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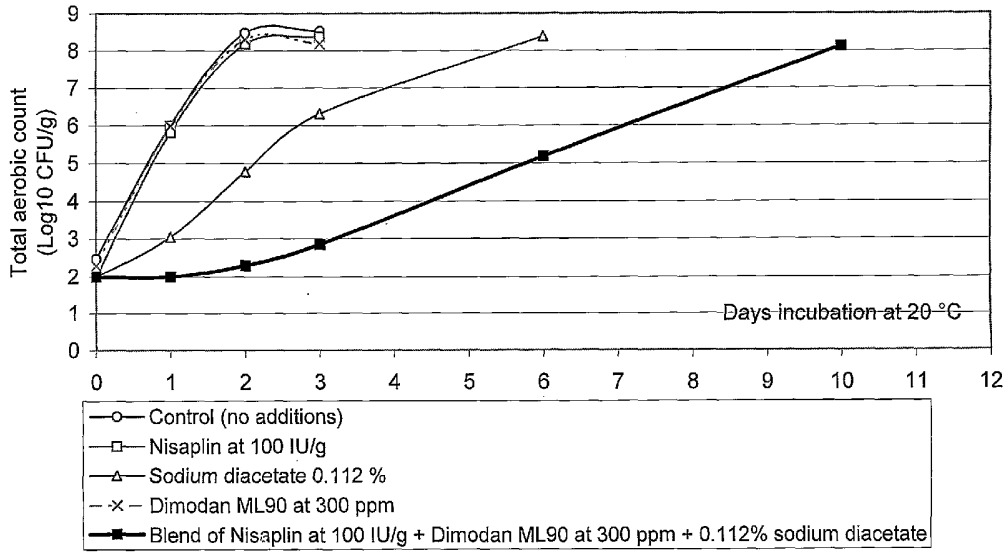
(54) Title: ANTI-MICROBIAL COMPOSITION



(57) Abstract: The present invention provides an antimicrobial composition comprising (i) an antimicrobial material; (ii) an organic acid or salt thereof; and (iii) an emulsifier.

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ANTI-MICROBIAL COMPOSITION

The present invention relates to a composition having an anti-microbial effect for use in a foodstuff.

Background

Pasteurised meat products, which are usually heat-treated at 72-85°C, have better texture and quality than sterilised meat products that are heated above 100°C. The lower processing temperatures of pasteurised meat products result in better mouthfeel, and ensure the meat will be more tender and juicy. These products are very perishable. To prevent spoilage and ensure the required shelf life, pasteurised meat products should be stored at 0-4°C. Heat resistant spore-forming Gram-positive bacteria are able to survive pasteurisation and many of these species will grow in the food even at low temperatures. Lactic acid bacteria are also often found in pasteurised meat products and have frequently been associated with spoilage. If, during the distribution and retail of pasteurised meat products, the food is subjected to periods of storage above refrigeration temperatures, these organisms will grow and spoilage will be accelerated. This is a potential problem during transport, distribution and retail storage of the meat, particularly in some parts of the world where refrigeration units are costly and unreliable. The ambient temperature in such countries may be high – increasing the rate of spoilage should refrigeration be inadequate. Spoilage represents a serious economic loss to the food manufacturers and retail outlets, but the consequences of unreliable refrigerated storage presents a potential threat to public health. When pasteurised food products are stored above 5°C, food pathogenic microorganisms as well as food spoilage organisms are also able to grow. An effective preserving system is needed to ensure shelf life and prevent this spoilage.

In many markets, for example China, the cold storage distribution chains for pasteurised meat products are not always reliable. The temperature of refrigerators in many supermarkets may be approximately 10°C; and some will be much higher than this. As a consequence many pasteurised meat products will experience storage at temperatures above 4°C and in summer months this temperature may become very high. In China, cooked meat products are considered spoiled if the total microflora reach levels of 3 x 10^4 CFU/g. In general, pasteurised meat products spoil after 3-5 days storage at ambient

room temperature, and after 15-20 days at 10-15°C. Effective preservation systems are urgently needed in China to ensure shelf life of these meat products when refrigerated storage cannot be guaranteed. With addition of 1-3% sodium lactate, pasteurised meat products (which may contain other chemical preservatives) usually have a shelf life of 15 days at 25°C, which increases to 60 days at 10°C. The shelf life for most pasteurised sausages (as indicated on the label) is 30-60 days.

Bacteriocins are antimicrobial proteins or peptides that can be produced by certain bacteria, which can kill or inhibit the growth of closely related bacteria. The bacteriocins produced by lactic acid bacteria are of particular importance since they have great potential for the preservation of food and for the control of foodborne pathogens. The most well known bacteriocin is nisin, which is the only bacteriocin currently authorised as a food additive. Nisin is produced by fermentation of the dairy starter culture bacterium *Lactococcus lactis* subsp. *lactis*, and has been used commercially in food as the preservative Nisaplin® (Danisco) since 1953. In 1969 a joint FAO/WHO expert committtee on food additives recognised nisin as a safe and legal biological food preservative. In 1988, the US Food and Drug Administration affirmed nisin as GRAS (generally recognised as safe) for use as a direct ingredient in human food. Nisin has an unusually broad antimicrobial spectrum for a bacteriocin, being active against most Gram-positive bacteria (e.g. species of *Bacillus*, *Clostridium*, *Listeria*, lactic acid bacteria). It is not normally effective against Gram-negative bacteria, yeasts or moulds. Nisin is allowed as a food preservative worldwide but its levels of use and approved food applications are strictly regulated, varying from country to country.

Other bacteriocins have since been discovered with potential as food preservatives, e.g. pediocin, lacticin, sakacin, lactococcin, enterococin, plantaricin, leucocin. These are also active, although usually with a more narrow spectrum, against Gram-positive bacteria. Their food use is at present restricted to production of the bacteriocin *in situ*, i.e. by growth of the producer organism within the food.

Nisin has been used successfully as a preservative in several meat products but, for reasons that are not fully understood, often does not perform well in meat so that high levels are often needed to ensure shelf life. The synergy between nisin and emulsifiers is well known and covered by certain patents. The synergies of nisin plus monolaurin, and nisin plus sucrose fatty acid esters are particularly strong and well documented (Jung et

al., 1992; Mansour et al. 1999; Thomas et al., 1998; US 5217950). A combination of nisin with such emulsifiers would be expected to improve nisin efficacy in meat products, and enable lower nisin levels to be used. However, despite this, the necessary addition levels required for efficacy may be uneconomic for food manufacturers, particularly in some parts of the world where food spoilage is more difficult to control due to poor quality raw ingredients, inadequate processing and/or unreliable refrigeration. If post-processing contamination occurs this may also introduce Gram-negative bacteria, whose growth cannot be controlled by nisin or other bacteriocins even in combination with emulsifiers.

Organic acids also can be used as part of a preservative system to control the growth of a range of microorganisms in food. These have sometimes been used in combination with nisin and other bacteriocins, and the effect is usually additive. Sorbic acid has also been used in combination with monolaurin (Bell and de Lacey, 1987) in pasteurised cured meat products to control spoilage.

The above extensive prior art does not address or solve the problems of providing an effective and broad range protection against microbial spoilage of food products.

The present invention alleviates the problems of the prior art.

In one aspect the present invention provides an antimicrobial composition comprising (i) an antimicrobial material; (ii) an organic acid or salt thereof; and (iii) an emulsifier.

In one aspect the present invention provides a synergistic antimicrobial composition comprising (i) an antimicrobial material; (ii) an organic acid or salt thereof; and (iii) an emulsifier.

In one aspect the present invention provides a antimicrobial composition comprising (i) an antimicrobial material; (ii) an organic acid or salt thereof; and (iii) an emulsifier, wherein each of (i), (ii) and (iii) are present in amounts to provide a synergistic antimicrobial effect.

In one aspect the present invention provides a process for preventing and/or inhibiting the growth of, and/or killing a micro-organism in a material, the process comprising the step of contacting the material with an antimicrobial composition comprising (i) an antimicrobial

material; (ii) an organic acid or salt thereof; and (iii) an emulsifier.

In one aspect the present invention provides use of an antimicrobial composition for preventing and/or inhibiting the growth of, and/or killing a micro-organism in a material; wherein the antimicrobial composition comprises (i) an antimicrobial material; (ii) an organic acid or salt thereof; and (iii) an emulsifier.

