PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A23B 4/20, 4/22, 4/24
A23B 5/14, 5/16, 5/18
A23C 3/08, C12N 9/36

A1

(11) International Publication Number: **WO 90/04331**

(43) International Publication Date: 3 May 1990 (03.05.90)

(21) International Application Number: PCT/US89/04576

(22) International Filing Date: 13 October 1989 (13.10.89)

(30) Priority data:
258,606 17 October 1988 (17.10.88) US

(60) Parent Application or Grant
(63) Related by Continuation
US 258,606 (CIP)
Filed on 17 October 1988 (17.10.88)

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(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.

Published
With international search report.

(54) Title: BACTERIOSTATIC AND BACTERICIDAL COMPOSITION AND METHODS OF USE THEREOF

(57) Abstract

A bactericidal and bacteriostatic composition capable of exerting its bactericidal and bacteriostatic effect on a number of bacteria is presented, the composition comprising lysozyme, a mineral component and an acid/acidimmunomodulating agent and further wherein the pH of the composition is no greater than about 5.0 for a thermolabile composition, and greater than about 8.0 for a thermostable composition. Also presented is a method of use of such composition for the reduction or inhibition of bacteriologically activity in a substrate, a method for the extension of the shelf life of fresh foods and a method for extending the shelf life of milk and milk products and inducing the curding thereof.

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DESCRIPTION

BACTERIOSTATIC AND BACTERICIDAL COMPOSITION AND METHODS OF USE THEREOF

Technical Field

The present invention relates to a composition having bactericidal and bacteriostatic activity. The composition comprises lysozyme, a mineral component and an acid or acid-immunomodulating agent, and is at a pH of no greater than about 5 for food products that do not require excessive heat during their manufacturing process -- or higher than 8 for products exposed to excessive/prolonged heating. This invention also encompasses a method of use of such composition for the reduction or inhibition of bacteriological activity in a substrate of a food and non-food nature, and a method for the extension of the shelf life of products like fresh foods including a method for extending the shelf life of milk and milk products and inducing the curding thereof.

Lysozyme is an antibiotic enzyme found in humans, animals and plants. In humans, it plays an important

role in the immune system, acting against bacterial and viral infections. It is generally found in tears, nasal mucus, milk, saliva, blood serum, in a great number of tissues and secretions of different animals, vertebrates and invertebrates, in egg white, in some molds and in the latex of different plants. It was first discovered in human tears in 1922 by Alexander Fleming and first isolated and characterized in 1967 by Alfred Sapse, B. Bonavida and E.E. Sercarz at the University of California at Los Angeles (Bonavida et al., *Human Tear Lysozyme; I. Purification, Physicochemical and Immunochemical Characterization*, J. Lab. Clin. Med. 70:951, 1967).

Lysozyme in body fluids, when used alone, has been found to have very limited bactericidal effects and as such, needs to be synergistically aided *in vivo* by certain proteins. Included among these is transferrin (a carrier of iron) and ceruloplasmin (a carrier of copper); the immunomodulator proteins immunoglobulin A (IgA) and immunoglobulin G (IgG); and, in tears, by tear prealbumin.

Hen egg white lysozyme is a lysozyme isolated from the whites of hen eggs and is readily available. As is the case with tear lysozyme, hen egg white lysozyme has a very low bactericidal and bacteriostatic activity in and of itself. What is needed, therefore, is a composition which causes the bactericidal and bacteriostatic activity of lysozyme *in vitro* to be increased to approximate that of human lysozyme *in vivo*.

Background Art

The antibacterial activity of lysozyme has been recognized both in terms of lysozyme alone, as in Japanese Patent No. 72-46336, as well as in combination with other ingredients such as glycine (in Japanese Patent No. 73-16613) and hydrogen peroxide with certain acids or acid salts (in German Patent No. 2,126,204). However, the synergistic effect of the combination of lysozyme with an immunomodulator acid and a mineral at variable pH's, be it a very acidic pH or very basic, on the antibacterial activity of lysozyme has never been recognized or disclosed.

Disclosure of Invention

As noted above, the present invention relates to a composition comprising lysozyme, most preferably hen egg white lysozyme, a mineral component and an acid/acid-immunomodulating agent and is at a pH of no greater than about 5.0, for certain foods/products, or higher than about 8.0 for others, the composition having bactericidal and bacteriostatic activity. This invention also encompasses a method of use of such composition for the reduction or inhibition of bacteriological activity in a substrate, food or non-food, a method for the extension of the shelf life of products like fresh foods including a method for inducing curding in milk and milk products. As used herein, the terms "bacteria", "bacterial", etc., shall also be understood to include "fungus", "fungal", etc.

