Where science
& creativity meet

Functionally proven commercial preparation of nisin

NISAPLIN® ANTIMICROBIAL

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One of the most effective and reliable label-friendly solutions available to the food industry to secure quality and improve safety over shelf life

For more than 60 years, food manufacturers in countries around the world have been using NISAPLIN®, IFF's commercial preparation of Nisin A, to delay outgrowth of undesirable microorganisms. Nisin A is a naturally occurring antimicrobial considered one of the most effective available solutions against Gram positive and sporeformers. It is an easy to use, label-friendly way to protect and extend product quality throughout the desired shelf life in a wide range of food and beverage applications.

With its regulatory approval continuing to expand, NISAPLIN® provides the food industry an unprecedented way to meet consumer needs. And by partnering with IFF, you gain access to the industry's most robust portfolio of food protection solutions combined with experts who can help you solve challenges and win in the market.

Your daily challenges

- Maintain product quality throughout desired shelf life
- Inhibit Gram-positive bacteria and sporeformers
- Formulate with effective, label-friendly solutions
- Protect your brand image and equity
- Comply with local regulatory guidelines

Features and benefits

NISAPLIN® is	NISAPLIN® helps you to
Naturally occurring antimicrobial Produced via fermentation with traditional starter culture	Satisfy consumer demand for simple and understandable ingredient label declarations
Best in class antimicrobial efficacy	Extend and secure your product shelf life reducing returns and waste Improve food safety to help protect your brand image
Not altering flavor, odor or appearance of food	Contribute to a positive eating experience
Easy and safe to use Stable through food processing heat treatment and storage conditions	Secure rapid development of new and innovative food products
Not produced from GMOs Entirely produced in Europe (from fermentation to packaging)	Capture loyalty of food consumers concerned with food quality, safety and sustainability

FAO/WHO approved applications[1]

Dairy	Culinary	Bakery
• Flavored fluid milk drinks [01.1.4] • Clotted cream (plain) [01.4.3] • Unripened cheese [01.6.1] • Ripened cheese [01.6.2] • Processed cheese [01.6.4] • Cheese analogues [01.6.5] • Whey-protein cheese [01.6.6] • Dairy-based desserts (e.g. pudding, fruit or flavored yoghurt) [01.7] • Cereal and starch-based desserts (e.g. rice pudding, tapioca pudding) [06.5]	• Heat-treated processed meat, poultry, and game products in whole pieces or cuts [08.2.2] • Heat-treated processed comminuted meat, poultry, and game products [08.3.2] • Edible casings (e.g. sausage casings) [08.4] • Liquid egg products [10.2.1] • Ready-to-eat soups and broths, including canned, bottled, and frozen [12.5.1]	• Fine bakery wares (sweet, salty, savory) and mixes [07.2]

[1] *Refer to regulatory guidelines for specific application criteria and usage levels from The "Codex General Standard for Food Additives" (GSFA, Codex STAN 192-1995) on GSFA Online@ http://www.fao.org/gsfaonline/additives/details.html?id=1*

Main microorganisms inhibited by NISAPLIN®

Gram-positive bacteria and sporeformers

Spoilage contaminants	Pathogens
• *Alicylobacillus acidoterrestris*	
• *Bacillus spp.*	• *Bacillus cereus*
• *Brochothrix thermosphacta*	
• *Clostridium spp.*	• *Clostridium botulinum*
• *Desulfotomaculum nigrificans*	
• *Enterococcus spp.*	• *Enterococcus faecium*
• *Geobacillus stearothermophilus*	
• *Lactobacillus spp.*	
• *Leuconostoc spp.*	
• *Listeria innocua*	• *Listeria monocytogenes*
• *Micrococcus spp.*	
• *Pediococcus spp.*	
• *Staphylococcus spp.*	• *Staphylococcus aureus*

DAIRY

Processed cheese

In processed cheese, the melt process typically involves a heat treatment that kills vegetative bacterial cells, yeasts and molds but not bacterial spores. Product composition, high pH and moisture content, as well as anaerobic conditions favor the outgrowth of anaerobic spore-forming bacteria like *Clostridium* spp. These bacteria are often present in raw cheese, butter, skimmed milk, whey powder, herbs and spices used on processed cheese recipes.