In one aspect the present invention provides a kit for preparing a composition as defined herein, the kit comprising (i) an antimicrobial material; (ii) an organic acid or salt thereof; and (iii) an emulsifier, in separate packages or containers; optionally with instructions for admixture and/or contacting and/or use.

In one aspect the present invention provides a foodstuff prepared by a process as defined herein.

In one aspect the present invention provides a foodstuff obtainable by a process as defined herein.

Aspects of the invention are defined in the appended claims.

We have found that a synergistic combination of an antimicrobial material, such as the bacteriocin nisin, with an emulsifier, applied together with an organic salt (or corresponding acid), such as sodium diacetate, gives a broad acting preservative that is effective at lower levels than expected for the equivalent bacteriocin levels. The present combination gives unexpectedly enhanced antibacterial activity. We have found that an unexpected enhancement of antibacterial activity from the combination of antimicrobial material with the organic acid salt, and the combination of the emulsifier with the organic acid salt. The antimicrobial composition is suitable for use in a range of foods. Use in pasteurised foods and in particular pasteurised meat products, has been found to be unexpectedly effective. Such foodstuffs are often subjected to temperature abuse during storage and/or subject to post processing contamination.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

PREFERRED ASPECTS

ANTIMICROBIAL MATERIAL

In one preferred aspect the antimicrobial material is an antibacterial material.

In one preferred aspect the antimicrobial material is a bacteriocin.

The antimicrobial material, such as a bacteriocin, may typically be selected from materials (bacteriocins) that can be used as preservatives in food

Preferably the antimicrobial material is selected from lanthionine containing bacteriocins, *Lactococcus*-derived bacteriocins, *Streptococcus*-derived bacteriocins, *Pediococcus*-derived bacteriocins, *Lactobacillus*-derived bacteriocins, *Carnobacterium*-derived bacteriocins, *Leuconostoc*-derived bacteriocins, *Enterococcus*-derived bacteriocins and mixtures thereof.

Preferably the antimicrobial material is selected from nisin, pediocin, lactocin and mixtures thereof.

Preferably the antimicrobial material is at least nisin.

Preferably the antimicrobial material consists of nisin.

Nisin is a lanthionine-containing bacteriocin (US 5691301) derived from *Lactococcus lactis* subsp. *lactis* (formerly known as *Streptococcus-lactis*) (US 5573801). In a preferred aspect of the present invention the bacteriocin used in the present invention is at least nisin.

As discussed in US 5573801 nisin is a polypeptide bacteriocin produced by the lactic acid bacteria, *Lactococcus lactis* subsp. *lactis* (formerly known as *Streptococcus lactis* Group N).

Nisin is reportedly a collective name representing several closely related substances which have been designated nisin compounds A, B, C, D and E (De Vuyst, L. and Vandamme, E. J. 1994. Nisin, a lantibiotic produced by *Lactococcus lactis* subsp. *lactis*: properties, biosynthesis, fermentation and applications. In: Bacteriocins of lactic acid bacteria. Microbiology, Genetics and Applications. Eds.: De Vuyst and Vandamme. Blackie Academic and Professional, London). . The structure and properties of nisin are also discussed in the article by E. Lipinska, entitled "Nisin and Its Applications", The 25th Proceedings of the Easter School in Agriculture Science at the University of Nottingham, 1976, pp. 103-130 (1977), which article is hereby incorporated by reference. In 1969 the FAO/WHO Joint Expert Committee on Food Additives set specifications for the purity and identity of nisin (FAO/WHO Joint Expert Committee on Food Additives. 1969. Specifications for identity and purity of some antibiotics. 12th Report. WHO Technical Report Series No. 430). This committee recognised nisin as a safe and legal preservative based on extensive toxicological testing. Nisin has the food additive number E234 and is classed as GRAS (Generally Recognised As Safe) (Food and Drug Administration. 1988. Nisin preparation: Affirmation of GRAS status as a direct human ingredient. Federal Regulations 53: 11247). The international activity unit (IU hereinafter) was defined as 0.001 mg of an international nisin reference preparation. Nisaplin® Natural Antimicrobial is the brand name for a nisin concentrate containing 1 million IU per g, which is commercially available from Danisco.

Nisin is an acknowledged and accepted food preservative with a long history of safe, effective food use. There have been several reviews of nisin, e.g. Hurst 1981; 1983; Delves-Broughton, 1990; De Vuyst and Vandamme, 1994; Thomas *et al.* 2000; Thomas & Delves-Broughton, 2001). Nisin was discovered over 50 years ago and the first commercial preparation, made in 1953, was Nisaplin®. Nisin has several characteristics that make it particularly suitable as a food preservative. It has undergone extensive toxicological testing to demonstrate its safety. It is heat-stable, acid-stable and effective against a broad spectrum of Gram-positive bacteria. It is not normally effective against Gram-negative bacteria, yeasts or moulds but activity against Gram-negative bacteria and yeasts has been reported in the presence of chelating agents (PCT/US 8902625. WO 89/12399). Nisin is an effective preservative in pasteurised and heat-treated foods (e.g. processed cheese, cheese, pasteurised milks, dairy desserts, cream, mascarpone and other dairy products, puddings such as semolina, tapioca etc., pasteurised liquid egg, pasteurised potato products, soy products, crumpets, pikelets, flapjacks, processed

meat products, beverages, soups, sauces, ready to eat meals, canned foods, vegetable drinks) and low acid foods such as salad dressings, sauces, mayonnaise, beer, wine and other beverages.

Although some loss of activity may be expected when used with processed foods, this may be ameliorated e.g. by increasing the amount of nisin applied. Effective levels of nisin to preserve foodstuffs reportedly range from 25-500 IU/g or more. Other effective levels would be appreciated by one skilled in the art. For example levels of 50-400 IU/g may be utilised.

Since the discovery of the first bacteriocin, nisin, many other bacteriocins have now been found (Hoover, 1993; Ray & Daeschel, 1994; Axelsen, 1998; Naidu, 2000; Ray *et al.* 2001; Ray & Miller, 2003). The bacteriocin pediocin, produced by *Pediococcus pentosaceus, P. acidilactici*, or *Lactobacillus plantarum*, may be used in the present invention. Like nisin, different structures of pediocin have been described. At present pediocin and other bacteriocins are not allowed as food additives but their antibacterial activity can be achieved by production of the bacteriocin *in situ*, as a consequence of the growth of the producer organism in the food. This is the purpose of commercial protective cultures such as HOLDBAC™ Listeria (Danisco). Pediocin has a more narrow antimicrobial spectrum compared to nisin, but there is much interest in its food safety ability to kill, prevent or control the growth of the food pathogen *Listeria monocytogenes* (Ray & Miller, 2000). Other bacteriocins may be used in the present invention, including those named generally as divercin, leucocin, mesentericin, sakacin, curvacin, bavaricin, acidocin, bifidocin, carnobacteriocin, pisicocin, piscicolin, mundticin, enterocin, thermophilin, lacticin, plantaricin, lactococcin, dricin, diplococcin, mesenterocin, leuconosin, carnosin, acidophilin, lactacin, brevicin, lactocin, helevticin, reutericin, propionicin.

EMULSIFIER

In one preferred aspect the emulsifier is synergistic with the antimicrobial material.

In one preferred aspect the emulsifier is selected from monoglycerides, diglycerides, acetic acid esters of mono-diglycerides, sucrose fatty acid esters, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides, diacetyl tartaric esters of

monoglycerides, tartaric acid esters of mono-diglycerides, citric acid esters of mono-diglycerides, glycerol monolaurate, polyphosphates, polyoxyethylene sorbitan esters (E432-E436) and polysorbates (e.g. Tween 80, Tween 20), and mixtures thereof.

In a highly preferred aspect the emulsifier is glycerol monolaurate.

It will be appreciated by one skilled in the art that for food application, the emulsifier is ideally a foodgrade emulsifier.

ORGANIC ACID

It will be appreciated that the organic acid or salt thereof may be in the acid form or in the salt form. In one preferred aspect the organic acid or salt thereof is an organic acid salt.

In one preferred aspect the organic acid or salt thereof may be selected from acetic acid, acetates, diacetates, benzoic acid, benzoates, citric acid, citrates, lactic acid, lactates, nitric acid, nitrites and nitrates, propionic acid, propionates, sorbic acid, sorbates, parabens (esters of p-hydroxybenzoic acid), sulfites, and mixtures thereof.

Preferablty the salt of the organic acid is a sodium salt.

Preferablty the organic acid or salt thereof is sodium diacetate.