Lysozyme (N-acetylmuramide glycanohydrolase; globulin G_1) is a mucolytic enzyme having antibiotic properties. Its molecular weight is about 14,500 and

its structure consists of a single polypeptide chain of 129 amino acid subunits of 20 different types, crosslinked by four disulfide bridges. Although not intending to be bound by any particular theory, it is thought that lysozyme exhibits its antibacterial activity (i.e., its bactericidal and bacteriostatic activity) by dissolving the bacterial cell wall mucopolysaccharides by hydrolyzing the beta-(1 through 4) linkages between the N-acetyl-D-muramic acid and 2-acetylamino-2-deoxy-D-glucose residues.

An antibacterial composition which comprises lysozyme advantageously comprises about 0.5 parts per million (ppm) to about 500 ppm of the lysozyme. More preferably, such a composition comprises about 5 ppm to about 250 ppm of the lysozyme, most preferably about 20 to about 200 ppm. The preferred lysozyme is hen egg white lysozyme due to its commercial availability, although any isolated lysozyme, such as others of animal origin (i.e., turkey egg-white), vegetable origin (i.e., yam/sweet potato), human (i.e., serum, placenta) or bioengineered, can be utilized.

The composition of this invention preferably also contains a mineral, most preferably zinc or iodine. It has been found that zinc and iodine each contribute synergistically to the activity of lysozyme. This is especially surprising for zinc, since it had been commonly thought that zinc, when added in certain concentrations to oral supplements or alimentation fluids, may contribute directly or indirectly to microbial growth or worsen infections.

Other minerals in addition to iodine and zinc which have found to be effective include, without limitation,

metals such as germanium, beryllium, chromium, tungsten, selenium, iron, copper, manganese, superoxide dismutase, magnesium and strontium. Mixtures of the above minerals may also be utilized. As noted, zinc and iodine are most preferred. Zinc can also be provided as zinc sulfate, zinc oxide, zinc gluconate and other salts and compounds of zinc and iodine can also be provided as sodium iodide, sodium iodohippurate, kelp and other salts and compounds of iodine.

The mineral component is preferably present in the composition of the present invention in an amount of about 0.05 ppm to about 50 ppm (which can also be expressed as milligrams/milliliters, mg/ml), most preferably about 0.1 ppm to about 25 ppm, for effectiveness. When zinc is used as the mineral, it is most preferably present at levels of above 2 to about 25 ppm; when iodine is used, the most preferred levels are about 0.125 to about 5 ppm. It will be recognized, though, that the upper limit on the mineral level is significant only in practical or economic terms and the inclusion of more than the indicated upper limit will not adversely impact on the efficacy of the inventive composition.

Also present in the composition of this invention is an acid or acid-immunomodulator, which, under certain conditions, can provide the synergistic benefits noted above which are provided by IgA and IgG. Preferred among acids is caprylic acid. Many suitable immunomodulators are also acids, the most preferred of which is ascorbic acid. Other suitable acids which may be included as the acid/acid-immunomodulator are acetic acid, pantothenic acid, folic acid, benzoic acid, tartaric acid and para-amino benzoic acid (PABA). Of

particular importance is the immunomodulator acetylsalicylic acid and its derivatives. Ascorbic acid can also be replaced in the inventive composition by amino acids such as glutamic acid, alanine, glycine, methionine, phenylalanine, tryptophan, etc., also organic and inorganic acids. The caprylic acid can also be replaced by other fatty acids such as saturated fatty acids like butyric, lauric, myristic, palmitic, stearic and arachidic and unsaturated fatty acids like oleic, elaidic, cis and trans vaccenic, linoleic, gammalinoleic linolenic and arachidonic. As is the case with the mineral component, the acid/acid-immunomodulator can also comprise a mixture of the above-mentioned acids. In addition, it will be recognized that the acid can be used, among others, in its hydroxy form or as methyl or cholesteryl esters.

The acid/acid-immunomodulator is advantageously present in an amount of about 6 to about 500 ppm, preferably about 50 to about 300 ppm. Most advantageously, the acid/acid-immunomodulator, especially if caprylic acid, as is most preferred, is present in an amount of about 15 ppm to about 150 ppm.

