, C. butyricum,* and *C. tyrobutyricum*. In trials with processed cheese products inoculated with a cocktail of spores of these *Clostridium* spp. at approximately 200 spores per gram, spoilage was prevented during storage at 37°C by 6.25 mg/kg nisin. Partial control was achieved with 2.5 mg/kg while control samples without nisin readily spoiled.

The potential for growth and toxin production by *C. botulinum* in processed cheese products, particularly spreads, is of considerable significance. In processed cheese spreads, nisin at levels 12.5 mg/kg and above is effective in delaying or preventing growth and subsequent toxin production by inoculated spores of *C. botulinum* types A and B. Facultative aerobic *Bacillus* spp. which can also cause spoilage in processed cheese products are also controllable using nisin at levels 5–20 mg/kg.

Other pasteurised dairy products

Other pasteurised dairy products, such as dairy desserts, cream, clotted cream and mascarpone cheese, often cannot be subjected to full sterilisation without damaging quality and are thus sometimes preserved with nisin. Tests on a chocolate dairy dessert resulted in a 20 day increase in shelf life at 7°C with 3.75 mg/kg nisin while the same nisin level gave a 30 day increase in shelf life at 12°C for a crème caramel dessert.

The addition of nisin to pasteurised milk is permitted in some countries. In trials at Reading University, UK, nisin added at 1 mg/L before pasteurisation at 72°C/15s, 90°C/15s or 115°C/2s resulted in significant shelf-life extension of the milk at 10°C.

Natural cheese

The first application of nisin was to prevent blowing problems in semi-hard ripe cheese such as Emmenthal and Gouda due to growth of *C. butyricum* and *C. tyrobutyricum*. Although promising results were obtained, a problem with inhibition of the starter cultures and consequent delay of the ripening process led to the work being discontinued. Using food-grade genetic transfer techniques of conjugation it has now been possible to develop nisin-producing, nisin-resistant starter cultures with the desired properties for cheese quality. Cheeses have been made with sufficient nisin content to provide protection against growth of *Clostridium* spp., *Staphylococcus aureus* and *Listeria monocytogenes.*

Yoghurt

The addition of nisin to stirred yoghurt post-production has an inhibitory effect on the starter culture (a mixture of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains), thereby preventing subsequent over-acidification of the yogurt. Thus an increase in shelf life is obtained by maintaining the flavour of the yoghurt (less sour) and preventing syneresis. Typical addition levels for this application are 0.5–1.25 mg/kg.

Egg products

Pasteurised liquid egg products (whole, yellow and white) and value-added egg products (eg omelettes, scrambled eggs, pancake mixes) receive heat treatments for destruction of *Salmonella*. In the UK for instance, liquid whole egg must be pasteurised for at least 2.5 min at 64.4°C. However, such heat treatment is insufficient to kill bacterial spores and the more heat-resistant non-spore forming Gram positive bacteria such as *Enterococcus faecalis*. Nisin at 2.5–5 mg/L gives significant increases in shelf life and protection against growth of the psychroduric food poisoning bacteria *B. cereus* and *L. monocytogenes*.

Pasteurised soups

A recent trend in soup manufacture has been production of fresh pasteurised products with relatively limited chilled shelf life. Heat resistant spores of *Bacillus* spp. are able to survive pasteurisation. Nisin at levels of 2.5–5.0 mg/L is effective at preventing or delaying outgrowth of psychroduric spoilage *Bacillus* spp. during prolonged storage.

Table 1. Typical addition levels of nisin and *Nisaplin*® in food applications

Food application	Typical target organisms	Level of nisin (mg/kg or mg/L)	Level of *Nisaplin*® (mg/L)
Processed cheese	*Clostridium* spp. *Bacillus* spp.	5–15	200–600
Pasteurised milk and milk products	*Clostridium* spp. *Bacillus* spp.	0.25–10.0	10–400
Pasteurised chilled soups	*B. cereus* *C. pasteurianum*	2.5–6.25	100–200
Crumpets	*B. cereus*	4–6.25	150–250
Canned foods (high acid)	*C. botulinum and thermosaccharolyticum*	2.5–5.0	100–200
Ricotta cheese	*Listeria monocytogenes*	2.5–5.0	100–200
Continental type cooked sausage	Lactic acid bacteria, *Brochothrix thermosphacta, L. monocytogenes*	5–25	200–1000
Dipping sauces	Lactic acid bacteria	1.25–6.25	50–250
Salad dressings	Lactic acid bacteria	1.25–5	50–200
Beer: pitching yeast wash	Lactic acid bacteria, eg. *Lactobacillus*, *Pediocococcus*	25.0–37.5	1000–1500
post fermentation		0.25–1.25	10–50