It will be appreciated by one skilled in the art that for food application, the emulsifier is ideally a foodgrade organic acid or salt.

MICROORGANISM

As discussed herein the present invention may prevent and/or inhibit the growth of, and/or kill a micro-organism in a material. This may be slowing or arresting a micro-organism, such a bacteria, or by killing the micro-organism present on contact with the present composition.

In one aspect the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect.

In one aspect the bacteriocin and the extract are present in an amount to provide a microbicidal or microbiostatic effect.

In a highly preferred aspect the microbicidal or microbiostatic effect is a bactericidal or bacteriostatic effect.

It is advantageous for the bactericidal or bacteriostatic effect to be in respect of Gram-positive bacteria and Gram-negative bacteria. Preferably the bactericidal or bacteriostatic effect is in respect of Gram-positive bacteria.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of a Gram-positive bacteria selected from species of *Bacillus, Brochothrix, Carnobacterium, Clostridium, Enterococcus, Listeria, Lactobacillus, Leuocostoc, Micrococcus, Pediococcus*, and *Streptococcus.*

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of an organism selected from species of *Clostridium, Bacillus, Listeria, Staphylococcus*, lactic acid bacteria, *Pseudomonas, Escherichia coli, Salmonella, Campylobacter, Yersinia.*

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of an organism selected from Gram-positive bacteria associated with food spoilage or foodborne disease including *Bacillus* species, *Bacillus subtilis, Bacillus cereus*, *Listeria* species, *Listeria monocytogenes*, lactic acid bacteria, lactic acid spoilage bacteria, *Lactobacillus species*, *Staphylococcus aureus*, *Clostridium* species, *C. sporogenes, C. tyrobutyricum* and *C. botulinum* (when the antimicrobial material is recognised as effective against *C. botulinum* or is part of a system effective against *C. botulinum*).

In a preferred aspect the bactericidal or bacteriostatic effect of the invention in combination with a chelating agent is in respect of an organism selected from other micro-organisms associated with food spoilage or foodborne disease, including yeasts, moulds and Gram-negative bacteria including *Escherichia coli*, *Salmonella* species, and *Pseudomonas* species.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of lactic acid

bacteria such as *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, and *Enterococcus*; *Listeria monocytogenes*, spore forming heat resistant bacteria such as *Bacillus* and *Clostridium*; and *Brochothrix thermosphacta*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Lactobacillus*, *Leuconostoc*, *Carnobacterium*, *Enterococcus*, *Listeria monocytogenes*, *Bacillus*, *Clostridium*; and *Brochothrix thermosphacta*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of an organism selected from *Listeria monocytogenes* and *Bacillus cereus*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Bacillus cereus*.

In a preferred aspect the bactericidal or bacteriostatic effect is in respect of *Listeria monocytogenes*.

APPLICATIONS

The antimicrobial composition in accordance with the present invention may be used in a broad range of application areas.

Pharmaceutical Formulations

The compositions of the present inventions may be incorporated in pharmaceutical compositions useful in the treatment of one or more diseases/infections,

The compositions of the present invention may be used as therapeutic agents – i.e. in therapy applications.

The term "therapy" includes curative effects, alleviation effects, and prophylactic effects.

In one aspect, the present invention provides a pharmaceutical composition, which comprises a compositions according to the present invention and optionally a pharmaceutically acceptable carrier, diluent or excipient (including combinations thereof).

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art

The compositions of the present invention may be used in combination with one or more other active agents, such as one or more other pharmaceutically active agents.

By way of example, the compositions of the present invention may be used in combination with other anti-microbial agents

In another embodiment the present compositions thereof can be used in therapeutic or personal hygiene formulation in combination with other anti-microbials, anti-fungal agents, ant-bacterial agents, or other pharmaceutically active compositions, such as anti-inflammatory agents, steroids, topical analgesics,

Parasitic Microbials:

The present compositions may be active against protozoan parasitic disorders such as malaria, toxoplasmosis and giardiasis.

The present compositions may be active in the treatment or prevention of parasitic disorders such as trypanocidal disorders.

Personal Hygiene/Cosmetics

Personal hygiene compositions are applied topically to the skin to treat skin conditions including acne, fine lines and age spots, itching and pain from insect bites, bee stings, fungi (including athletes foot and jock itch), flaking and/or scaly skin (including dandruff, seborrheic dermatitis, psoriasis and heat rash), and burns. Different compositions are presented for use as an acne treatment, a face and body wash, a dermatophyte (nail fungus) treatment. Still another is intended for use in makeup, and another in lipstick. The present compositions may be found to be active in these applications.

The present invention provides topical antimicrobial compositions containing a cosmetically acceptable diluent or carrier, and an antimicrobially effective amount of the present composition.

Feedstuffs

The present composition can be included in an animal feed itself to prevent or reduce pathogen contamination, and/or as a disinfectant for use in decontaminating premises for food production, and including slaughter houses, milk and dairy production facilities, other food production and processing facilities, commercial and domestic kitchens. The present composition can also be used as a disinfectant for personal use, particularly for people who prepare food stuffs, thereby preventing or reducing bacterial contamination.

Food

In a preferred aspect the material is a foodstuff.

Many foodstuffs may be protected by the present invention. Typical foodstuffs are raw, processed or pasteurised foods including raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood products, [raw or cooked meat, poultry and seafood products], , sausages, frankfurters, soups, sauces, dressings ready to eat meals, pasta sauces, pasteurised soups, mayonnaise, salad dressings, marinades, oil-in-water emulsions, margarines, low fat spreads, water-in-oil emulsions, dairy products, cheese spreads, processed cheese, dairy desserts, flavoured milks, cream, fermented milk products, cheese, butter, condensed milk products, cheese spreads, pasteurised liquid egg,, ice cream mixes, soya products, pasteurised liquid egg, bakery products (such as crumpets), confectionery products, fruit products, and foods with fat-based or water-containing fillings.

In one preferred aspect the foodstuff is selected from raw meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood products [raw or cooked meat, poultry and seafood products] and raw or cooked foodstuffs prone to surface bacterial growth.

In one preferred aspect the foodstuff is a processed meat product such as processed

meat products selcted from cured sausages, frankfurters, and hot dogs.

ADDITIONAL COMPONENTS

The antimicrobial composition may contain one or more additional components. However, in some aspects the antimicrobial composition contains no additional components or contains no additional components that materially affect the properties of the composition.

In one preferred aspect the composition further comprises a second emulsifier.

In one preferred aspect the second emulsifier is a high melting point emulsifier. The term "high melting point emulsifier" preferably means an emulsifier having a dropping point of greater than 40°C. Preferably the high melting point emulsifier has a dropping point of greater than 60°C. Preferably the high melting point emulsifier is an emulsifier having an iodine value of less than 40. Preferably the high melting point emulsifier is an emulsifier having an iodine value of less than 5.

In a further preferred aspect the second emulsifier is selected from saturated and unsaturated monoglycerides (such as distilled monoglycerides) having a fatty acid chain length of from 16 to 22 carbons and mixtures thereof.

In a further preferred aspect the second emulsifier is selected from saturated and unsaturated monoglycerides (such as a distilled monoglyceride) having a fatty acid chain length of 16 carbons, saturated and unsaturated monoglycerides (such as a distilled monoglyceride) having a fatty acid chain length of 18 carbons and mixtures thereof.

In a further preferred aspect the second emulsifier is selected from saturated monoglyceride (such as a distilled monoglyceride) having a fatty acid chain length of 16 carbons, saturated monoglyceride (such as a distilled monoglyceride) having a fatty acid chain length of 18 carbons and mixtures thereof.

In a further preferred aspect the second emulsifier is a mixture of (i) saturated monoglyceride (such as a distilled monoglyceride) having a fatty acid chain length of 16 carbons and (ii) saturated monoglyceride (such as a distilled monoglyceride) having a

fatty acid chain length of 18 carbons. Preferably (i) is present in an amount of 30-70 wt% and (ii) is present in an amount of 70-30 wt%. Preferably (i) is present in an amount of 40-60 wt% and (ii) is present in an amount of 60-40 wt%. Preferably (i) is present in an amount of approximately 45 wt% and (ii) is present in an amount of approximately 55 wt%.

In one preferred aspect the second emulsifier is a monoglyceride distilled from palm oil. In one preferred aspect the second emulsifier is Dimodan™ HP, available from Danisco A/S, Denmark.

