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(54) Title: USE OF NONANOIC ACID AS AN ANTIMICROBIAL AGENT, IN PARTICULAR AN ANTIFUNGAL AGENT

(57) Abstract: The invention relates to the use of nonanoic acid as an antimicrobial, in particular antifungal, agent or additive, in particular in or for foods, such as dairy products or fruit juices. A particular aspect of the invention comprises the use of nonanoic acid i a cheese coating. The invention also relates to a cheese coating in which nonanoic acid has been incorporated as antifungal agent; a cheese that has been provided with such a coating; and a nonanoic acid-containing composition for applying such a coating. The nonanoic acid is used in particular on or close to the surface of the food, or uniformly distributed through the food, in an amount of 10 - 10,000 ppm, in particular 100 - 1,000 ppm. The nonanoic acid can furthermore be used as an antimicrobial agent for treating substrates or surfaces, in particular substrates or surfaces that come into contact with foods; for protecting foods, cut flowers and bulbs during transport and/or during storage; in disinfectants and cleaning agents; to protect or treat wood; in cosmetics or skin care products; and in pharmaceutical compositions to prevent and treat fungal infections and yeast infections , such as *Candida*.

Use of nonanoic acid as an antimicrobial agent, in particular an antifungal agent

The present invention relates to the use of nonanoic acid as an antimicrobial agent, in particular an antifungal agent.

More particularly, the invention relates to the use of nonanoic acid as an antimicrobial agent, in particular an antifungal agent, in foods and in particular in dairy products such as cheese and products based on fruit, such as fruit juices.

The invention furthermore relates to foods which contain nonanoic acid as an antimicrobial agent.

Particular aspects of the invention lie in the use of nonanoic acid in (solutions or suspensions for) cheese coatings, in the nonanoic acid-containing cheese coatings thus obtained and in the cheeses coated with these nonanoic acid-containing coatings.

The use of the nonanoic acid in food products is known. For instance, it is used as a synthetic flavouring in, for example, non-alcoholic drinks, ice cream, confectionery, gelatine, milk puddings and bakery products.

US Patent 2 154 449 describes the antifungal properties of C_3 - C_{12} carboxylic acids and salts thereof, in particular the incorporation of calcium propionate in bread dough in order to prevent the formation of mould on bread.

European Patent Application EP 0 244 144 A1 teaches the addition of glyceryl fatty acid esters in combination with one or more C_6 - C_{18} carboxylic acids as preservatives to, inter alia, food compositions.

International application WO 96/29895 describes a method for improving the shelf/storage life of perishable products by treating surfaces, equipment and materials, which come into contact with the products during the processing thereof, with an antimicrobial aromatic compound. WO 96/29895 states that fatty acids, including nonanoic acid, can also be used in combination with the aromatic compound.

International application WO 92/19104 teaches the use of C_7 - C_{20} carboxylic acids, including nonanoic acid, for controlling infections in plants caused by bacteria and moulds.

European Patent Application EP 0 022 289 relates to the incorporation of C_3 - C_{11} carboxylic acids in polymers for the production of medical instruments, such as catheters.

European Patent Application EP 0 465 423 describes antimicrobial pharmaceutical preparations containing C_4 - C_{14} carboxylic acids. US Patent 4 406 884 describes

antimicrobial pharmaceutical preparations for topical use which contain C_5 - C_{12} carboxylic acids.

US Patent 3 931 413 teaches the treatment of plants with C_6 - C_{18} carboxylic acids to combat infections by moulds which overwinter in the buds of the plants.

Nonanoic acid is also used in some meat products to adjust the acidity. For instance, US Patent 4 495 208 describes a dog or cat food with good storage/shelf life which has a high moisture content (A_w > 0.9 and a water content of 50 - 80 %) that contains 4 -15 % (m/m) fructose, 0.3 - 3.0 % (m/m) of an edible organic acid, sufficient inorganic acid to obtain a pH in the range of 3.5 - 5.8 and an antifungal agent. The organic acid is preferably chosen from heptanoic acid, octanoic acid, nonanoic acid or a combination thereof.

In the animal feed according to US Patent 4 495 208 the edible organic acid is always present alongside a sugar (fructose) and an antifungal agent (antimycotic) known per se, such as sorbic acid and/or the salts thereof. It is stated that the combination of these three constituents in the indicated amounts gives a synergistic bactericidal action.

US Patent 3 985 904 describes a food based on meat which has a high moisture content and is suitable for human consumption or as an animal feed. This food has a moisture content of at least approximately 50 % (m/m) and a water activity A_w of at least approximately 0.90 and contains more than 50 % (m/m) of a ground, boiled, protein-like chicken, fish or meat material. 1 - 35 % (m/m) of a gelatine-like filler based on starch, between 1.7 and 3.8 % of an edible, non-toxic acid and an effective amount of an antifungal agent.

The edible organic acid is incorporated in this food in an amount which is sufficient to bring the pH of the food to a value in the range from 3.9 to 5.5. Although US-A 3 985 904 mentions various suitable edible acids in column 6, nonanoic acid is not explicitly mentioned here.

According to US-A 3 985 904, the antifungal agent is chosen from benzoates, propionates and sorbate salts.

EP-A 0 876 768 describes the use of fatty acid monoesters of polyglycerol to improve the storage/shelf life of foods. Here the fatty acid radicals can be chosen from caproic acid, caprylic acid, lauric acid or myristic acid.

The use of nonanoic acid in herbicidal compositions for agricultural use is described, inter alia, in US Patents 5 098 467, 5 035 741, 5 106 410 and 5 975 4110.

US Patents 4 820 438, 5 330 769 and 5 391 379 describe the use of nonanoic acid in soap and cleaning agents.

None of the above literature citations describes or suggests unambiguously that nonanoic acid can be safely incorporated in foods and/or can be used on foods in order to inhibit the growth of bacteria, moulds and yeasts. In particular, none of these literature citations teaches the dosage at which nonanoic acid can safely be used for this purpose.

Currently, natamycin is used as antifungal agent in cheese making. This compound, which is also designated pimaricin or "antibiotic A5283" and is marketed under the trade names Delvocid® and Natamax® (inter alia), is a metabolic product of *Streptomyces natalensis* and *S. chattanoogensis*.

However, the use of natamycin has a number of disadvantages. For instance it is fairly expensive. Moreover, it has been found that the mould *Penicillium discolor* is able to grow on (the surfaces of) cheeses treated with natamycin. This is particularly disadvantageous in the cheese industry, since *P. discolor* is widespread in cheese warehouses.

It has now been found that nonanoic acid displays an antimicrobial action, in particular an antifungal action, especially when it is used in amounts which can suitably be incorporated in food products. More particularly, it has been found that nonanoic acid can advantageously be used as an antimicrobial agent, in particular antifungal (fungicidal) agent, in dairy products such as cheese and products based on fruit, such as fruit juices.

The antimicrobial action of nonanoic acid found according to the invention is partly surprising because it is known that some types of mould (such as *Aspergillus niger, Synchephalastrum racemosus, Geotrichum candidum, Penicillium expansum, Rhizopus stolonifer* and *Mucor plombus*) naturally produce nonanoic acid.

In addition, it has been found according to the invention that nonanoic acid is also able to inhibit the development of yeasts, which can likewise arise in cheese warehouses.

In a first aspect the invention therefore relates to the use of nonanoic acid (n-octane-1-carboxylic acid, pelargonic acid, n-nonylic acid) as an antimicrobial agent, in particular antifungal agent (additive) in or for foods and/or other products which have to be protected against perishing caused by microorganisms. The invention also relates to the use of salts of nonanoic acid as an antimicrobial agent.

The invention further relates to foods which contain nonanoic acid as an antimicrobial agent, in particular antifungal agent.

The food can be any substance that is suitable for consumption by humans or animals, in particular for human consumption, and can be either a ready-to-eat food product or a constituent that can be incorporated in or processed to give a food product.

The food or food product is in particular a product or substance that is susceptible to perishing caused by microorganisms, including bacteria, yeasts and in particular moulds (that is to say when no antimicrobial agent is added), such as, for example, a substance or product which will keep for between a few days and a few weeks (for example from 3 days to 3 weeks) under the customary conditions for storage of the product, such as a temperature in the range from room temperature (20 - 25 °C) down to refrigerator temperature (approximately 4 °C). However, the invention is not restricted to these.

In this context the nonanoic acid is used to inhibit microbial growth, in particular the formation of mould, and thus to extend the storage/shelf life. For instance, microbial growth can be retarded by the use of nonanoic acid. The degree of retardation will be dependent on, inter alia, the food, the nonanoic acid concentration, the conditions under which the food is stored (temperature, atmospheric humidity), the types of microorganisms to which the food is exposed and the degree of loading. In the case of mould formation, the mould formation (i.e. the point in time at which the first growth of mould is discernible to the naked eye) will in general be delayed by at least one day, preferably at least 5 - 7 days, that is to say at the temperature at which foods are usually stored – usually room temperature (20 °C) or in the refrigerator (4 °C) – compared with the untreated food. For instance, in the case of cheese that was coated with a nonanoic acid-containing coating according to the invention the first discernible formation of mould was postponed from 60 to 67 days. In this context reference is made to Example 1 below, as well as the results given in Figure 1.

For the purposes of the invention, "inhibiting mould formation" and/or "antifungal" is preferably also understood to mean that the development of yeasts is (also) inhibited.

Moreover, it has been established according to the invention that nonanoic acid also has an antibacterial action, for example against bacteria which cause food to perish or otherwise reduce the quality thereof, and/or against pathogens such as *Listeria, Legionella, Salmonella* and *E.coli* O157, *Staphylococcus.*

This inhibitory action of nonanoic acid on (the growth of) bacteria can also advantageously be employed in (the preparation of) fermented dairy products such as yoghurt. This will be explained in more detail below.

The food can be a solid, semi-solid or fluid food and can be a fermented or non-fermented food.

A few non-limiting examples of foods in which nonanoic acid can be used according to the invention as an antimicrobial agent, in particular antifungal agent, are:

- ready-to-eat food products, including dough products such as pre-baked bread, noodles, pasta, soups and the like; fish and meat products such as sausage, and products based on vegetables or fruit, such as fruit juices and canned fruit or combinations of fruit (juices) with dairy products; flour; nuts and (dried) southern fruits; and also products such as pre-prepared meals, diet foods, complete foods and baby food;
- foods and constituents for further processing, such as mayonnaise, ketchup and similar sauces; jam, marmalade and similar fruit preparations; and the like.

According to the invention nonanoic acid can also be used outside the food sector as an antimicrobial agent, in particular antifungal and/or antibacterial agent, and examples of this will be given below.