NISAPLIN® has been used in processed cheese since the 1950s and is widely accepted by the industry and regulatory authorities around the world for this application. The antimicrobial effectiveness of NISAPLIN® allows processed cheese to be stored at ambient temperatures for a longer period and reduces the risk of *C. botulinum* outgrowth and toxin formation.

Even after UHT treatment, processors are adding NISAPLIN® to ensure a long shelf life, particularly for product stored at high ambient temperature.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Block cheese Sliced cheese Spread cheese	From 100 to 600 mg/kg	• CODEX: 480 mg/kg • EU: 500 mg/kg • US: 250 mg/kg • CN: 450 mg/kg • Mercosur: 500 mg/kg • CA: 1,200 mg/kg (only unstandardized processed cheese products)

Unripened cheese

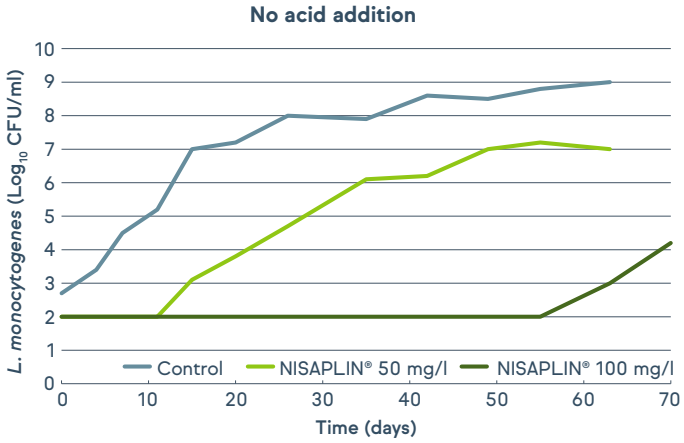
Soft, white, fresh cheeses going through minimal processing before packaging are highly perishable and have a short shelf life, even at refrigerated temperature. Additionally, they are highly susceptible to *Listeria monocytogenes* contamination risk.

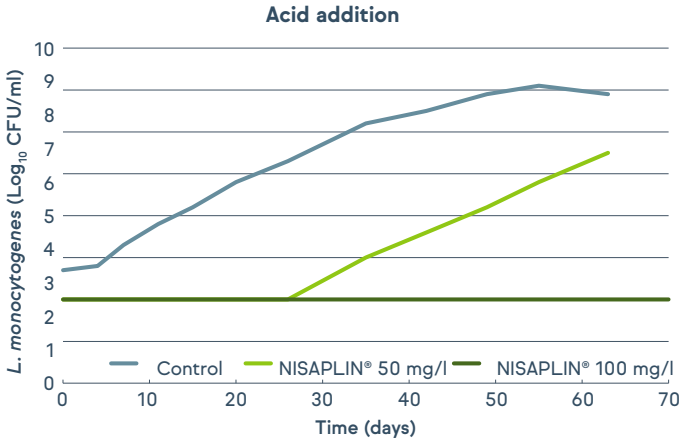
NISAPLIN® can be used to extend the shelf life of these unripened cheeses. Now the main interest of using NISAPLIN® in unripened cheese is to effectively control *Listeria monocytogenes* contamination.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Cottage cheese Quark Feta cheese Ricotta Fresh mozzarella	From 100 to 500 mg/kg	• CODEX: 480 mg/kg • EU: 400 mg/kg (Mascarpone) • US: 250 mg/kg • CN: 450 mg/kg • Mercosur: 500 mg/kg

NISAPLIN® effectively controls the growth of *Listeria monocytogenes* in ricotta-type cheese[2]

***Listeria monocytogenes* challenge study:** In ricotta-type cheese production, NISAPLIN® may extensively increase the shelf life of product when stored at 6–8°C. A pool of strains of *L. monocytogenes* was effectively controlled, when present in low numbers, for a period of 8 weeks or even longer with acetic acid addition.