Flour based products

Crumpets are popular products in UK, Australia and New Zealand, produced on a hot plate from a flour batter with yeast or an aerating agent or both to give them a raised profile and open texture. They are toasted before eating. Crumpets have a non-acid pH (pH 6), high moisture (48–54%) and high water activity (0.95–0.97). The product is sold at ambient temperature and has a shelf life of five days. There have been a number of food poisoning outbreaks due to the growth of *Bacillus cereus* in crumpets, particularly in Australasia. Flour used in the manufacture of crumpets invariably contains low numbers of *B. cereus* spores that are not killed during the hot plate cooking process. During the 3–5 day ambient shelf life of the product, *B. cereus* can increase from undetectable levels to > 10^5 cfu/g – sufficient bacteria to cause food poisoning. Addition of nisin to the batter mix at ≥ 3.75 mg/kg to prevent the growth of *B. cereus* has received regulatory approval in Australia and New Zealand.

Canned foods

Nisin is used in canned foods mainly for the control of thermophilic spoilage. It is mandatory in most countries that low acid canned foods (pH>4.5) receive a minimum heat process of $F_0 = 3$ to ensure the destruction of *C. botulinum* spores. Low acid foods processed at $F_0 \geq 3$ are susceptible to spoilage from surviving heat-resistant spores of thermophilic bacterial species of *B. stearothermophilus* (cause of flat sour spoilage) and *C. thermosaccharolyticum* (cause of blown cans). Nisin addition can facilitate prolonged storage of canned vegetables at warm ambient temperatures by inhibiting spore outgrowth of these thermophilic organisms and can also allow a reduction in the process down to the minimum of F_0=3 without increasing the risk of thermophilic spoilage. Nisin usage levels in low-acid canned vegetables are 2.5–5.0 mg/kg. Residual nisin levels in canned foods after high temperature processing can be as low as 2% of the addition level. However, the fact that heat-resistant thermophilic spores are highly sensitive to nisin combined with the heat damage enhancing their sensitivity means that extremely low levels of residual nisin can still be effective in this application. Pre-acidification of the brine with citric acid improves nisin retention with minimal effect on the pH of the vegetables after processing.

Examples of use are canned peas, carrots, peppers, potatoes, mushrooms, okra, baby sweet corn, and asparagus. Nisin is also used in canned dairy puddings containing semolina and tapioca.

Bacterial spoilage of canned high acid foods (pH below 4.5) is restricted to non-pathogenic spoilage species such as *C. pasteurianum, B. macerans* and *B. coagulans.* Nisin addition levels of 1.25–2.50 mg/kg are used in high acid tomato-based products.

Meat products

Concern regarding the high levels of nitrite in cured meat has resulted in research investigating the use of nisin as a partial replacement for nitrite. Only high (and uneconomic) levels of nisin, achieved good control of *C. botulinum.* Further work is necessary before a case for such an application is demonstrated. More encouraging results have been obtained in vacuum-packed cooked continental type sausages where lactic acid bacteria can cause spoilage. Nisin in the mix at 1.25–6.25 mg/kg or dipping the cooked sausage into nisin solutions of 5.0–25.0 mg/L has increased shelf life at 6–12°C. Nisin has better inhibitory effects against lactic acid bacteria in sausages with lower fat levels, and in sausages containing diphosphate compared to those with orthophosphate. This application has achieved regulatory approval in USA.

Seafoods

The potential hazard of botulism in both vacuum-packed and modified atmosphere packed fish led to work at the Torry Research Station in the UK where application of nisin by spray to fillets of cod, herring and smoked mackerel inoculated with *C. botulinum* Type E spores resulted in a significant delay in toxin production at 10 and 26°C. Another problem in smoked fish is growth of the psychroduric pathogen *L. monocytogenes*, especially in fresh and lightly preserved products. Nisin is an effective antilisterial agent in smoked salmon, especially when packed in a carbon dioxide atmosphere.

Nisin at 25 mg/kg in combination with a reduced heat process, that does not cause product damage of lobster meat, achieved a *Listeria* kill significantly better than either heat or nisin alone. Washing crabmeat with nisin reduced levels of *L. monocytogenes.*

Salad dressings

Reduced acidity may improve the flavour of cold blended salad dressings but using reduced levels of acetic acid and raising the pH from 3.8 to 4.2 can make salad dressings prone to lactic acid bacterial spoilage. Such growth has been successfully controlled by nisin at 2.5–5.0 mg/L.

Alcoholic beverages

Acid tolerant lactic acid bacteria of the genera *Lactobacillus, Pediococcus,* and *Leuconostoc* can spoil beer and wine and nisin, at levels of 0.25–2.5 mg/L, is effective in preventing such spoilage. Yeasts are unaffected by nisin, thus the preservative can be added during the fermentation. Nisin can be added to fermenters to prevent or control contamination and can also be used to increase the shelf-life of unpasteurised and bottle-conditioned beers. Furthermore, nisin can be used in the pitching yeast wash, at 25.0–37.8 mg/L, as an alternative to acid washing for the control of lactic acid bacteria.

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