One example that is worthy of mention at this juncture is the use of nonanoic acid or a nonanoic acid-containing coating to improve the storage/shelf life of fruit such as oranges, lemons, grapefruit, apples, pears and also nuts and (dried) southern fruits, coffee, tea, tobacco and the like, in particular before or during transport and/or during long-term storage, for example in a warehouse or a fruit store (which may or may not be air-conditioned).

When used as an antifungal agent according to the invention, the nonanoic acid will be used in an amount effective for the inhibition of moulds, yeasts and bacteria, which as a rule will be between 1 and 10,000 mg nonanoic acid per kg food, in particular 10-1,000 mg nonanoic acid per kg food and more particularly 100–500 mg nonanoic acid per kg food. Thus, for example, nonanoic acid can be used in yoghurt in an amount of approximately 200 milligram (mg) nonanoic acid per kilogram (kg) yoghurt. The lower limit for the effective amount of nonanoic acid will preferably be chosen from the series 10, 25, 50 or 100 mg nonanoic acid per kg food, whilst the upper limit is preferably chosen from the series 10,000, 5,000, 2,500, or 1,000 mg nonanoic acid per kg food. Preferably, these amounts are based on the water content of the food. Thus, in the case of a food having a water content of 80 %, 80 % of the abovementioned amounts of nonanoic acid can also be added per kg food.

The precise amount of nonanoic acid will, however, be dependent on the intended food and the way in which the nonanoic acid is used in the food. Thus, the nonanoic acid can be uniformly distributed throughout the entire food but, for example - especially in the case of solid or semi-solid foods – can also be present essentially only on or near the surface of the food, for example in the form of a nonanoic acid-containing antimicrobial, in particular antifungal, coating or surface layer, or as a result of treatment of the surface of the food with nonanoic acid. In these latter cases the concentration of nonanoic acid, based on the complete food, can be low (that is to say lower than the amounts indicated above), provided that sufficient nonanoic acid is present at or close to the surface in order to achieve the desired antimicrobial, in particular antifungal, action.

In general the presence of nonanoic acid in amounts of 10 - 10,000 ppm, in particular 100 - 2,000 ppm – i.e. locally or uniformly throughout the entire food – will be adequate to obtain the desired antimicrobial, in particular antifungal, action. The same concentrations of nonanoic acid – i.e. locally or uniformly throughout the entire food – will as a rule be sufficient to inhibit and/or to prevent the growth of yeast and/or of bacteria.

In a preferred aspect the food product is a dairy product, which in general is defined as a food based on milk or constituents of milk, in particular based on cows milk or constituents thereof.

The dairy product is in particular a fermented dairy product that can be solid, semi-solid or fluid. A few non-limiting examples are cheese, butter, cream, yoghurt or yoghurt products (for example yoghurt drinks, such as, for example, milk/fruit juice drinks), cottage cheese, kefir, milk puddings and the like. The invention can also be employed in food products in which such dairy products have been incorporated/processed, such as sauces, pastries, desserts, foods (including complete food and baby food), snacks (for example containing cheese), meat products (such as ham in which proteins have been incorporated), powdered milk and coffee whiteners, and the like.

Use in cheese, and in particular in cheeses which have a low salt content (that is to say less than 4 %, in particular less than 3 %) and a high moisture content (that is to say 30 % or more, in particular 40 % or more) is to be particularly preferred. This can be carried out in particular by treating the surface of the cheese with nonanoic acid. Thus, the invention can (also) be used with feta, cheese spread and similar products.

The fermented dairy product preferably has a pH of 3.5 to 5.5, for example in the

range of 5.1 - 5.5 for cheese and of 3.9 - 4.4 for yoghurt. Although it is not precluded that addition of nonanoic acid according to the invention makes some (usually minor) contribution to achieving this value, the final pH will as a rule be the result of the fermentation process and the buffer action possibly associated with this.

In another preferred embodiment the food product is a fruit juice or similar drink, such as, for example, products in which dairy products such as milk or yoghurt and fruit juices have been processed, which have a limited shelf-life.

The nonanoic acid can be used in a manner known per se for antimicrobial agents, in particular antifungal agents, that is to say by adding the nonanoic acid or a nonanoic acid-containing additive to the food or food product, or incorporating the nonanoic acid or a nonanoic acid-containing additive in the food or food product, during and/or after the preparation thereof. During this operation the nonanoic acid can be uniformly mixed or distributed through the food and/or used on the surface of the food, for example by spraying or brushing with nonanoic acid (for example in the form of an aqueous solution), by immersing (in particular cheese) in a solution of nonanoic acid or by applying a nonanoic acid-containing coating. For this operation it is possible to use, for example, an aqueous solution or suspension of nonanoic acid or another nonanoic acid-containing, preferably liquid, mixture, which contains 100 - 5,000 ppm, in particular 200 to 3,000 ppm nonanoic acid and which furthermore can contain all constituents known per se for solutions for applying a cheese coating, such as (the constituents of) synthetic coatings known per se (for example based on copolymers) and/or coatings based on foodstuffs.

For instance – in a 140 gram coating for a 12.8 kg cheese – the nonanoic acid concentration in the coating can be 5,000 ppm (which corresponds to 49.2 mg nonanoic acid per kg cheese), 1,000 ppm (which corresponds to 9.8 mg/kg cheese) or 100 ppm (which corresponds to 0.98 mg/kg cheese).

The nonanoic acid-containing cheese coating thus obtained, the cheeses which have been provided with such nonanoic acid-containing cheese coatings and the nonanoic acid-containing solutions which are used in this operation form further aspects of the invention.

In this context a further advantage of nonanoic acid is that it is also able to counteract and/or prevent too extensive development of the surface flora on the cheese (coating) – which can lead to the cheese rind being adversely affected – (this is in contrast to natamycin, that essentially is not able to exert any influence on bacterial growth).

As a rule the nonanoic acid will be used to replace the one or more antimicrobial, in

particular antifungal, additives already used in a food known per se. In addition, the nonanoic acid can advantageously be used in those foods for which the known antimicrobial agents are unsuitable or less suitable. For such applications the use of nonanoic acid can form an alternative to the sterilisation treatments and/or similar antimicrobial treatment (that is to say other than the use of an antimicrobial additive) which are otherwise required.

Usually a single treatment of the food with nonanoic acid – such as the application of a nonanoic acid-containing coating – will be sufficient to obtain the desired antimicrobial action. However, repeated treatment of the food with nonanoic acid is not precluded.

According to the invention nonanoic acid is used in particular to replace natamycin, in particular in applications in the dairy and cheese industries. In this regard reference is made, for example, to the applications of natamycin which are described by J. Stark in *De Ware(n) Chemicus,* 27 (1997), 173-176.

According to the invention nonanoic acid is highly preferentially compatible with the food, that is to say the use of nonanoic acid according to the invention has no adverse effect on the flavour, odour, consistency, pH or other desired characteristics of the food, at least not during the time that the food has to be or can be kept or stored prior to end use or consumption. As a rule this means that the food must be acid-resistant to a certain extent, that is to say at least must be able to withstand the pH that is obtained by the use of the nonanoic acid in the abovementioned amounts. In the event of possible problems with the compatibility, the use of a separate nonanoic acid-containing coating can offer a solution.

The food can furthermore contain all other additives known per se for the food, provided that these are compatible with nonanoic acid and do not adversely affect the antimicrobial action thereof.

When nonanoic acid is used as antimicrobial agent according to the invention, as a rule no further antimicrobial agent will be required and according to one embodiment of the invention the food essentially contains exclusively nonanoic acid as antimicrobial agent, that is to say in the amounts specified above (in per cent by mass or ppm). However, it can not be entirely precluded that in addition to the nonanoic acid minor amounts of one or more further antimicrobial agents known per se are present, such as the agents which are mentioned below. Therefore, "essentially exclusively" is defined as meaning that the nonanoic acid makes up at least 80 % (m/m), preferably at least 90 % (m/m) and more preferentially at least 95 - 99 % (m/m) of all antimicrobial constituents

present (that is to say added to the food in order to achieve an antimicrobial action).

Furthermore it is possible to use nonanoic acid in a mixture with one or more antimicrobial agents which are known per se and are compatible with nonanoic acid, a synergistic effect possibly being able to be obtained. In this case – compared with the use of the known agent as such – the nonanoic acid will as a rule replace some of the quantity of the known antimicrobial agent usually used. Nonanoic acid will as a rule make up at least 30 % (m/m), preferably at least 50 % (m/m) and more preferentially at least 70 % (m/m) of the total antimicrobial constituents in such mixtures.

A few non-limiting examples of antimicrobial agents that can be used according to the invention in combination with nonanoic acid are: sorbic acid and salts thereof, benzoic acid and salts thereof, para-hydroxybenzoic acid or esters thereof, propionic acid and salts thereof, pimaricin, polyethylene glycol, ethylene/propylene oxides, sodium diacetate, caprylic acid (octanoic acid), ethyl formate, tylosin, polyphosphate, metabisulphite, nisin, subtilin and diethyl pyrocarbonate.

The nonanoic acid can furthermore be used in combination with agents for adjusting the acidity, including the acids acceptable for foods, such as citric acid, acetic acid and the like. In this context the nonanoic acid can, in particular, protect the food (which in this case can have a pH in the range from 2 to 6) against acid-resistant moulds. Examples of such acid-resistant moulds are, but are not restricted to, *Penicillium roqueforti, P. carneum, P. italicum, Monascus ruber* and/or *Paecilomyces variotii* (which occur, for example, in rye bread); and *Penicillium glandicola, Penicillium roqueforti, Aspergillus flavus, Aspergillus candidus* and/or *Aspergillus terreus* (which, for example, occur in products which have been preserved by acid, such as sour and/or sweet-sour preserves).

More generally, according to the invention it is preferable that at least some, and preferably an appreciable proportion, of the nonanoic acid is present in the undissociated form in the food. The general rule in this context is that the amount of undissociated nonanoic acid increases at lower pH: for instance, approximately 90 % of the nonanoic acid is present in undissociated form at a pH of approximately 3.8.

According to one aspect of the invention, nonanoic acid is therefore also used in foods which have a low pH, such as a pH in the range 2 to 6, preferably 3 to 5.8, or 4 to 5.6. For instance, for example, the pH of cheese rind is around 4.8 - 5.3.

In addition to the antimicrobial, in particular antifungal, action described above, the use of nonanoic acid according to the invention can also yield the following further

advantages:

- nonanoïc acid is a stable molecule in both the dissociated and undissociated form. The long alkyl chain is inert and renders the molecule barely reactive.
- nonanoic acid is a natural substance which occurs in plants, inter alia;
- nonanoic acid has been approved for use in foods (inter alia by the FDA);
- nonanoic acid remains stable under the majority of processing steps/processes for food products;
- nonanoic acid is less susceptible to UV light than is, for example, natamycin;
- nonanoic acid is stable in the presence of metals in metallic form;
- nonanoic acid is stable under heating.