[2] Graphs reproduced from publication: Davies, E. A. et al. (1997) The use of the bacteriocin, nisin, as a preservative in ricotta type cheeses to control the food-borne pathogen Listeria monocytogenes. Letters in Applied Microbiology 24: 343–346.

Dairy desserts

Dairy desserts are manufactured using either a pasteurization or a UHT process. Pasteurized products have a limited shelf life even when chilled, while UHT products have a longer shelf life at ambient temperature. Ingredients such as sugar, milk powders, cream, starches and flavoring ingredients may contain heat-resistant spores of Gram-positive bacteria. These spores can survive pasteurization processes, thus, limiting shelf life when products are stored at refrigerated temperatures. Even in UHT processed dairy desserts, the survival of thermophilic spores can cause spoilage problems, particularly if products are stored at high ambient temperatures.

NISAPLIN® is an effective antimicrobial in pasteurized and UHT processed dairy desserts preventing the outgrowth of heat-resistant bacterial spores. Greatest effectiveness occurs in desserts with homogenous composition or in desserts that are hot filled and sealed after pasteurization or aseptically filled into sterile containers.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Dairy desserts	From 75 to 250 mg/kg	• CODEX: 480 mg/kg • EU: 120 mg/kg (Semolina, tapioca puddings and similar products) • US: 600 mg/kg • CN: 450 mg/kg

Cream products

This product category is quite diverse but present similar bacteriological problems to other dairy products. The stability and characteristic of cream products depend on the heat regime and other process treatments used. Compared to other dairy categories, heat treatments are often limited, making the use of NISAPLIN® even more essential to improve product shelf life or reduce storage constraints.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Cream products	From 50 to 200 mg/kg	• CODEX: 400 mg/kg (clotted cream) • EU: 400 mg/kg (clotted cream) • US: 250 mg/kg • CN: 450 mg/kg

Examples of shelf life extension in dairy products

Ricotta cheese (3-5°C)

Control	+20mg/kg NISAPLIN®
10 days	40 days
4 times extension	

Pasteurized hot filled cream caramel (12°C)

Control	+50mg/kg NISAPLIN®
6 days	35 days
More than 5 times extension	

Pasteurized aseptic cold filled chocolate dairy dessert (7°C)

Control	+50mg/kg NISAPLIN®	+150mg/kg NISAPLIN®
7 days	21 days	31 days
From 3 to 4.5 times extension		

CULINARY

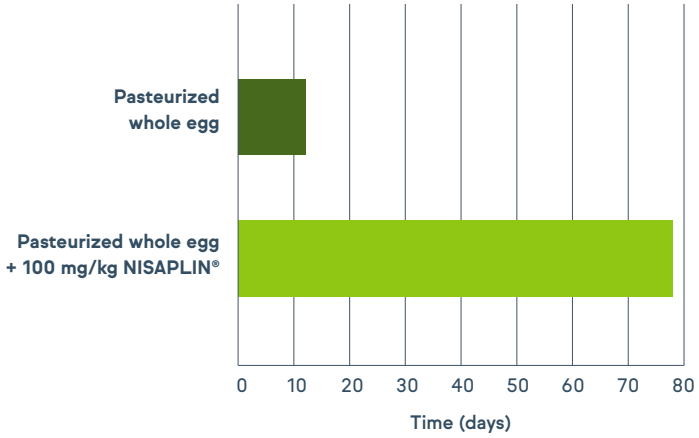
Liquid egg and batters/mixes

Liquid egg products are widely used by various sectors of the food industry. They are popular in hotels, restaurants and institutions because of their convenience and versatility. The presence of *Salmonella* and *Listeria* in commercially broken raw liquid egg products and *Bacillus* as well as lactic acid spoilage bacteria in commercially pasteurized liquid egg products are well documented. As egg proteins have low thermostability, pasteurization regimes mandated in various countries for processed liquid eggs are limited to conditions sufficient to destroy all pathogenic types of microorganisms present (vegetative cells). As a result, non-pathogenic thermophiles and endospores of sporeformers may survive the heat treatment. Surviving bacteria include both Gram-negative and Gram-positive species such as the spore-forming *Bacillus*. Many of these surviving bacteria are capable of growth at refrigeration temperatures.