The presence of a high melting point emulsifier such as a distilled monoglyceride having a fatty chain length of from 16 to 22 carbons is advantageous. We have found that the presence of such a material overcomes handling problems of some synergistic emulsifiers. For example, Dimodan™ ML 90 (a synergistic emulsifier) is found to be lumpy to handle and difficult to disperse in food material. The presence of the second emulsifier raises the melting point of the synergistic emulsifier of the present composition. The raised melting point allows for the combined emulsifiers to prepared in powder form easing handling. The powder material is also more easily dispersed.

An alternative manner for improving the handling properties of "lumpy" synergistic emulsifiers is to plate them on to the organic acid/salt component. We have found that in one preferred aspect the handling problems of Dimodan™ ML 90 are overcome by plating it onto sodium diacetate.

In one preferred aspect the composition is prepared as a powder combining the bacteriocin-synergistic emulsifier with the organic acid/salt component (sodium diacetate) such that the dispersal of the emulsifier is facilitated within the food matrix prior to heating.

In one preferred aspect the antimicrobial composition further comprises a chelator. Preferably the chelator is selected from EDTA, citric acid, monophosphates, diphosphates, triphosphates and polyphosphates.

Further suitable chelator are taught in US 5573801 and include carboxylic acids, polycarboxylic acids, amino acids and phosphates. In particular, the following

compounds and their salts may be useful:

Acetic acid, Adenine, Adipic acid, ADP, Alanine, B-Alanine, Albumin, Arginine, Ascorbic acid, Asparagine, Aspartic acid, ATP, Benzoic acid, n-Butyric acid, Casein, Citraconic acid, Citric acid, Cysteine, Dehydracetic acid, Desferri-ferrichrysin, Desferri-ferrichrome, Desferri-ferrioxamin E, 3,4-Dihydroxybenzoic acid, Diethylenetriaminepentaacetic acid (DTPA), Dimethylglyoxime, O,O-Dimethylpurpurogallin, EDTA, Formic acid, Fumaric acid, Globulin, Gluconic acid, Glutamic acid, Glutaric acid, Glycine, Glycolic acid, Glycylglycine, Glycylsarcosine, Guanosine, Histamine, Histidine, 3-Hydroxyflavone, Inosine, Inosine triphosphate, Iron-free ferrichrome, Isovaleric acid, Itaconic acid, Kojic acid, Lactic acid, Leucine, Lysine, Maleic acid, Malic acid, Methionine, Methylsalicylate, Nitrilotriacetic acid (NTA), Ornithine, Orthophosphate, Oxalic acid, Oxystearin, B-Phenylalanine, Phosphoric acid, Phytate, Pimelic acid, Pivalic acid, Polyphosphate, Proline, Propionic acid, Purine, Pyrophosphate, Pyruvic acid, Riboflavin, Salicylaldehyde, Salicyclic acid, Sarcosine, Serine, Sorbitol, Succinic acid, Tartaric acid, Tetrametaphosphate, Thiosulfate, Threonine, Trimetaphosphate, Triphosphate, Tryptophan, Uridine diphosphate, Uridine triphosphate, n-Valeric acid, Valine, and Xanthosine

Many of the above sequestering agents are useful in food processing in their salt forms, which are commonly alkali metal or alkaline earth salts such as sodium, potassium or calcium or quaternary ammonium salts. Sequestering compounds with multiple valencies may be beneficially utilised to adjust pH or selectively introduce or abstract metal ions e.g. in a food system coating. Additional information chelators is disclosed in T. E. Furia (Ed.), CRC Handbook of Food Additives, 2nd Ed., pp. 271-294 (1972, Chemical Rubber Co.), and M. S. Peterson and A. M. Johnson (Eds.), Encyclopaedia of Food Science, pp. 694-699 (1978, AVI Publishing Company, Inc.) which articles are both hereby incorporated by reference.

The terms "chelator" is defined as organic or inorganic compounds capable of forming co-ordination complexes with metals. Also, as the term "chelator" is used herein, it includes molecular encapsulating compounds such as cyclodextrin. The chelator may be inorganic or organic, but preferably is organic.

Preferred chelator are non-toxic to mammals and include aminopolycarboxylic acids and

their salts such as ethylenediaminetetraacetic acid (EDTA) or its salts (particularly its di- and tri-sodium salts), and hydrocarboxylic acids and their salts such as citric acid. However, non-citric acid and non-citrate hydrocarboxylic acid chelators are also believed useful in the present invention such as acetic acid, formic acid, lactic acid, tartaric acid and their salts.

As noted above, the term "chelator" is defined and used herein as a synonym for sequestering agent and is also defined as including molecular encapsulating compounds such as cyclodextrin. Cyclodextrins are cyclic carbohydrate molecules having six, seven, or eight glucose monomers arranged in a donut shaped ring, which are denoted alpha, beta or gamma cyclodextrin, respectively. As used herein, cyclodextrin refers to both unmodified and modified cyclodextrin monomers and polymers. Cyclodextrin molecular encapsulators are commercially available from American Maize-Products of Hammond, Ind. Cyclodextrin are further described in Chapter 11 entitled, "Industrial Applications of Cyclodextrin", by J. Szejtli, page 331-390 of Inclusion Compounds, Vol. III (Academic Press, 1984) which chapter is hereby incorporated by reference.

Preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin. More preferably the chelator enhances the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin in respect of Gram-negative bacteria and other micro-organisms.

We have found that the provision of a chelator is particularly effective in view of the enhancement of the antimicrobial activity and/or antimicrobial spectrum of the bacteriocin provided.

COMPOSITION

The composition odf the present invnetion may contain each of thre three components in any amount. The three components of the composition may make up any amount of the composition. In preferred aspects

- the antimicrobial material is present in an amount of at least 1% of the total composition
- the antimicrobial material is present in an amount of at least 2% of the total composition

- the antimicrobial material is present in an amount of at least 5% of the total composition
- the antimicrobial material is present in an amount of at least 10% of the total composition
- the antimicrobial material is present in an amount of at least 20% of the total composition
- the antimicrobial material is present in an amount of at least 30% of the total composition
- the organic acid/salt is present in an amount of at least 1% of the total composition
- the organic acid/salt is present in an amount of at least 2% of the total composition
- the organic acid/salt is present in an amount of at least 5% of the total composition
- the organic acid/salt is present in an amount of at least 10% of the total composition
- the organic acid/salt is present in an amount of at least 20% of the total composition
- the organic acid/salt is present in an amount of at least 30% of the total composition
- the emulsifier is present in an amount of at least 1% of the total composition
- the emulsifier is present in an amount of at least 2% of the total composition
- the emulsifier is present in an amount of at least 5% of the total composition
- the emulsifier is present in an amount of at least 10% of the total composition
- the emulsifier is present in an amount of at least 20% of the total composition
- the emulsifier is present in an amount of at least 30% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 1% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 2% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 5% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 10% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 20% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 30% of the total composition

- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 40% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 50% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 60% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 80% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 90% of the total composition
- the combined total of antimicrobial material, organic acid/salt and emulsifier is at least 95% of the total composition

In one preferred aspect the composition is in powder form. In one preferred aspect the powder is combined with a food grade anti-caking agent. The presence of the anti-caking agent facilitates the dispersal of the emulsifier within the food matrix prior to heating.

PROCESS

It will be appreciated by one skilled in the art that when the components on the present invention are contacted with a material they may be contacted simultaneously or separately or a combination of both for example the order of contact may be

- antimicrobial material, organic acid/salt and emulsifier simultaneously
- antimicrobial material and organic acid/salt simultaneously followed by emulsifier
- antimicrobial material and emulsifier simultaneously followed by organic acid/salt
- organic acid/salt and emulsifier simultaneously followed by antimicrobial material
- antimicrobial material followed by organic acid/salt followed by emulsifier
- antimicrobial material followed by emulsifier followed by organic acid/salt
- organic acid/salt followed by antimicrobial material followed by emulsifier
- organic acid/salt followed by emulsifier followed by antimicrobial material
- emulsifier followed by antimicrobial material followed by organic acid/salt
- emulsifier followed by organic acid/salt followed by antimicrobial material

HIGHLY PREFERRED ASPECTS

Some highly preferred aspects of the present invention are set out below

Preferably the antimicrobial material is nisin, the organic acid or salt thereof is sodium diacetate; and the emulsifier is glycerol monolaurate.

Preferably the antimicrobial material is nisin, the organic acid or salt thereof is sodium diacetate; and the emulsifier is glycerol monolaurate and the composition comprises a second emulsifier comprising or consisting of a mixture of (i) saturated monoglyceride (such as a distilled monoglyceride) having a fatty acid chain length of 16 carbons and (ii) saturated monoglyceride (such as a distilled monoglyceride) having a fatty acid chain length of 18 carbons.