The invention has been described above with reference to a preferred embodiment thereof; that is to say use in foods, in particular in dairy products. However, it will be clear to those skilled in the art from the above description that nonanoic acid can also find use outside the food sector as an antifungal, yeast-inhibiting and/or antibacterial agent.

In this context it will, in particular, be an advantage that nonanoic acid has been approved for use in foods, so that it can be used in applications where it can come into contact with foods or the human body, such as with the skin.

A number of possible, non-limiting applications are:

- use as or in disinfectant(s), cleaning agent(s) and the like, for both domestic and industrial applications;
- disinfection and/or cleaning (including preventive treatment) of conveyor belts, pallets and the like;
- disinfection and/or cleaning (including preventive treatment) of apparatus, products and/or surfaces which come into contact with foods, such as cutting machines, mixers, stirrers, sorting equipment, filling machines and other equipment from the food processing industry; vats, dishes, tanks, plates, containers and other holders; and also worktops, sink units and the like; both domestic and industrial;
- disinfection and/or cleaning (including preventive treatment) of areas which may or may not be enclosed, in particular areas in which food products are processed and/or stored, such as cupboards, refrigerators, kitchens, factory areas, freight areas, warehouses and the like (both domestic and industrial); and in particular cheese warehouses and other commercial premises where *P. discolor* can occur;
- coating and/or (preventive) treatment of packaging for, for example, foods (such as

fruit, vegetables, cheese and the like), for example made of materials such as plastic, paper, cardboard or shaped cardboard;

- protection of fruit, such as oranges, lemons, grapefruit, apples, pears; nuts and (dried) southern fruits, coffee, tea, tobacco and the like, and also of cut flowers and bulbs, against moulds and/or bacteria, before or during transport and/or during (long-term) storage, for example in a warehouse or in an (optionally) air-conditioned fruit store;
- disinfection and/or cleaning (including preventive treatment) of, for example, tents or tarpaulins, and also indoors (for example on walls) to prevent or to counteract mould growth, for example as a consequence of damp;
- protection and/or treatment of wood and similar materials;
- use in cosmetics and skincare products;
- use for pharmaceutical applications, for example to prevent and treat fungal infections and yeast infections, such as *Candida*.

These aspects of the invention in general comprise the treatment of a surface or substrate that is susceptible to mould formation, or that can be contaminated or infected by a mould and/or the spores thereof, with an amount of nonanoic acid which has an effective antifungal and/or antibacterial action.

This amount will differ depending on the application and the way in which the nonanoic acid is used on the surface or substrate. As a rule the presence of nonanoic acid in amounts of 10 - 10,000 ppm, in particular 100 - 2,000 ppm, will again be sufficient to achieve an antimicrobial, in particular antifungal, action, although higher concentrations can be used for some applications. The nonanoic acid can be used on the surface or substrate in any suitable way, such as, once again, spraying or brushing with nonanoic acid (for example in the form of an aqueous solution), by applying a nonanoic acid-containing coating or by use of an atomised spray containing nonanoic acid. This treatment can optionally be repeated.

In this context the nonanoic acid can once again be used instead of, or together with, disinfectants which may be known for the envisaged application, as well as in combination with other agents or constituents customary for the envisaged application. For these applications, the nonanoic acid and any other constituents can optionally be marketed in a suitable container, for example in a bottle or in the form of a spray.

A particular application of nonanoic acid according to the invention furthermore

relates to the control – in particular the inhibition – of bacterial growth during fermentation processes, such as the preparation of fermented food products such as yoghurt. For this application use is made in particular of the antibacterial action of nonanoic acid. For instance, nonanoic acid can be used to control the pH during or after such fermentation processes and in particular to prevent and/or reduce post-acidification of, for example, yoghurt, as explained in more detail in the examples. The taste of the yoghurt is retained for longer as a result. In addition, the antimicrobial, in particular antifungal, action according to the invention will also be obtained.

The invention will now be explained with reference to the following non-limiting examples and the figures, in which:

- Figure 1 is a graph (time against visible intensity of mould formation) in which the effect of nonanoic acid on mould formation on Gouda cheese is shown;
- Figure 2 is a graph (time against number of bacteria) which shows the effect of nonanoic acid (pelargonic acid) on the development of yoghurt bacteria at 7 °C;
- Figure 3 is a graph (time against pH) which shows the effect of nonanoic acid (pelargonic acid) on the post-acidification of yoghurt at 7 °C;
- Figure 4 is a graph (time against number of bacteria) which shows the effect of nonanoic acid (pelargonic acid) on the development of yoghurt bacteria at 32 °C;
- Figure 5 is a graph (time against pH) which shows the effect of nonanoic acid (pelargonic acid) on the post-acidification of yoghurt at 32 °C;
- Figure 6 is a plot (time against number of bacteria) that shows the influence of nonanoic acid (pelargonic acid) on the development of surface flora on cheese rind;
- Figure 7 is a plot (time against number) that shows the effect of nonanoic acid (pelargonic acid) on the development of *D. hansenii, S. cereviseae, C. lipolytica* and *R. rubra*;
- Figures 8A and 8B are photographs which show the effect of natamycin (Figure 8A) and nonanoic acid (Figure 8B), respectively, on the inhibition of the growth of *P. discolor* on blocks of cheese rind;
- Figure 9 is a graph (time against number of bacteria) which shows the effect of nonanoic acid on the growth of *Bacillus cereus* in soup;
- Figure 10 is a graph (time against number of bacteria) which shows the effect of nonanoic acid on the growth of *Staphylococcus aureus* in soup;
- Figure 11 is a graph (time against number of cells) which shows the effect of

nonanoic acid on the growth of *Debaromyces hansenii* in a milk/fruit juice drink;

- Figure 12 is a graph (time against number of cells) which shows the effect of nonanoic acid on the growth of *Penicillium italicum* in a milk/fruit juice drink.

Experimental

Example 1: Use of nonanoic acid in Gouda cheese

A trial production of Gouda cheeses was made. In this batch of cheeses one series was treated with 1000 ppm nonanoic acid (nonanoic acid) and the other series was not treated with a fungicide (blank). The two series were inoculated with spores of the mould *P. discolor* (0.1 spore/cm^2) and stored at 13 °C and 88 % relative humidity. All individual cheeses were assessed visually at frequent intervals for the extent of the presence of mould. The following scale was used for the optical assessment of the intensity of visible moulds;

0 = no mould
1 = some mould
2 = distinct mould
3 = considerable mould
4 = very considerable mould or overgrown with mould.

The results are shown diagrammatically in Figure 1. In the case of the cheeses without fungicide slight mould growth (intensity 1) was detectable after about 60 days. In the case of the series of cheeses treated with nonanoic acid it was 66 days before mould growth (intensity 1) was observed.

Example 2: Use of nonanoic acid in yoghurt to prevent post-acidification

In an experiment various concentrations of nonanoic acid were added to freshly prepared yoghurt. One series was monitored for 8 hours at the culture temperature (filling, 32 °C) and another series was incubated for 14 days at 7 °C (refrigerator temperature). This was carried out to investigate the extent to which nonanoic acid has an effect during yoghurt fermentation and/or during storage of the filled packs of yoghurt. For both series the pH was determined and the number of yoghurt bacteria.

The results are shown in Figures 2 - 5. Addition of 1,000 ppm nonanoic acid substantially prevented post-acidification (32 °C) and the number of yoghurt bacteria was reduced by 2 log units. At 7 °C an effect on the post-acidification was already detectable at

lower nonanoic acid contents (200 ppm). Addition of 1,000 ppm prevented post-acidification virtually completely when storing at refrigerator temperature and the number of yoghurt bacteria decreased by 4 log units.

Example 3: Effect of nonanoic acid on the surface flora of cheese rind

The effect of nonanoic acid on the surface flora on cheese rind was determined. The results (time against number of bacteria) are shown in Figure 6.

The effect of nonanoic acid (pelargonic acid) on the development of *D. hansenii, S. cereviseae, C. lipolytica* and *R. rubra* was also determined. The results (time against number) are shown in Figure 7.

Example 4: Use on blocks of cheese rind

In this experiment blocks of cheese rind were inoculated with *P. discolor*. The blocks were incubated at 20 °C and high relative humidity (95 %). These conditions were employed to provide the mould with the optimum opportunity to grow and are therefore more severe than the usual conditions for maturing cheese.

The results are given in Figure 8, which shows photographs of the blocks of cheese rind taken two weeks after inoculating with *P. discolor*. One series was treated with natamycin (Figure 8A) and the other series with nonanoic acid (Figure 8B). It can clearly be seen that after 2 weeks mould formation was inhibited in the blocks treated with nonanoic acid.

Example 5: Use in soup

In this experiment a creamy mushroom soup with parsley (chill-fresh product obtained from the Albert Heijn delicatessen in March 2000) was inoculated with 10^4 CFU/ml (colony-forming units per ml soup) of *Bacillus cereus* (NIZO B443) or with 10^4 CFU/ml *Staphylococcus aureus* (NIZO B1211). The soup was then incubated at 20 °C, without and with increasing concentrations of nonanoic acid (100, 500 and 1,000 ppm). Samples were taken at the times indicated in Figures 9 and 10 (Figure 9 for *B. cereus* and Figure 10 for *S. aureus*). From each sample a series of dilutions was plated to determine the number of CFU/ml soup. The *B. cereus* samples were plated on mannitol egg yolk polymyxin agar (MYP) and incubated for 24 hours at 30 °C; the *S. aureus* samples were plated on Baird-Parker egg yolk tellurite agar (BP) and incubated for 48 hours at 37 °C.

The results are shown in Figures 9 and 10. The addition of 100 ppm nonanoic acid to the soup has a slightly inhibiting effect on the growth of both *B. cereus* and *S. aureus*, whilst with the addition of 500 or 1,000 ppm nonanoic acid the growth of both bacteria is virtually completely inhibited.