NISAPLIN® added prior to the pasteurization of liquid egg products can prevent or delay the outgrowth of heat-resistant bacterial spores, including those of *Bacillus cereus*. It can also control the growth of other Gram-positive bacteria that can survive the heat process, e.g. *Enterococcus faecalis* and *Listeria monocytogenes*. The results of trials and commercial usage feedback indicate significant increases in shelf life (double or more), as well as the control of food-poisoning pathogens like *Bacillus cereus* and *Listeria monocytogenes*.

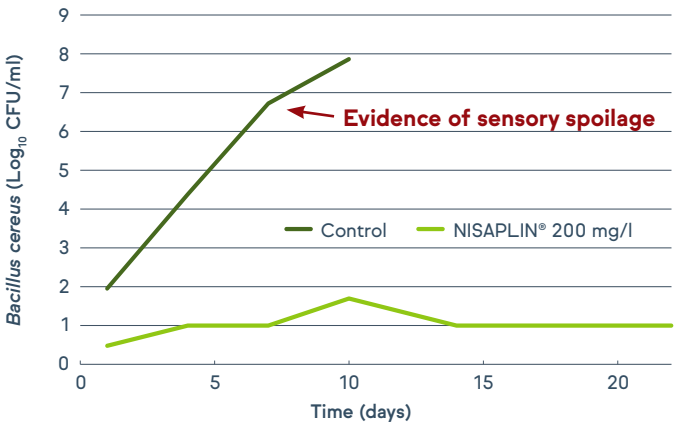
Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Whole egg Egg white (Albumen) Scrambled egg Omelet Reduced/free cholesterol products Batters Pancake mixes	From 100 to 250 mg/kg	• CODEX: 240 mg/kg • EU: 250 mg/kg (only pasteurized liquid egg (white, yolk or whole eggs)) • US: 600 mg/kg (only liquid eggs and egg substitutes) • CN: 225 mg/kg (only egg products (changed physical properties)) • CA: 600 mg/kg

Shelf life[3] extension of pasteurized whole egg with NISAPLIN®



[3] Days before reaching total plate count > 10^6 CFU/g (microbial deterioration limit) when stored at 8°C.

NISAPLIN® controls the growth of *Bacillus cereus* in pasteurized liquid whole egg



Spoilage *Bacillus cereus* contamination test: In pasteurized liquid whole egg production, NISAPLIN® may extensively increase the shelf life of product when stored at 6–8°C. *Bacillus cereus* cell count of NISAPLIN® treated samples remains below 10^2 CFU/ml for 22 days while control samples reached 10^6 CFU/ml after 7 days and show total discoloration and strong off odors after 10 days.

Processed meat products (including poultry meat)

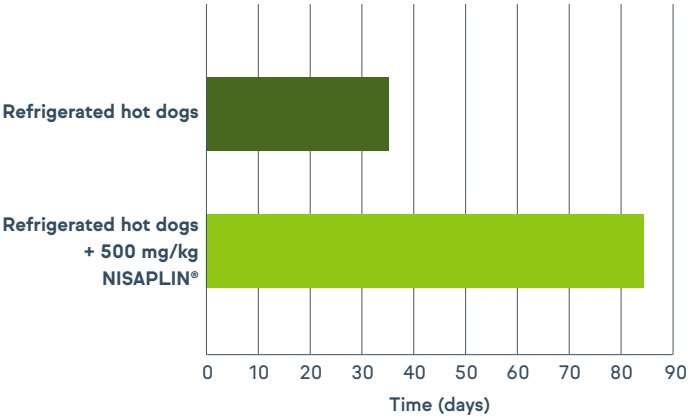
Cooked, sliced and pre-packaged meat products are popular convenience foods. The microbiological stability of these products is impacted by product composition, packaging method and storage temperature. Cooked ready-to-eat (RTE) meat & poultry products are typically chilled-stored in modified atmosphere parks or distributed unpacked. In retail shops, product can be sliced thus increasing contamination risk.