The present invention will now be described in further detail in the following examples.

EXAMPLES

Example 1 - Demonstration of efficacy and ease of use of a triple antimicrobial combination blend, enabling reduced nisin levels to be used

An antimicrobial powder blend was prepared by combining an emulsifier synergistic with nisin (Dimodan™ ML 90, Danisco) with sodium diacetate such that the emulsifier was in the form of a powder. This process was facilitated by inclusion of a further, non-synergistic emulsifier. Nisin (Nisaplin®; Danisco) was also added. The antimicrobial blend was then dispersed easily and homogeneously into a chicken soup, which was then pasteurised. The pH of the samples was as follows: Control (no additions): pH 6.12; nisin at 100 IU/g: pH 6.14; sodium acetate at 0.112%: pH 5.42; Dimodan® ML90 at 300 ppm: 6.13; the antimicrobial blend at 0.2%: pH 5.42. After cooling the soup was inoculated with approximately 10^3 cfu/g of a mixed *Listeria monocytogenes* cocktail comprising *L. monocytogenes* strains 272; NCTC 12426; 358 and S23. The soup was then incubated at 20°C, a temperature representative of grossly inadequate storage of such a pasteurised product. Samples were microbiologically analysed by total aerobic viable count enumeration at regular intervals. Results are shown in Figure 1. Previous results had shown that the second emulsifier had no antimicrobial activity, so this was

not included as a control. At this inoculation level, and such a high incubation temperature, the nisin and Dimodan ML90 showed no antilisterial effect alone. The sodium diacetate alone showed efficacy that was partly due to the drop in pH that it caused. The antimicrobial blend showed significantly greater activity than any of the components alone. This enhanced the nisin to show significant antilisterial activity at 100 IU/g.

Example 2 - Demonstration of efficacy of a triple antimicrobial combination blend in a Chinese sausage

The antimicrobial blend was tested in a Chinese type sausage containing 14-16% protein, 18-22% fat and 58-60% water. As is common in China, the sausages contained 1% sodium lactate, primarily as a humectant. One batch of sausages was tested with no preservative or lactate addition. The raw meat was treated, ground and cured. It was then emulsified with addition of ice, at a temperature not exceeding 14°C. The meat emulsion was then filled into sausage casings, pasteurised at a core temperature of 75°C for 20 minutes. After cooling at ambient temperature for 30 minutes, the sausages were cooled in water at 4°C.

Sausages were stored at a range of temperatures representing the extremes of refrigeration failure and environmental conditions that could be experienced during distribution and in retail outlets. No inoculation was performed. Sausages were analysed regularly by microbiological enumeration of total aerobic viable count (TAVC).

Results are shown in Table 1.

a) Incubation at 37°C

Addition	Nisin addition within this addition (IU/g)	Days until 10^5 cfu/g for TAVC
No additions	0	1
1% sodium lactate	0	3.5
1% sodium lactate + 150 IU/g nisin	150	5.5
1% sodium lactate + 0.11 % antimicrobial	37	7
1% sodium lactate + 0.13 % antimicrobial blend	43	9
1% sodium lactate + 0.17 % antimicrobial blend	56	10

b) incubation at 25 °C

Addition	Nisin addition within this addition (IU/g)	Days until 10^5 cfu/g for TAVC
No additions	0	5
1% sodium lactate	0	13
1% sodium lactate + 150 IU/g nisin	150	17
1% sodium lactate + 0.11 % antimicrobial blend	37	17
1% sodium lactate + 0.13 % antimicrobial blend	43	21
1% sodium lactate + 0.17 % antimicrobial blend	56	30

c) incubation at 10 °C

Addition	Nisin addition within this addition (IU/g)	Days until 10^5 cfu/g for TAVC
No additions	0	55
1% sodium lactate	0	75
1% sodium lactate + 150 IU/g nisin	150	70
1% sodium lactate + 0.11 % antimicrobial blend	37	> 100
1% sodium lactate + 0.13 % antimicrobial blend	43	> 100 (one aberrant point)
1% sodium lactate + 0.17 % antimicrobial blend	56	> 100

Example 3 - Demonstration of enhanced antibacterial efficacy by a combination of an emulsifier (Dimodan ML90) and sodium diacetate

The three components of the novel antimicrobial blend were tested separately and in dual combinations in a chicken soup system. This was a rich, nutritious pasteurised broth, stored at refrigeration temperatures and containing poultry, vegetables, flavourings and cream. Additions of an emulsifier (Dimodan ML90, Danisco), a bacteriocin (nisin, Nisaplin, Danisco) and an organic acid salt (sodium diacetate, Danisco) were made as appropriate. Addition of sodium diacetate caused a drop in pH, so the soups were re-adjusted back to pH 6.0 after this addition. All the tests were pH 6.0. The soups was then pasteurised and inoculated with a cocktail of *Listeria monocytogenes strains* (strains 272; NCTC12426; 358; CRA3930). The tests were incubated at 8°C and analysed at regular

intervals.

The results are shown in Figure 2.

Controls and tests with 200 ppm Dimodan ML90 reached 10^6 CFU/g by 4.5 days. 0.1% sodium diacetate extended this threshold to 11 days, but the combination of sodium diacetate and Dimodan ML90 achieved this after 14 days, demonstrating that the combination had a better antimicrobial effect.

Example 4 - Demonstration of enhanced efficacy between nisin and sodium diacetate

The experiment was conducted as described above. Results are shown in Figure 3. The enhanced activity of the combination can be assessed by comparing the time taken for the total aerobic counts in the samples to reach 10^6 CFU/g: Control = 4.5 d; nisin at 50 IU/g = 6 days; sodium diacetate at 0.1% = 10.5 days. The combination of 0.1% sodium diacetate and 50 IU/g nisin reached this threshold after 16.5 days. This demonstrates that the combination had a better antimicrobial effect.

Example 5 - Demonstration that the triple combination is more effective than the double synergistic combination of nisin + emulsifier

The experiment was conducted in the chicken soup model. Additions were made to the soup, the pH recorded but not adjusted and the soup pasteurised. When cool the soup was inoculated with a cocktail of *Listeria monocytogenes* as before, and incubated at 20 °C. The higher incubation temperature resulted in fast listerial growth, which nisin alone at the test level failed to control. The results are shown in Table 2. The combined effect of the triple combination was greater than any of the double combinations.

Test additions	Days until 10^6 CF/g
Control (no additions) (pH 6.23)	1
Nisin (150 IU/g) (pH 6.19)	1
Sodium diacetate (0.114%) (pH 5.26)	3
Dimodan ML90 (284 ppm) (pH 6.19)	1
Nisin (150 IU/g) + sodium diacetate (0.114%) (pH 5.20)	6.5
Nisin (150 IU/g) + Dimodan ML90 (284 ppm) (pH 6.23)	1.5
Sodium diacetate (0.114%) + Dimodan ML90 (pH 5.23)	1
Nisin (150 IU/g) + diacetate (0.114%) + ML 90 (284 ppm) (pH 5.22) (pH 5.22)	10.5

Example 6 - Demonstration of the enhanced efficacy of the triple antimicrobial blend against *Listeria monocytogenes*

The antimicrobial combination of an emulsifier (Dimodan ML90), a bacteriocin (nisin) and an organic acid salt (sodium diacetate) was tested in a pasteurised Bolognese pasta sauce. After addition of the antimicrobials the soup was re-adjusted to approximately pH 5.88. After pasteurisation the sauce was inoculated with a cocktail of *Listeria monocytogenes* strains (strains NCIMB 12426, 358, 272, CRA3930) and the sauce samples were incubated at 8°C, a chilled abuse temperature. Results are shown in Figure 4. The triple combination achieved complete control of Listeria for the duration of the experiment (53 days) whereas the individual components of the combination showed much less efficacy.

Example 7 - Demonstration of the enhanced efficacy of the triple antimicrobial blend against *Bacillus cereus*

The antimicrobial combination was also tested against a cocktail of *Bacillus cereus* spores (strains 204, 199, ABC4/9, 3.046). The sauce was incubated at 20 °C. Results are shown in Figure 5. They show again the greatly enhanced activity of the triple combination.

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All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, biology, food science or related fields are intended to be within the scope of the following claims

CLAIMS

1. An antimicrobial composition comprising
(i) an antimicrobial material;
(ii) an organic acid or salt thereof; and
(iii) an emulsifier.