Example 6: Use in a milk/fruit juice product

In this experiment a milk/fruit juice drink ("Milk & Fruit"™ from Coberco, obtained from Albert Heijn; "Milk & Fruit"™ is a chilled-fresh, pasteurised product without preservatives, consisting of 80 % drinking yoghurt and 20 % pineapple juice and has a pH value of 4.0) was inoculated with 10^2 CFU/ml *Debaromyces hansenii* (NIZO F937) or *Penicillium italicum* (CBS 278.58). The milk/fruit juice drink was then incubated at 20 °C, without and with increasing concentrations of nonanoic acid (100, 500 and 1,000 ppm). Samples were taken at the times indicated in Figures 11 and 12 (Figure 11 for *D. hansenii* and Figure 12 for *P. italicum*). For each sample a series of dilutions was plated in order to determine the number of CFU/ml drink. The samples were plated on oxytetracycline glucose yeast agar (OGY) and incubated for 5 days at 25 °C. The results are shown in Figures 11 and 12. Addition of 100 ppm nonanoic acid gives complete inhibition of the growth of *D. hansenii*. Addition of 100 or 500 ppm inhibits the growth of *P. italicum* and addition of 1,000 ppm nonanoic acid gives complete inhibition of the growth of *P. italicum* for up to 6 days.

CLAIMS

1. Use of nonanoic acid as an antimicrobial, in particular antifungal, agent or additive in or for foods.

2. Use according to Claim 1, wherein the nonanoic acid is present in an amount of 10 - 10,000 ppm, in particular 100 - 1,000 ppm.

3. Use according to Claim 1 and/or 2, wherein the food is a solid or semi-solid food and wherein the nonanoic acid is essentially present on or close to the surface of the food.

4. Use according to Claim 3, wherein the nonanoic acid is present in the form of a nonanoic acid-containing coating or surface layer.

5. Use according to one of Claims 1 - 4, wherein the food is a dairy product.

6. Use according to one of Claims 1 - 5, wherein the dairy product is cheese.

7. Use according to Claim 1 and/or 2, wherein the nonanoic acid is essentially uniformly distributed through the food and is present in an amount of 1 - 10,000 mg, preferably 10 - 1,000 mg and more preferentially 100 - 500 mg nonanoic acid per kg of the food.

8. Use according to Claim 7, wherein the food is a dairy product.

9. Use according to Claim 7, wherein the food is a fruit juice.

10. Dairy product that contains nonanoic acid as an antimicrobial agent, in particular antifungal agent.

11. Dairy product according to Claim 10, wherein the nonanoic acid is present in an amount of 10 - 10,000 ppm, in particular 100 - 1,000 ppm.

12. Dairy product according to Claim 10 or 11, being a solid or semi-solid dairy product, the surface of which has been treated with nonanoic acid or a solution of nonanoic acid and/or that has been provided with a nonanoic acid-containing coating.

13. Dairy product according to one of Claims 10 - 12, being cheese.

14. Dairy product according to Claim 10 or 11 that contains nonanoic acid in an amount of 1 - 10,000, preferably 10 - 1,000 and more preferentially 100 - 500 mg nonanoic acid per kg of the dairy product, wherein the nonanoic acid is essentially uniformly distributed through the dairy product.

15. Fruit juice that contains nonanoic acid as an antimicrobial agent, in particular antifungal agent, in particular in an amount of 1 - 10,000, preferably 10 - 1,000 and more preferentially 100 - 500 mg nonanoic acid per kg of the fruit juice.

16. Cheese coating in which nonanoic acid has been incorporated as antimicrobial agent, in particular antifungal agent.

17. Cheese coating according to Claim 16, in which nonanoic acid is present in an amount of 10 - 10,000 ppm, in particular 100 - 1,000 ppm.

18. Cheese, in particular a cheese having a low salt content and/or a high moisture content, having a cheese coating according to Claim 16 or 17.

19. Composition for coating and/or treating the surface of a cheese, comprising a solution of 100 – 5,000 ppm, in particular 200 ppm to 3,000 ppm, nonanoic acid and optionally one or more constituents, known per se, of solutions for coating and/or treating the surface of a cheese.

20. Use of nonanoic acid as an antifungal agent, comprising the treatment of a surface or substrate that is susceptible to mould formation, or that can be contaminated or infected by a mould and/or the spores thereof, with an amount of nonanoic acid that

has an effective antifungal action.

21. Use according to Claim 20, wherein the surface or substrate is a surface or substrate that can come into contact with a food and/or with the human body, such as the skin.

22. Use of nonanoic acid according to Claim 20 or 21, wherein the nonanoic acid is used for:
- disinfection and/or cleaning (including preventive treatment) of conveyor belts, pallets and the like;
- disinfection and/or cleaning (including preventive treatment) of apparatus, products and/or surfaces which come into contact with foods;
- disinfection and/or cleaning (including preventive treatment) of areas that may or may not be enclosed;
- coating and/or (preventive) treatment of packaging for, for example, foods, for example made of materials such as plastic, paper, cardboard or shaped cardboard.

23. Use of nonanoic acid according to one of Claims 20 - 22 in or for cheese warehouses.

24. Use of nonanoic acid to protect fruit, nuts and (dried) southern fruits, coffee, tea, tobacco and the like, and also cut flowers and bulbs, against attack by moulds and/or bacteria, before or during transport and/or during (long-term) storage, for example in a warehouse or in an (optionally) air-conditioned fruit store.

25. Use according to Claim 24, wherein the nonanoic acid is used as a nonanoic acid-containing coating.

26. Use of nonanoic acid as an antimicrobial, in particular antifungal, constituent in disinfectant(s), cleaning agent(s) and the like, for both domestic and industrial use.

27. Use of nonanoic acid to protect and/or treat wood and/or similar materials against

attack by mould and/or as an antifungal constituent in compositions for the treatment of wood and/or similar materials.

28. Use of nonanoic acid as an antimicrobial, in particular antifungal, constituent in cosmetics and/or skin care products.

29. Use of nonanoic acid as an antimicrobial constituent, in particular as a fungicide, in the preparation of a pharmaceutical composition to prevent and treat fungal infections and yeast infections, such as *Candida*.

Fig 1

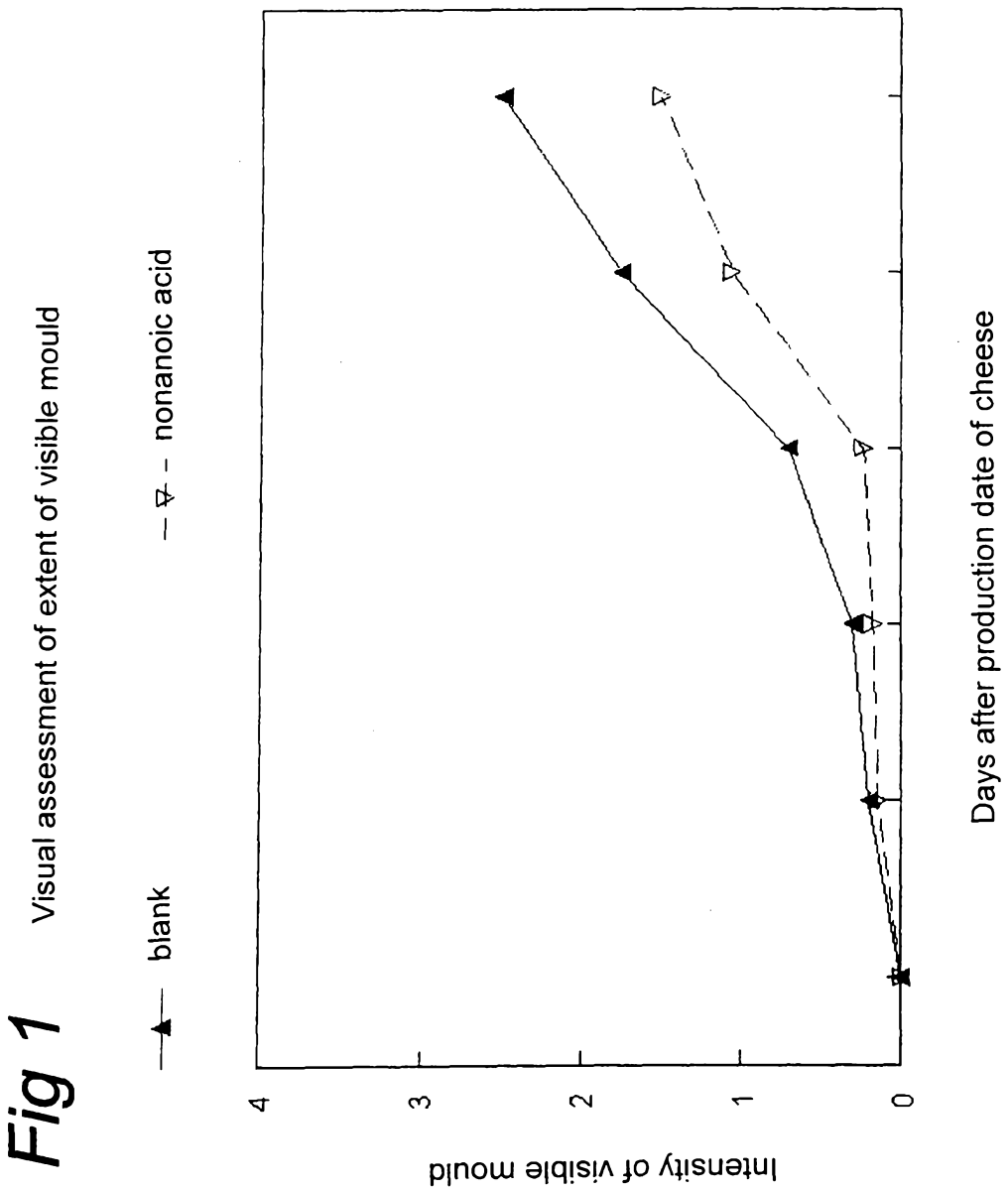

Visual assessment of extent of visible mould
blank
nonanoic acid
Intensity of visible mould
0
1
2
3
4
Days after production date of cheese