Lactic acid bacteria (LAB) are the major bacterial group associated with spoilage of refrigerated vacuum or modified atmosphere packaged cooked, cured and uncured meat products. During cold storage, psychrotrophic LAB reaching high cell counts will cause sour off-flavors, discoloration, gas production, slime production and/or drop in pH. Beyond spoilage, post process contamination by *Listeria monocytogenes* presents a greater public health concern risk. Its ability to grow at refrigerated temperatures and the lack of a heat kill step prior to consumption by consumers has resulted in documented food safety incidences.

Studies show that NISAPLIN® reduces initial *Listeria monocytogenes* counts and extend its lag phase, thereby hindering *Listeria monocytogenes* outgrowth in the processed meat products over shelf life. NISAPLIN® also inhibits the growth of Gram-positive spoilage bacteria common to processed meat products.

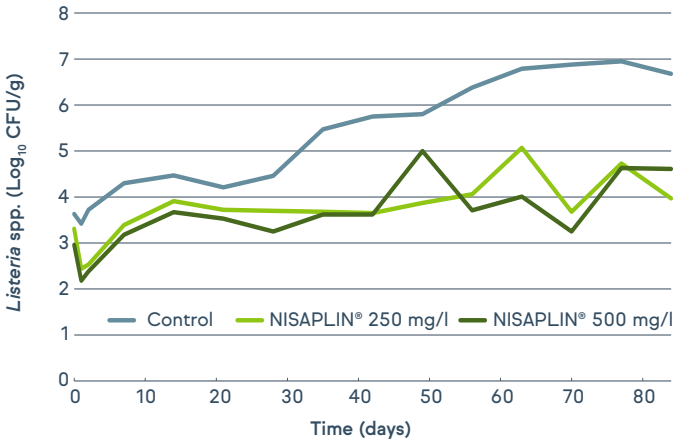
Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Hot dogs/frankfurters Bologna Other sausage types Cooked hams Roast	From 250 to 500 mg/kg	• CODEX: 1,000 mg/kg • US: 400 mg/kg • CN: 450 mg/kg • Mercosur: 1,000 mg/kg • CA: 1,000 mg/kg

Refrigerated hot dogs treated with NISAPLIN® stayed fresh for more than two times longer than the control



Spoilage LAB contamination test: LAB count of NISAPLIN® treated samples were 1 Log lower than control after 1 day. After 35 days (day when control reach quality alteration indicator), it remained at least 2 Log below control until the end of the study (84 days) never reaching quality alteration indicator.

NISAPLIN® effectively reduces *Listeria* load and outgrowth over shelf life in hot dog/frankfurter products



***Listeria* challenge test:** Initial log reductions in *Listeria* for hot dogs treated with 250 mg/kg and 500 mg/kg NISAPLIN® were 0.87 and 0.78 respectively, much greater than that of the control (0.21).

OTHER APPLICATIONS

Examples of additional applications approved in some countries

Culinary

- RTE smoked fish
- Sauces, dips & spreads
- Salad dressings
- RTE vegetable sides (including potato-based products)

Beverages

- Fruit juices (including concentrates)
- Beverages containing fruit juices
- Malt-based non-alcoholic drinks

Smoked fish

Listeria monocytogenes has been isolated from many types of seafood products with a relatively high prevalence in vacuum-packed cold smoked fish, particularly smoked salmon and trout. Despite considerable efforts to improve process hygiene and sanitation procedures in production facilities, the complete elimination of the pathogen in which smoked fish are produced is extremely challenging. Typical product characteristics like pH, water activity, salt, and smoke components are insufficient to prevent the growth of *L. monocytogenes* in chilled vacuum-packed products. As such it represents an important public health concern and exposes consumers to the risk of listeriosis.