2. An antimicrobial composition according to claim 1 wherein the antimicrobial material is a bacteriocin.

3. A process according to claim 2 wherein the bacteriocin is selected from lanthionine containing bacteriocins, *Lactococcus*-derived bacteriocins, *Streptococcus*-derived bacteriocins, *Pediococcus*-derived bacteriocins, *Lactobacillus*-derived bacteriocins, *Carnobacterium*-derived bacteriocins, *Leuconostoc*-derived bacteriocins, *Enterococcus*-derived bacteriocins and mixtures thereof.

4. An antimicrobial composition according to claim 3 wherein the antimicrobial material is selected from nisin, pediocin, lactocin and mixtures thereof.

5. An antimicrobial composition according to any one of the preceding claims wherein the antimicrobial material is nisin.

6. An antimicrobial composition according to any one of the preceding claims wherein the an organic acid or salt thereof is selected from acetates, diacetates, lactates, citrates, sorbates, propionates and mixtures thereof.

7. An antimicrobial composition according to any one of the preceding claims wherein the an organic acid or salt thereof is sodium diacetate.

8. An antimicrobial composition according to any one of the preceding claims wherein the emulsifier is synergistic with the antimicrobial material.

9. An antimicrobial composition according to any one of the preceding claims wherein the emulsifier is selected from monoglycerides, glycerol monolaurate, citric acid esters of mono-diglycerdies, sucrose fatty acid esters, diacetyl tartaric esters of

monoglycerides, lactic acid esters of monoglycerides, acetic acid esters of monoglycerides, polysorbates and mixtures thereof.

10. An antimicrobial composition according to any one of the preceding claims wherein the antimicrobial material is nisin, the organic acid or salt thereof is sodium diacetate; and the emulsifier is glycerol monolaurate.

11. An antimicrobial composition according to claim 10 further comprising a monoglyceride distilled from palm oil.

12. An antimicrobial composition according to any one of the preceding claims further comprising a second emulsifier

13. An antimicrobial composition according to any one of the preceding claims wherein the antimicrobial material is present in an amount to provide a microbicidal or microbiostatic effect.

14. An antimicrobial composition according to claim 13 wherein the microbicidal or microbiostatic effect is a bactericidal or bacteriostatic effect.

15. An antimicrobial composition according to claim 14 wherein the bactericidal or bacteriostatic effect is in respect of Gram-positive bacteria.

16. An antimicrobial composition according to claim 15 wherein the bactericidal or bacteriostatic effect is in respect of an organism selected from species of *Clostridium, Bacillus, Listeria, Staphylococcus*, lactic acid bacteria, *Pseudomonas, Escherichia coli, Salmonella, Campylobacter, Yersinia.*

17. An antimicrobial composition according to claim 16 wherein the bactericidal or bacteriostatic effect is in respect of *Listeria monocytogenes* and/or *Bacillus cereus.*

18. A process for preventing and/or inhibiting the growth of, and/or killing a micro-organism in a material, the process comprising the step of contacting the material with an antimicrobial material as defined in any one of claims 1 to 17.

19. A process according to claim 18 wherein the antimicrobial material, organic acid or salt thereof, and emulsifier are added to the material together.

20. A process according to claim 19 wherein two or more of the antimicrobial material, organic acid or salt thereof, and emulsifier are added to the material sequentially.

21. A process according to any one of claims 18 to 20 wherein the material is a foodstuff.

22. A process according to claim 19 wherein the foodstuff is a processed meat product such as processed meat products selcted from cured sausages, frankfurters, and hot dogs.

23. A process according to any one of claims 18 to 22 characterised by the features of any one of claims 2 to 17.

24. Use of an antimicrobial composition for preventing and/or inhibiting the growth of, and/or killing a micro-organism in a material; wherein the antimicrobial composition comprises

(i) an antimicrobial material;

(ii) an organic acid or salt thereof; and

(iii) an emulsifier.

25. Use according to claim 24 wherein the material is a foodstuff.

26. Use according to claim 24 or 25 for synergistically preventing and/or inhibiting the growth of, and/or killing a micro-organism in a material.

27. Use according to any one of claims 24 to 26 characterised by the features of any one of claim 2 to 17.

28. A kit for preparing a composition as defined in any one of claims 1 to 17, the kit comprising

(i) an antimicrobial material;

(ii) an organic acid or salt thereof; and

(iii) an emulsifier,

in separate packages or containers; optionally with instructions for admixture and/or contacting and/or use.

29. A foodstuff prepared by a process as defined in any one of claims 18 to 23.

30. A foodstuff obtainable by a process as defined in any one of claims 18 to 23.

31. A composition as substantially hereinbefore described with reference to any one of the Examples.

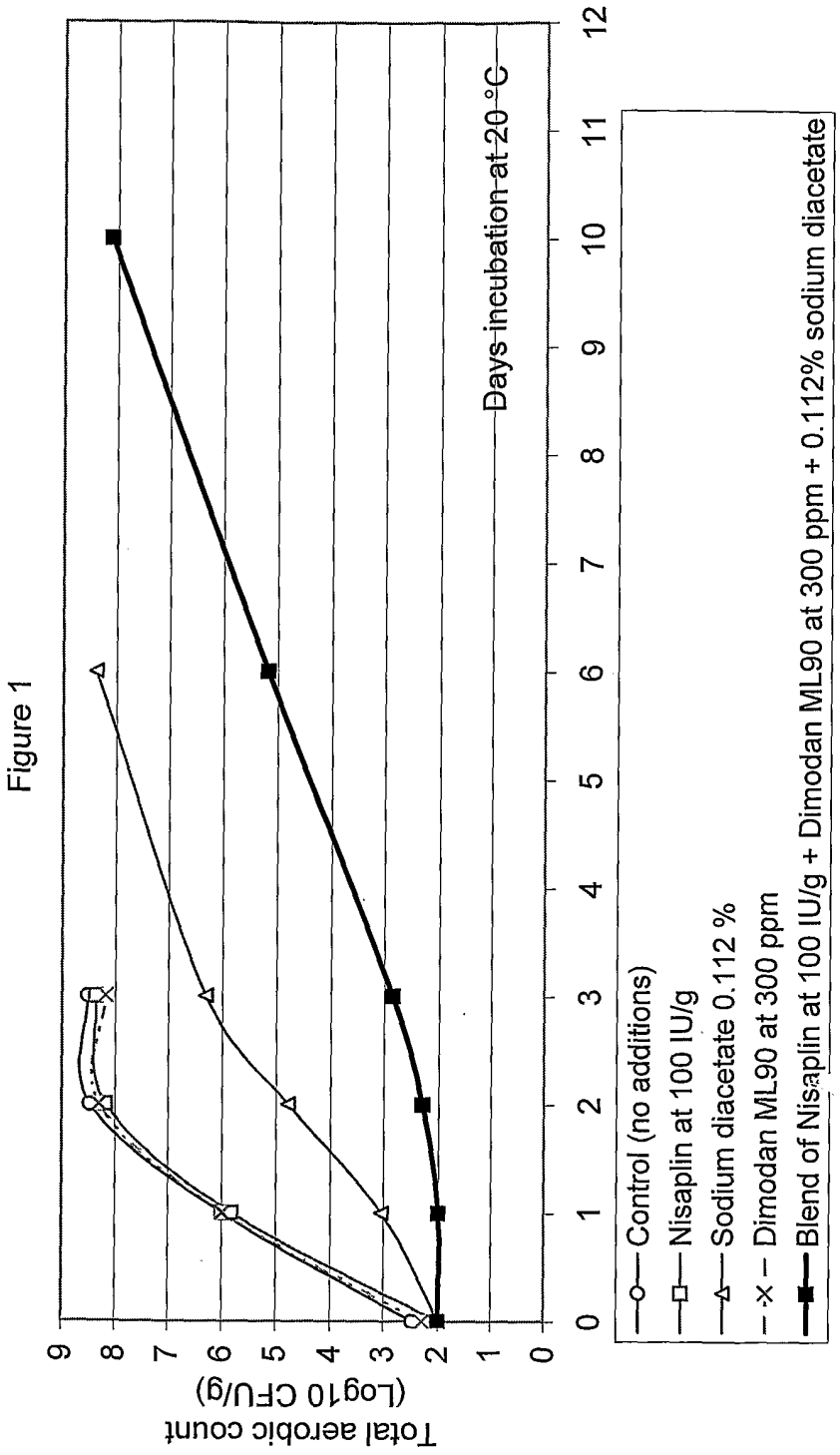
32. A process as substantially hereinbefore described with reference to any one of the Examples.