Fig 2

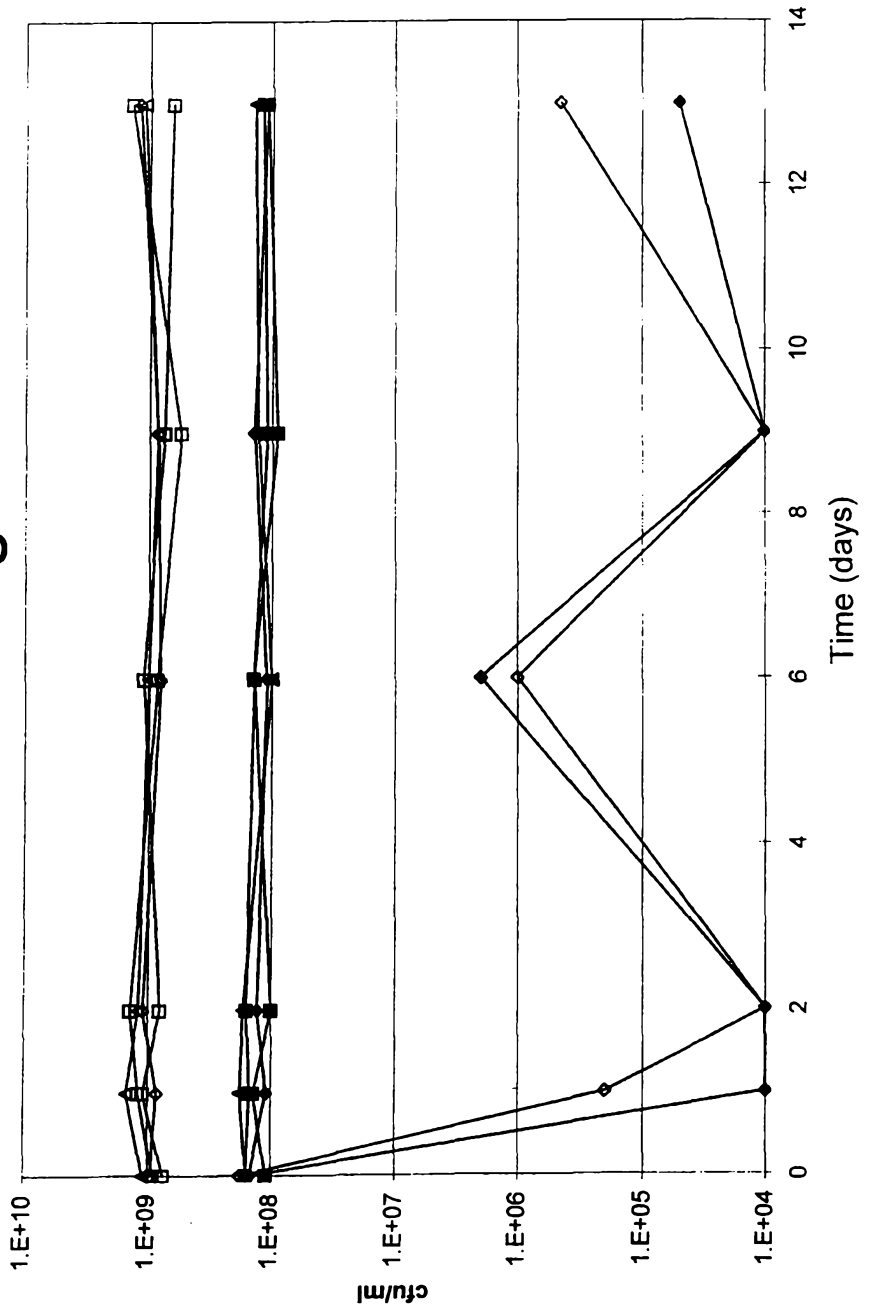

0 mg/l pelargonic acid Lb.acidophilus
50 mg/l pelargonic acid Lb.acidophilus
200 mg/l pelargonic acid Lb.acidophilus
500 mg/l pelargonic acid Lb.acidophilus
1000 mg/l pelargonic acid Lb.acidophilus
0 mg/l pelargonic acid Strep. thermophilus
50 mg/l pelargonic acid Strep. thermophilus
200 mg/l pelargonic acid Strep. thermophilus
500 mg/l pelargonic acid Strep. thermophilus
1000 mg/l pelargonic acid Strep. thermophilus
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
cfu/ml
0
2
4
6
8
10
12
14
Time (days)

Fig 3

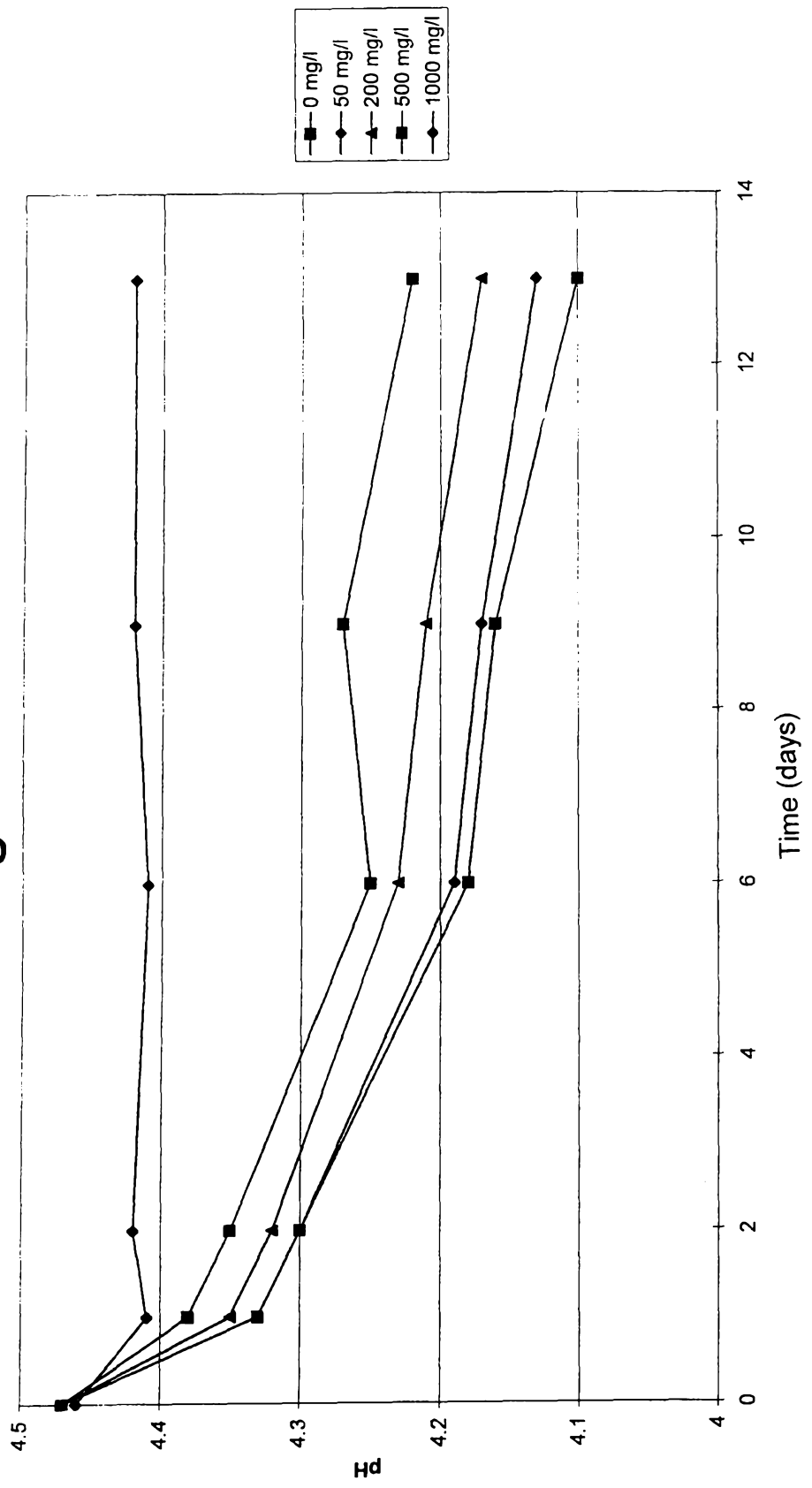

0 mg/l
50 mg/l
200 mg/l
500 mg/l
1000 mg/l
pH
4.5
4.4
4.3
4.2
4.1
4
0
2
4
6
8
10
12
14
Time (days)

Fig 4

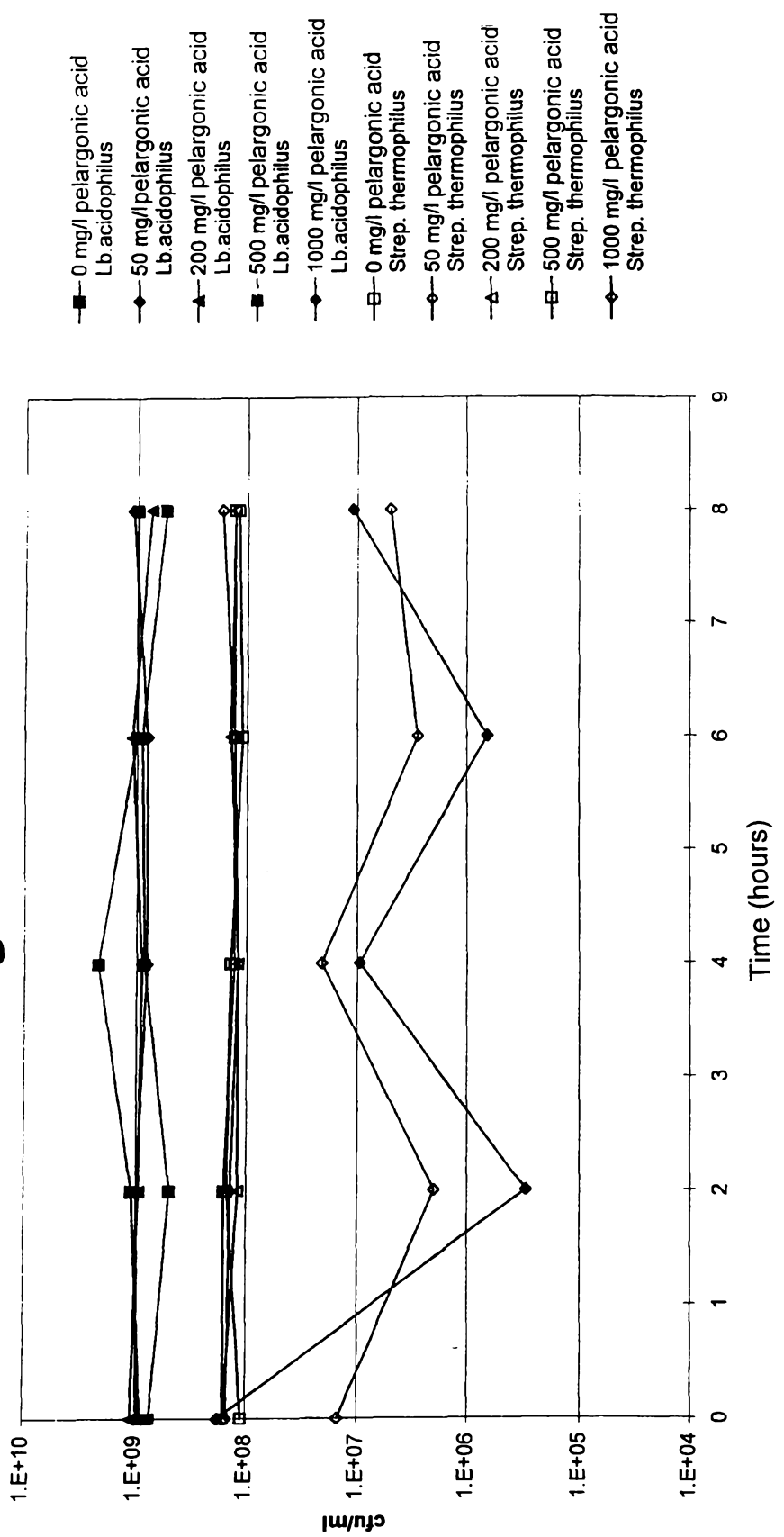

0 mg/l pelargonic acid Lb.acidophilus
50 mg/l pelargonic acid Lb.acidophilus
200 mg/l pelargonic acid Lb.acidophilus
500 mg/l pelargonic acid Lb.acidophilus
1000 mg/l pelargonic acid Lb.acidophilus
0 mg/l pelargonic acid Strep. thermophilus
50 mg/l pelargonic acid Strep. thermophilus
200 mg/l pelargonic acid Strep. thermophilus
500 mg/l pelargonic acid Strep. thermophilus
1000 mg/l pelargonic acid Strep. thermophilus
cfu/ml
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
0
1
2
3
4
5
6
7
8
9
Time (hours)