NISAPLIN® can inhibit *L. monocytogenes* growth in the smoked fish over the product's shelf life. It also inhibits the growth of Gram-positive spoilage bacteria common to smoked fish.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Smoked fish	From 250 to 500 mg/kg	• US: 400 mg/kg • CN: 450 mg/kg (all fully preserved fish and fish products) • CA: 1,000 mg/kg

Processed potatoes

Processed potatoes, simply by nature of their source and processing conditions, are subject to significant contamination by spoilage and/or potentially pathogenic organisms, namely lactic acid bacteria (LAB), spore-forming bacilli and clostridia. Common potato processing practices usually involve initial treatment with water rinses containing antimicrobials followed by steam heating after which the potatoes are cooled and further processed. The heating steps kill most vegetative cells, but thermally resistant bacterial spores may survive. Further processed potato products like shreds or hash browns generally suffer from spoilage caused by lactic acid bacteria. Other ingredients used in the formulation can be the source of contamination or can be a potential growth environment for toxin producing spore formers such as *B. cereus*.

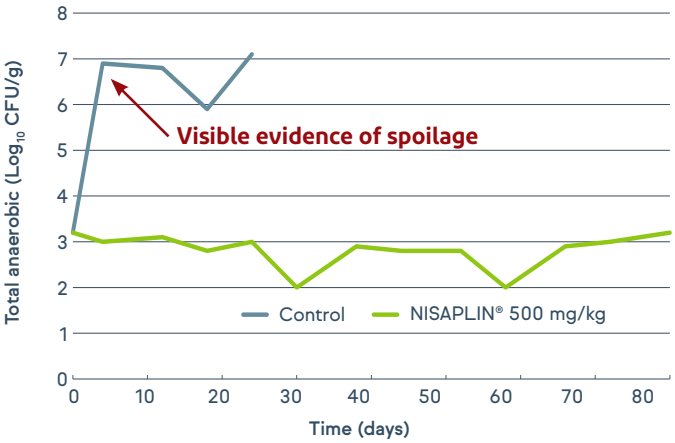
NISAPLIN® is an effective inhibitor of both spore-forming and spoilage LAB in potato processing.

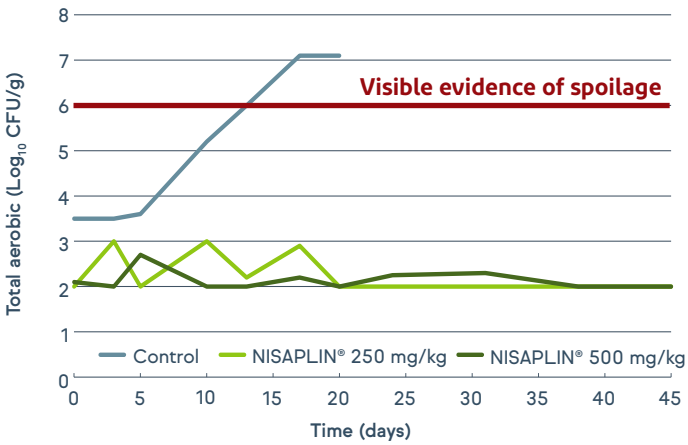
Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Minimally processed sliced potatoes Minimally processed shredded potatoes Minimally processed diced potatoes Fully cooked potatoes Refrigerated mashed potatoes Gratin Hash browns	From 250 to 500 mg/kg	• US: 300 mg/kg • CA: 500 mg/kg (only refrigerated cooked potato-based products)

When used in pasteurized mashed potatoes NISAPLIN® contributes to a great sensory experience throughout shelf life[4]

In a series of experiments, IFF investigated the efficacy of nisin in controlling the growth of *Clostridium* and *Bacillus* spores, which can survive the cooking process and grow in mashed potatoes.