33. A use as substantially hereinbefore described with reference to any one of the Examples.

34. A kit as substantially hereinbefore described with reference to any one of the Examples.

35. A foodstuff as substantially hereinbefore described with reference to any one of the Examples.

Figure 1


Total aerobic count
(Log10 CFU/g)
0
1
2
3
4
5
6
7
8
9
Days incubation at 20 °C
0
1
2
3
4
5
6
7
8
9
10
11
12
Control (no additions)
Nisaplin at 100 IU/g
Sodium diacetate 0.112 %
Dimodan ML90 at 300 ppm
Blend of Nisaplin at 100 IU/g + Dimodan ML90 at 300 ppm + 0.112% sodium diacetate

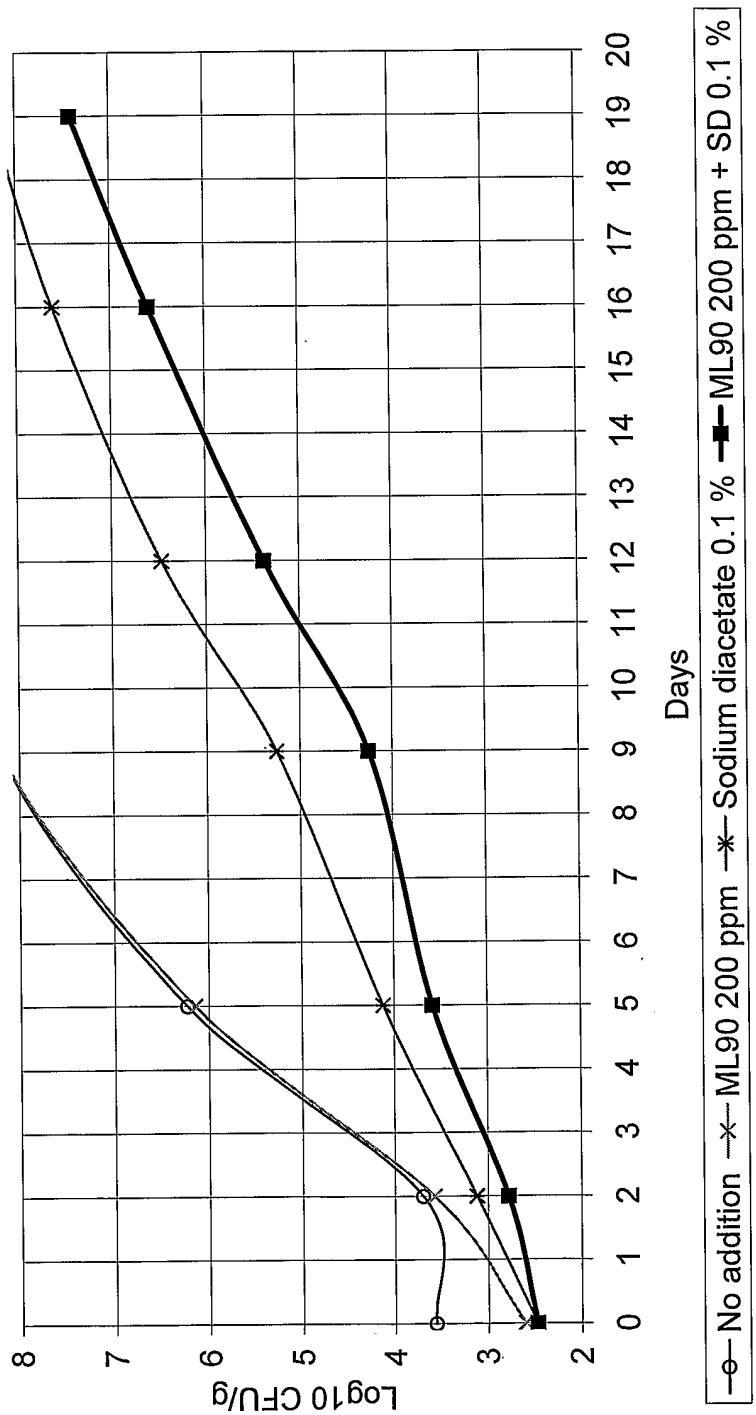


Fig 2. The enhanced effect of Dimodan ML90 and sodium diacetate against growth of *L. monocytogenes* in chicken soup at 8 °C. (Minimum detection 1.0 x 10^2 CFU/g, TAVC on MPCA).

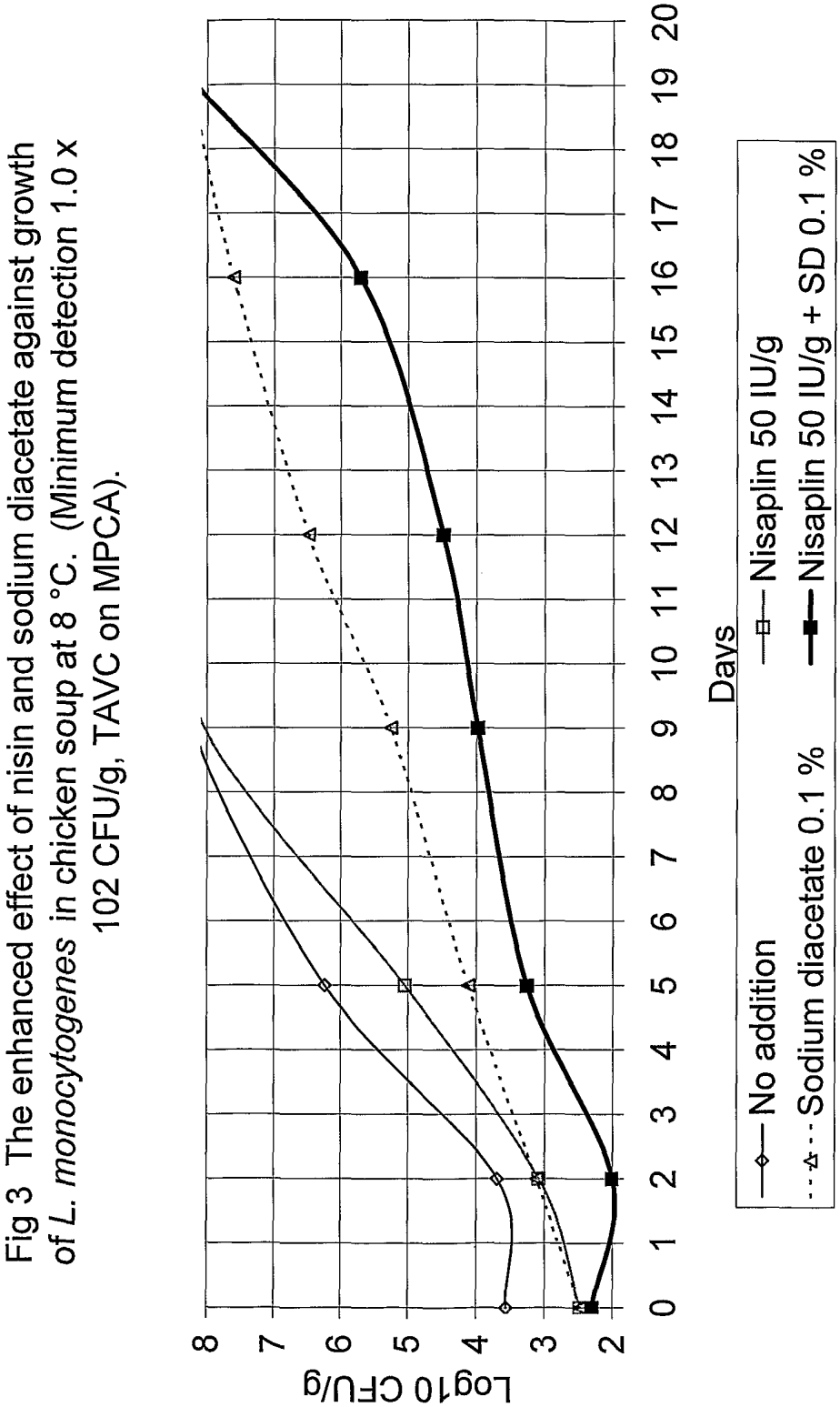
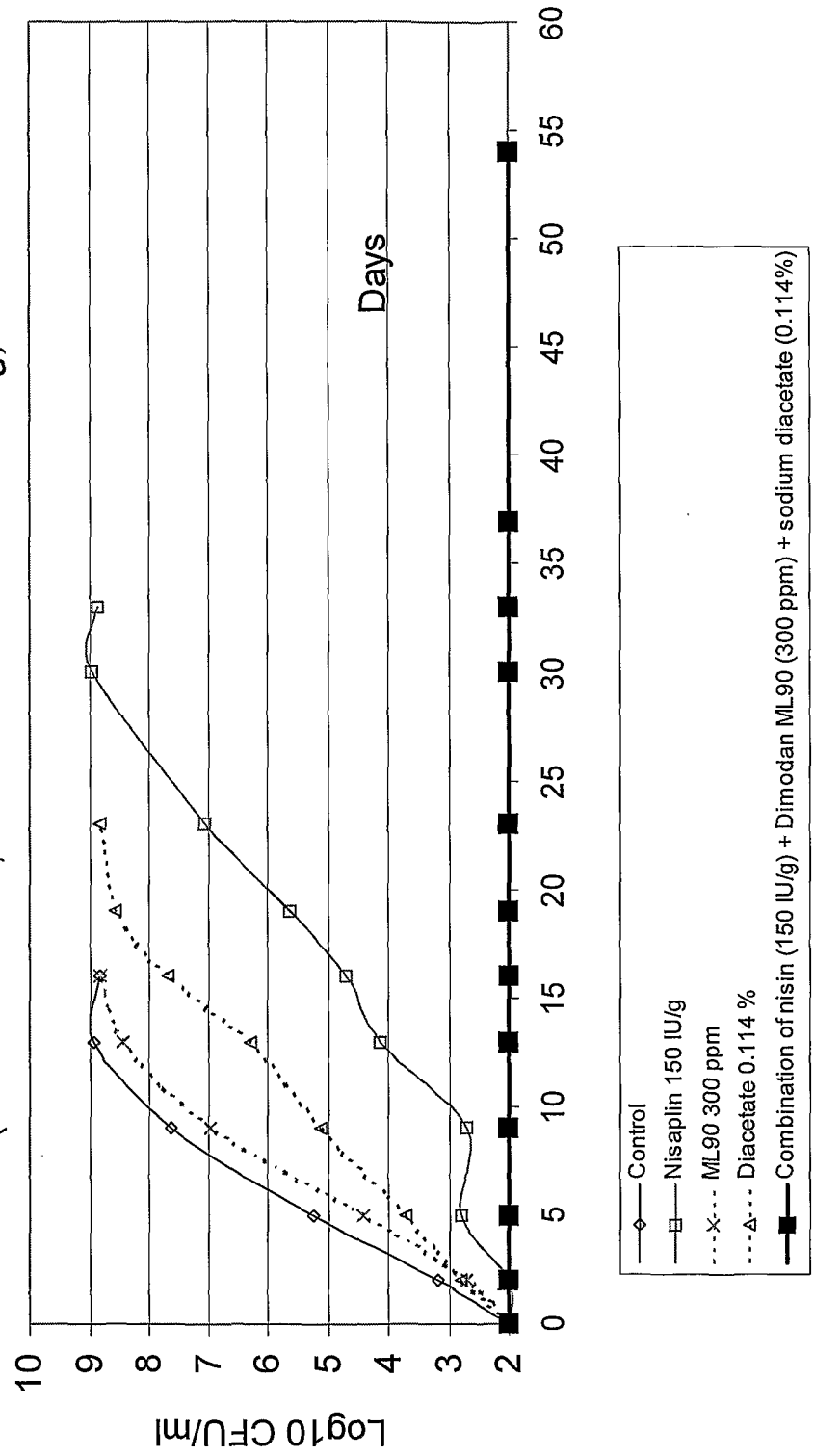