Fig 5

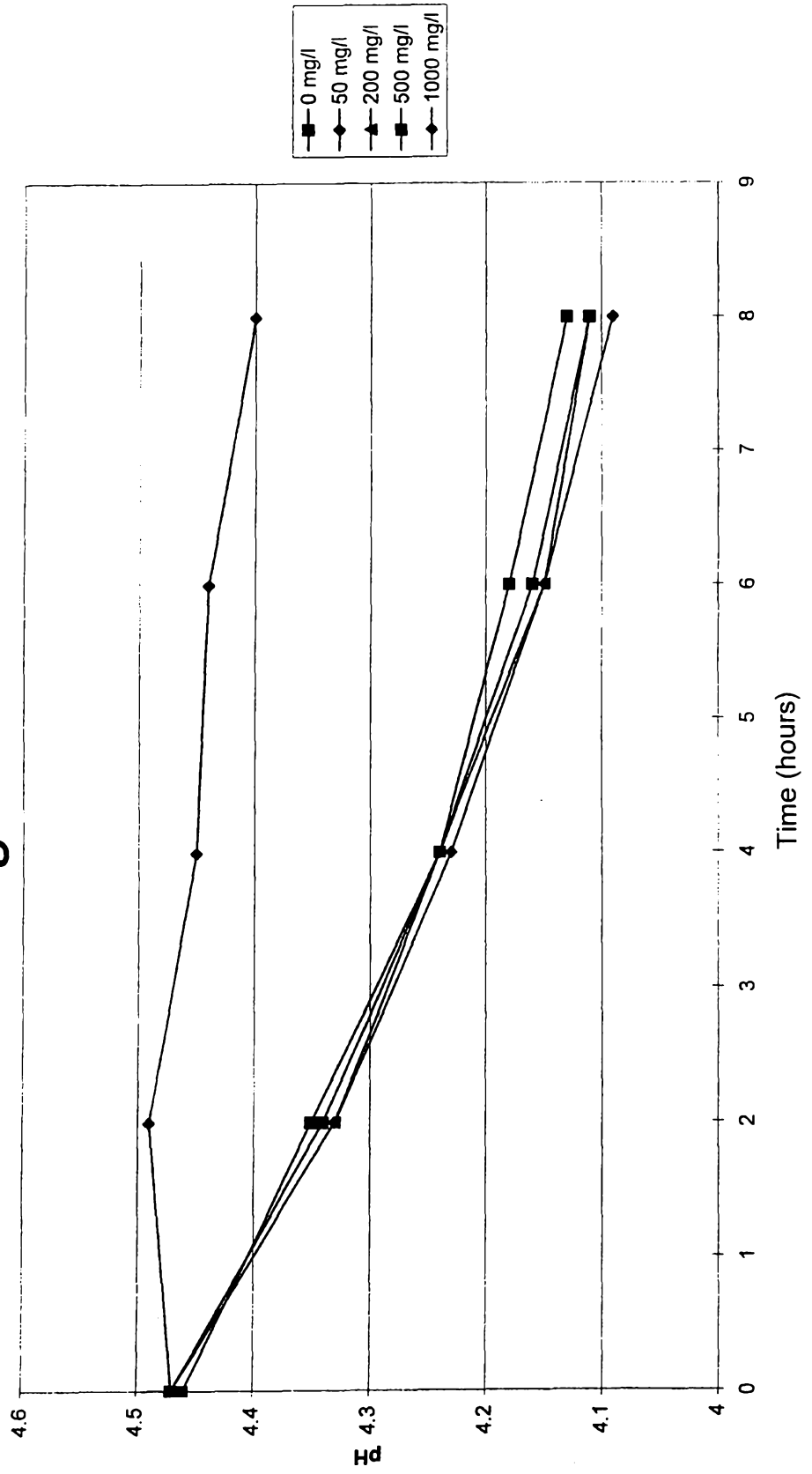
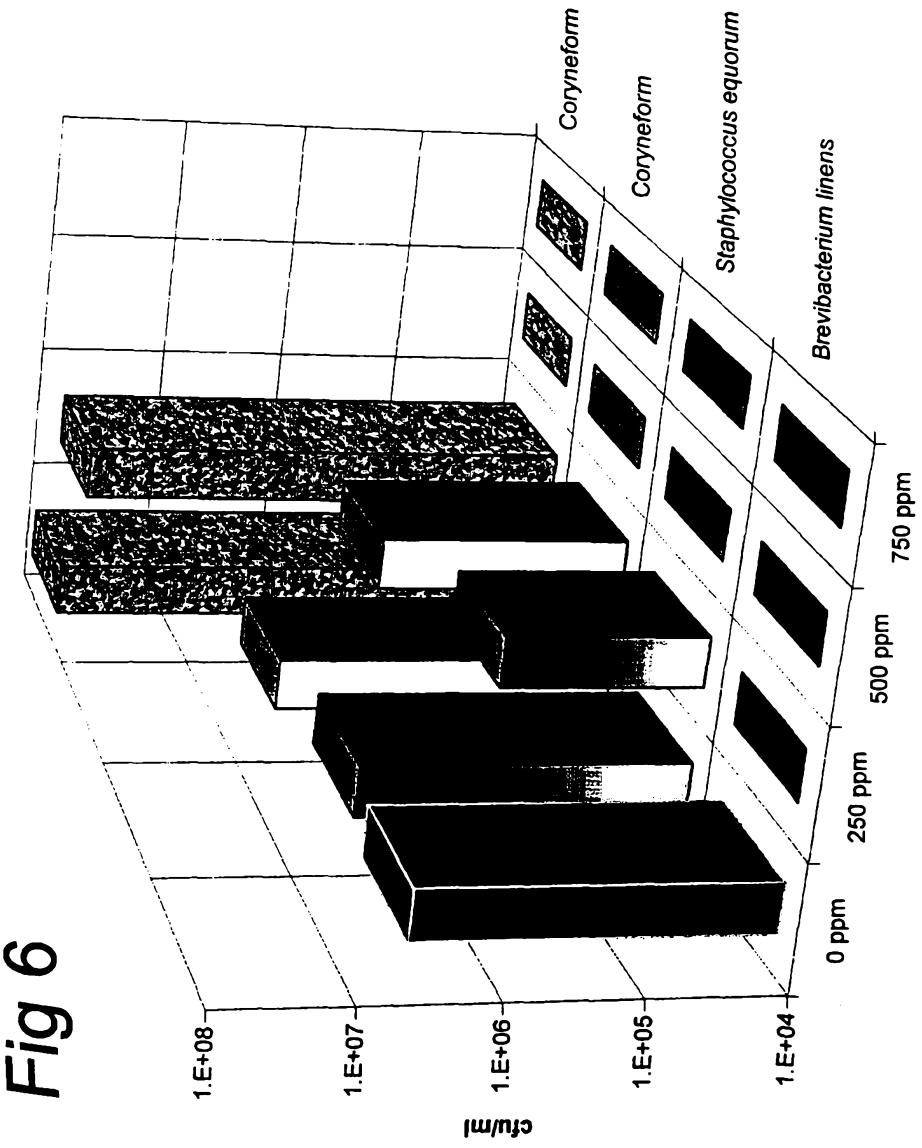

0 mg/l
50 mg/l
200 mg/l
500 mg/l
1000 mg/l
pH
4.6
4.5
4.4
4.3
4.2
4.1
4
0
1
2
3
4
5
6
7
8
9
Time (hours)

Fig 6


Coryneform
Coryneform
Staphylococcus equorum
Brevibacterium linens
0 ppm
250 ppm
500 ppm
750 ppm
1.E+04
1.E+05
1.E+06
1.E+07
1.E+08
cfu/ml

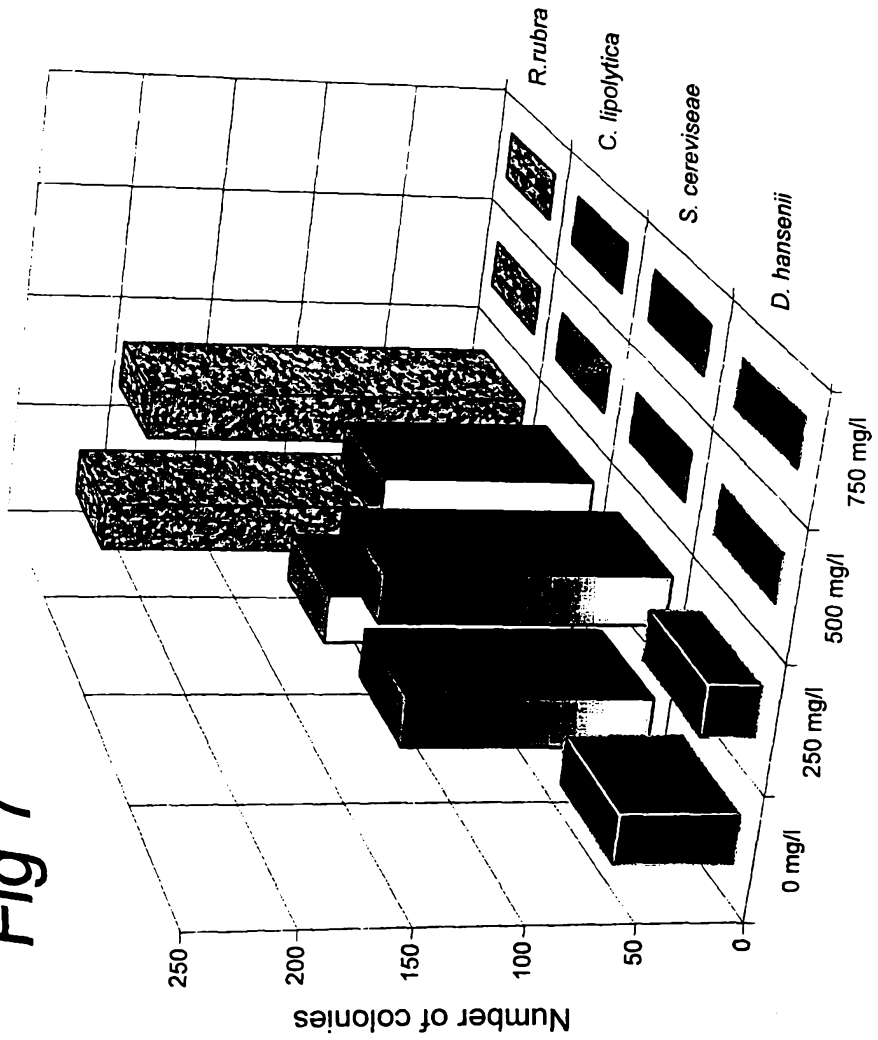

R.rubra
C. lipolytica
S. cereviseae
D. hansenii
0 mg/l
250 mg/l
500 mg/l
750 mg/l
Number of colonies
0
50
100
150
200
250