[4]Graph and text reproduced from publication: Thomas, L. V. et al (2002) Effective use of Nisin to control Bacillus and Clostridium Spoilage of a pasteurized mashed potato product. Journal of Food Protection, 65(10): 1580-1585.





***Clostridia* challenge test:** After only 2 days, mashed potato inoculated with a *Clostridium* cocktail (4 strains of *C. sporogenes* and 1 strain of *C. tyrobutyricum*) and stored @ 25°C had a total anaerobic viable count reaching 10^7 CFU/g and showing visible evidence of spoilage while NISAPLIN®-treated samples show no *Clostridia* growth over the course of the experiment (40 days).

Bacilli challenge test: Mashed potato samples were inoculated with a *Bacillus* cocktail (2 strains of *B. cereus* & 1 strain of *B. subtilis*) and stored @ 8°C. The total aerobic viable count reaching 10^6 CFU/g after 12 days (peaking over 10^7 CFU/g after 18 days) showing visible evidence of spoilage while NISAPLIN®-treated samples (both dosages) no show *Bacilli* growth over the course of the experiment (45 days).

Salad dressings

Salad dressings are emulsified and usually texturized products with a low pH, mainly composed of vegetable oil, acidulants, egg yolk and stabilizers. Vinegar (or acetic acid) is traditionally used to lower the pH and inhibit lactic acid bacteria spoilage. Water activity is also important in preventing bacterial growth. Predominant spoilage microorganisms are acid-tolerant lactobacilli, yeasts and molds. With consumers demand for cleaner label products, food manufacturers have looking for additional hurdles to secure product shelf life and prevent spoilage. NISAPLIN® is an effective antimicrobial solution in salad dressings since it inhibits most of the bacteria isolated from these products.

NISAPLIN® added at 200mg/l extend salad dressing's shelf life by 1.5 and up to 4 times.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Salad dressings	From 250 to 500 mg/l	• US: 600 mg/l

Sauces

Heat treated, low-acid sauces can sustain growth of *Listeria monocytogenes* and spoilage organisms, such as lactic acid bacteria. With NISAPLIN® effectively inhibiting these bacteria, food manufacturers can increase their products safety as well as their shelf life. IFF NISAPLIN® efficacy evaluation on commercially available Bolognese sauce stored at 8°C reveals that 250 mg/kg prevent the growth of a 4-strain cocktail of *L. monocytogenes* for 55 days and a 4-strain cocktail of *B. cereus* spores for 45 days.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Sauces	From 250 to 500 mg/kg	• US: 600 mg/kg • CN: 180 mg/kg (only soy, condiment, clear and fish sauces) • CA: 250 mg/kg

Fruit juices

Acid tolerant and heat resistant spore forming bacterium *Alicyclobacillus acidoterrestris* can survive normal pasteurization conditions in fruit juices and grow in acidic conditions (pH 2.5 to 6.0) at temperatures ranging from 25 to 60°C. The flat sour spoilage caused by this microorganism produces an offensive smelling compound, guaiacol, and other taint compounds. It has been implicated in numerous fruit juice spoilage incidents. NISAPLIN® is active against *Alicylobacillus acidoterrestris* and therefore lengthens the shelf life of fruit juices and fruit juice containing products.

Product examples	Typical NISAPLIN® usage	Max. NISAPLIN® level
Fruit juices	From 50 to 100 mg/l	• US: 50 mg/l • CN: 180 mg/l (beverages except packaged water) • CA: 100 mg/l

Technical services and customer support

The technical support team offers customers the following services:

- Technical advice
- Support for trials – including site visits and product formulation
- Analysis of food samples for NISAPLIN® activity
- Challenge and shelf-life studies
- Spoilage analysis

In nature, antimicrobial sensitivity of microorganisms within the same genera-species can vary and be strain-dependent. In food, antimicrobial activity is dependent on a variety of factors like dosage, process parameters, storage conditions and the food matrix. For these reasons, IFF recommends customers to validate the efficacy of IFF products in their process and production environment under good manufacturing practices.