Fig 3 The enhanced effect of nisin and sodium diacetate against growth of *L. monocytogenes* in chicken soup at 8 °C. (Minimum detection 1.0 x 102 CFU/g, TAVC on MPCA).

Fig 4. Enhanced antimicrobial efficacy of the triple blend against Listeria monocytogenes in Bolognese sauce at 8 °C.
(TAVC on MPCA, min. detection 1.0 x 10^2 CFU/g).



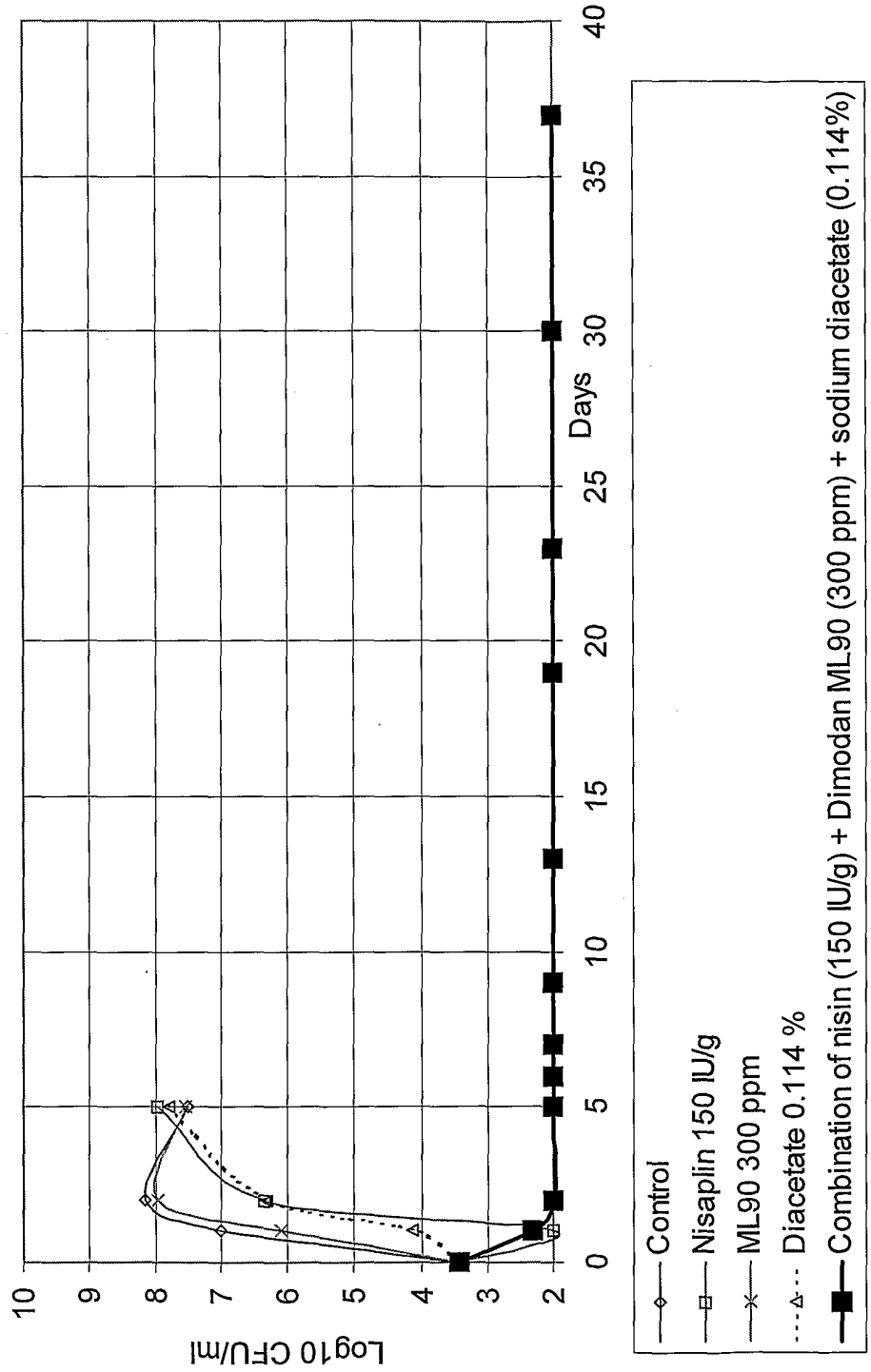


Figure 5. Efficacy of nisin/emulsifier/acetate blends against Bacillus cereus spores in Bolognese sauce at 20 °C. (TAVC on MPCA, min. detection 1.0 x 10^2 CFU/g).

INTERNATIONAL SEARCH REPORT

PCT/GB2005/001700

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L3/3463 A23L3/3508 A23B4/20 A23B4/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
22 July 2005	05/08/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Boddaert, P

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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