Fig 7

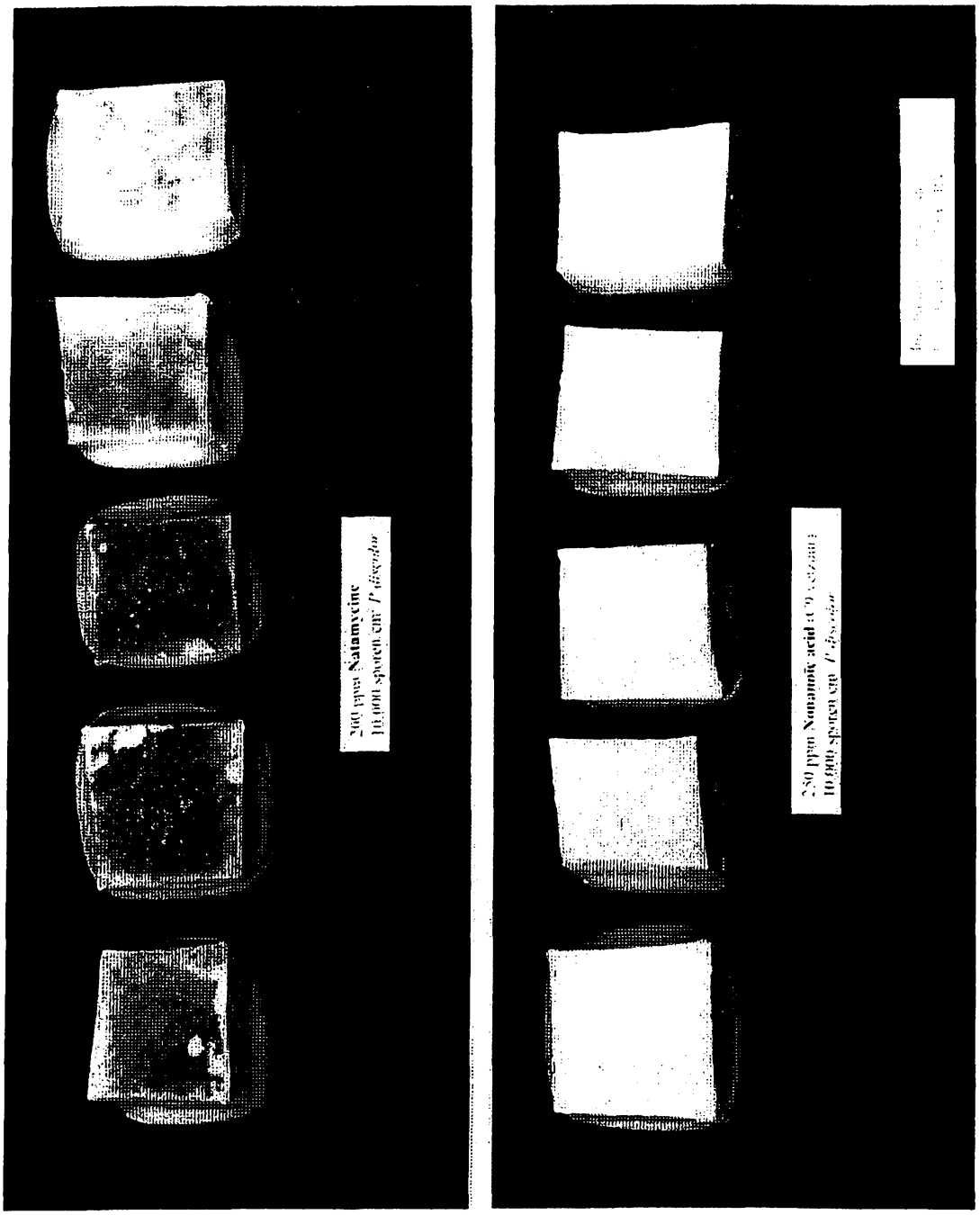

200 ppm Natamycine
10.000 sporen/ml P. digitatum
250 ppm Nonanoic acid
10.000 sporen/ml P. digitatum

Fig 8

Fig 9

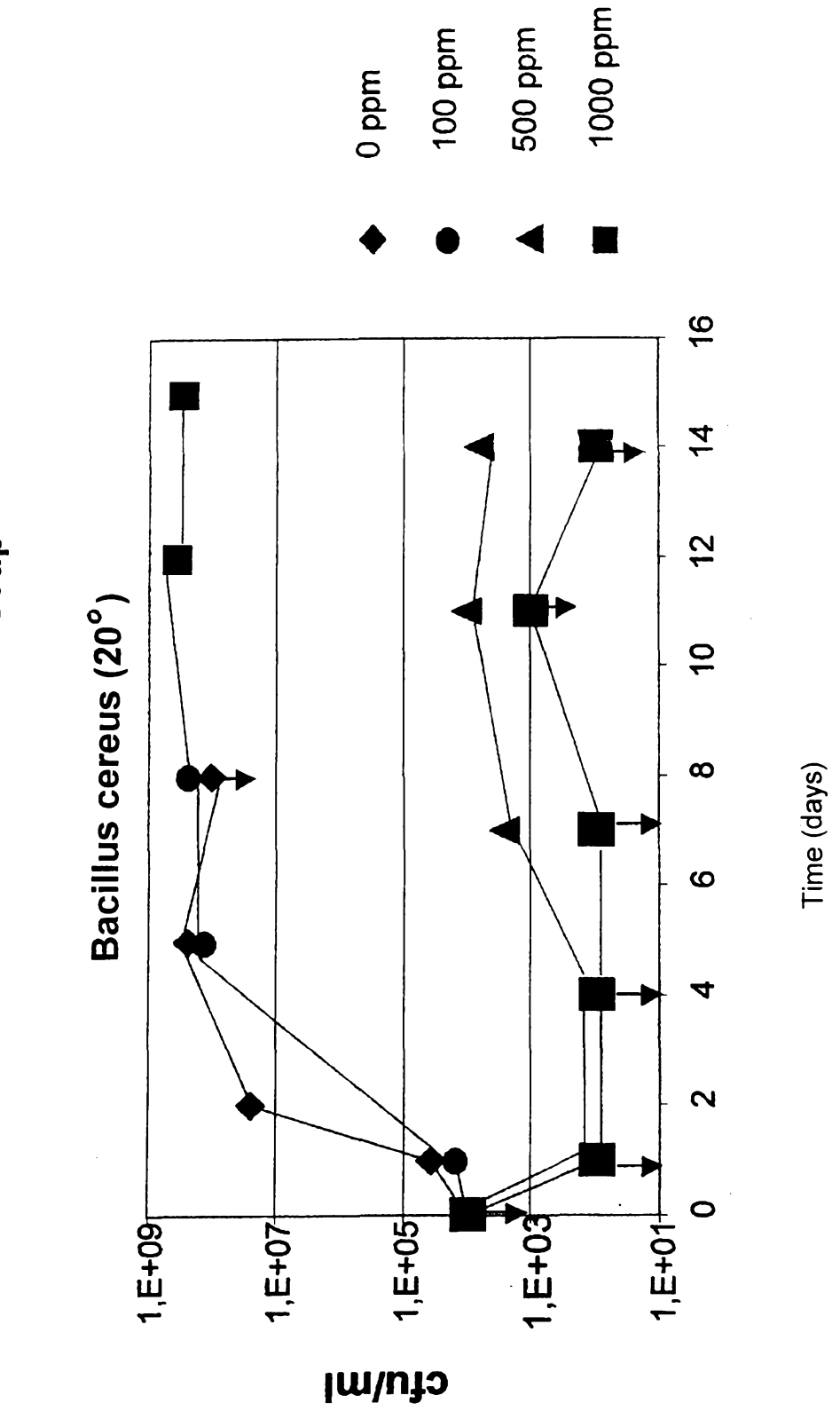

Use of nonanoic acid in soup
Bacillus cereus (20°)
0 ppm
100 ppm
500 ppm
1000 ppm
cfu/ml
1,E+09
1,E+07
1,E+05
1,E+03
1,E+01
0
2
4
6
8
10
12
14
16
Time (days)