What else to know about NISAPLIN®?

IFF has manufactured Nisin A (commercial name NISAPLIN®) in Europe for more than 60 years ago. Even today, all the industrial scale production steps (from fermentation and downstream processing) are done in Beaminster in the UK. Starting as Aplin & Barrett, IFF played a pioneering role in performing food application studies (including retention in foods and stability on food processes).

Example of NISAPLIN® retention in foods

Food application	NISAPLIN® retention after production	NISAPLIN® retention after storage
Pasteurized liquid eggs	70-90%	
Pork meat w/various fat/lean ratio (core temperature of 75°C for 2-3 mins)	95-100%	28 days @ 8°C = 45-75% 35 days @ 8°C = 45-50%
Pasteurized mashed potato	70-90%	60 days @ 8°C = 60% 40 days @ 25°C = 50%
Processed cheese	70-80%	30 weeks @ 20°C = 80% 30 weeks @ 25°C = 60% 30 weeks @ 30°C = 40%

Example of NISAPLIN® retention after autoclaving at various pH*

pH	Product examples	NISAPLIN® retention after autoclaving	NISAPLIN® retention after storage
1.0		7%	2 weeks @ 20-25°C = 5%
2.0	Cranberry / lemon / lime juice	72%	2 weeks @ 20-25°C = 67%
3.0	Grape / grapefruit juice	98%	2 weeks @ 20-25°C = 95%
3.5	Apple / orange juice	82%	2 weeks @ 20-25°C = 83%
4.0	Apple / orange / tomato juice	79%	2 weeks @ 20-25°C = 83%
5.0		44%	2 weeks @ 20-25°C = 40%

**Davies, E.A. et al (1998). The effect of pH on the stability of nisin solution during autoclaving. Letters of Applied Microbiology 27: 186-187.*

Nisin/NISAPLIN® dosage conversion table

NISAPLIN® is the commercial preparation of pure nisin A standardized to the specific nisin concentration of 1 x 10^6 IU/g.

Nisin (mg/kg)	NISAPLIN® (mg/kg	Nisin (mg/kg)	NISAPLIN® (mg/kg)
1.00	40	10.0	400
1.25	50	12.5	500
2.50	100	15.0	600
3.00	120	25.0	1,000
3.75	150	30.0	1,200
5.00	200	100.0	4,000
6.25	250	250.0	10,000
7.50	300		

This work along with safety assessment supported key regulatory approvals around the world. And today as IFF we continue to lead food application approval expansion to support food manufacturers with additional means to improve their product safety and shelf life.

Food regulation information

- Recognized as safe by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)
- Codex Alimentarius General Standard for Food Additives (CODEX GSFA) listed (INS234)
- GRAS status in the US (FDA)
- Approved as a food additive in the EU as E234
- Approved food additive in Canada and in their List of permitted preservatives
- Food Chemical Codex (FCC) Grade

Using NISAPLIN® Antimicrobial from IFF helps to foster consumer confidence in your brand while keeping environmental impact at a minimum. The product is in alignment with the objectives of the United Nations' Sustainable Development Goals.

Other food protection solutions from IFF

Antimicrobials & fermentates : NATAMAX®, MICROGARD®, BIOVIA®, FERM and NOVAGARD®.
Natural oxidation control solutions: GUARDIAN® with sub-brands AQUAROX™, CHELOX, INOLENS, SYNEROX, VITAGREEN (LOD), and VIVOX.
Traditional antioxidants & blends: GRINDOX® containing ascorbyl palmitate, naturally sourced mixed tocopherols, TBHQ, BHA, BHT and propyl gallate.

PB 00333-0 E A 10.2021

Danisco, now part of the IFF family