Fig 10

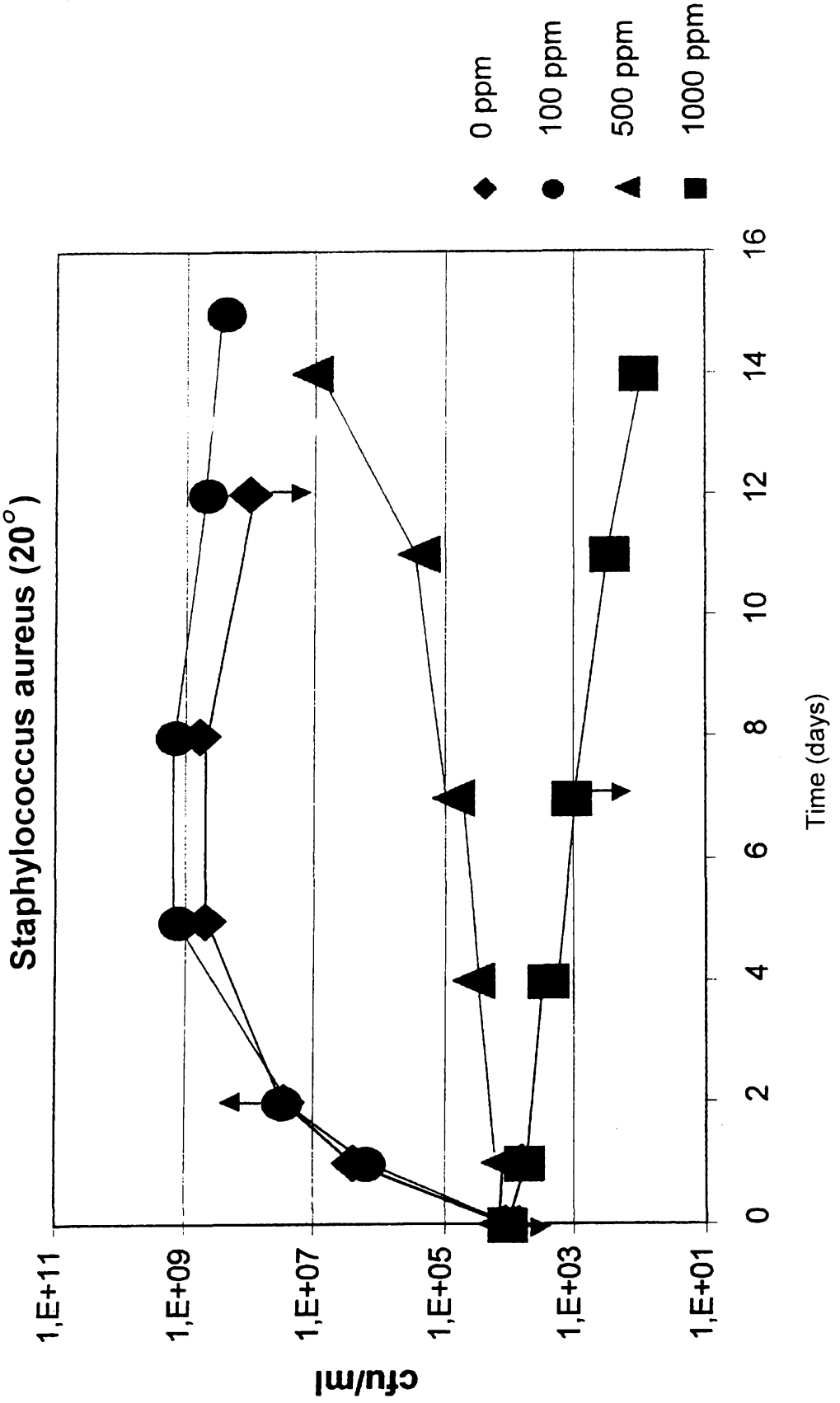

Use of nonanoic acid in soup
Staphylococcus aureus (20°)
0 ppm
100 ppm
500 ppm
1000 ppm
cfu/ml
1,E+11
1,E+09
1,E+07
1,E+05
1,E+03
1,E+01
0
2
4
6
8
10
12
14
16
Time (days)

Fig 11 Use of nonanoic acid in milk/fruit juice product

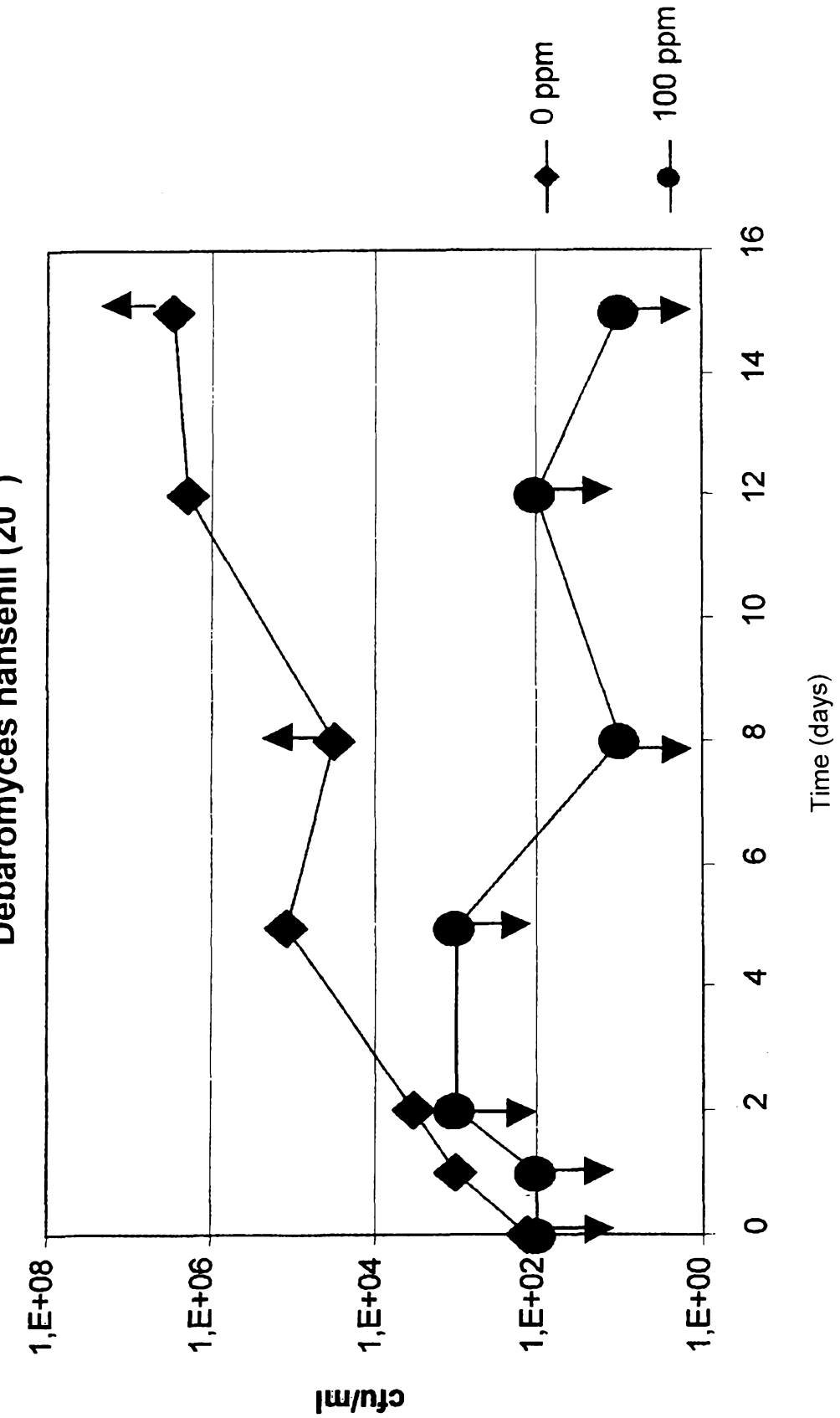
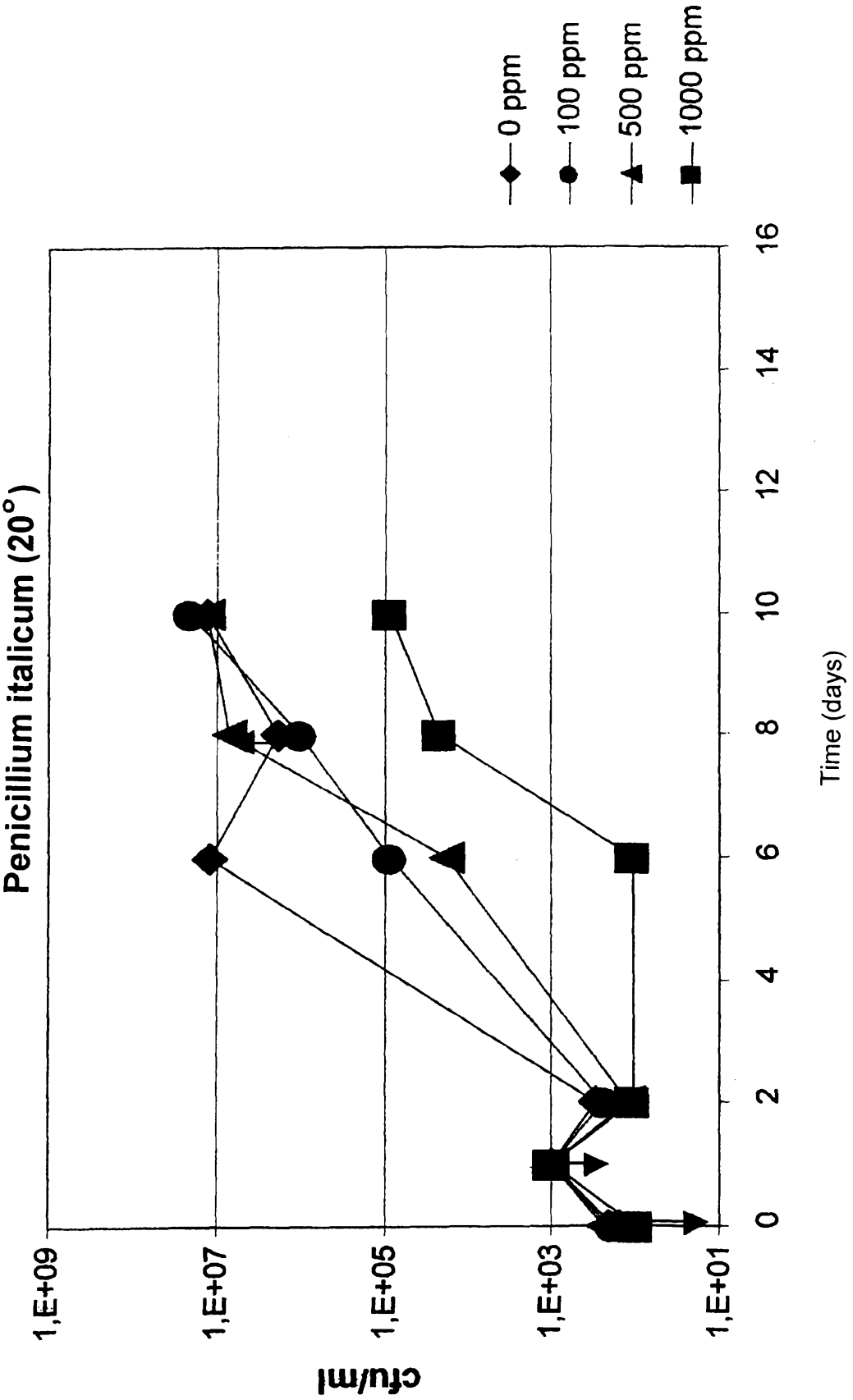


Fig 12


Use of nonanoic acid in milk/fruit juice product
Penicillium italicum (20°)
0 ppm
100 ppm
500 ppm
1000 ppm
cfu/ml
1,E+09
1,E+07
1,E+05
1,E+03
1,E+01
0
2
4
6
8
10
12
14
16
Time (days)