Surprisingly, it has been found that the composition of the present invention exhibits its highest levels of antibacterial activity, depending on the acid employed, at either a highly acidic pH, i.e., at a pH of no greater than about 5.0, preferably a pH of no greater than about 4.0 when the substrate is not exposed to excessive or prolonged heating during manufacture or at any time after application of the inventive composition (thermolabile). By excessive or prolonged heating is meant temperatures substantially above room temperature (i.e., about 25°C) for greater than about one hour.

When the substrate is exposed to such heating during manufacture (thermostable), the optimum pH, it has been found, is higher than about 8.0, preferably up to about pH 12.0, especially when caprylic acid is used. This runs counter to accepted belief that lysozyme is at its peak activity at a pH of 6.6 (slightly acidic) and is not effective under strongly acidic or strongly basic conditions. In order to maintain the pH at the desired levels, the pH of the lysozyme solution can be adjusted by a suitable agents, such as hydrochloric acid (HCl), if the composition is not brought to the preferred pH by, for instance, the acid/acid-immunomodulator.

Also surprising is the unique heat resistance of the compositions of the present invention. Although lysozyme is known to be readily inactivated by heat, it has been found that the activity of the inventive compositions is not diminished, even after 48 hours at 80°C.

Advantageously, to prepare the lysozyme composition of the present invention, the desired mineral component is mixed, and preferably dissolved, into a suitable solvent, most preferably a buffer solution such as a 0.1 molar (M) solution of citric acid, sodium citrate, or both, in water. The desired amounts of lysozyme and acid/acid-immunomodulating agent are then added to the mineral/solvent mixture and the pH adjusted with a suitable agent (if necessary). The inventive lysozyme composition can be prepared in liquid form or in spray, dip or solid form, and additionally, as a mixture in fodder for poultry, cattle, other animals, and fish.

It may be desired that the lysozyme as well as the mineral component be included as a salt. Such may have

advantageous effects on the activity of the inventive composition. Included among useful salts are lysozyme acetate, butyrate, caprylate, laureate, linolinate and the other individual fatty acids mentioned previously, also salts can be produced from mixing the mineral with a fatty acid resulting in, for instance, zinc acetate, butyrate, caprylate, laureate, etc., or, conversely, iodine acetate, butyrate, caprylate laureate, linolinate, etc., and finally mixing the two types of salts at pH 3.5 to 12.0 at 20-50°C, preferably at 25°C.

In order to reduce and/or inhibit the bacterial activity of a substrate (in other words, in order to have a bactericidal and/or bacteriostatic effect on something infected with bacteria), a bactericidal and/or bacteriostatic amount of the composition of this invention is added to the substrate. Substrates capable of being treated with the inventive composition include, but not by way of limitation, meats and meat products, like hen, turkey, ham, beef, especially ground beef, salami, sausages and smoked meat products; mayonnaise; dairy products like cheese, milk, yogurt, etc.; oils; fish and fish products like fish, shrimp, lobster and frogs legs; eggs and egg products; soft drinks including natural juices; animal feeds; and other high protein products. In addition, the compositions of the present invention can also be used to at least partially replace sulfites, in foods such as wines, corn derived products, including but not limited to corn syrup and corn starch, also mustard and fresh and dried fruits and vegetables. Moreover, the composition can be used to, at least partially, replace nitrites and nitrates in products such as hams, salami, sausages, game meats, and others.

In addition to the uses in foods, the inventive compositions have many non-food uses, including in

mouthwashes to destroy mouth infection-inducing bacteria such as Streptococcus salivarius, Bacteroides vulgatus, Candida albicans, etc.; denture cleaners; contact lens solutions; cosmetics such as ointments, creams, shampoos, talcum and other powders, deodorants and antiperspirants, nail polishes, mascara and eyelid products; feminine hygiene products including vaginal douches and use in disinfecting/decontaminating panties; diapers and urinary pants used by incontinent individuals; also as antibacterial/antifungus preparations in coatings for rooms or tanks used for processing foods. Other uses include as antifungus preparations to be sprayed on trees and agricultural products; in veterinary and for human use, as antibacterial/antifungus agents with antibiotic-like activity and bedsore treatment or prevention products.

Generally, a bactericidal and/or bacteriostatic amount of the inventive composition (or the amount which would generally be applied to about 1 kilogram (kg) or liter (l) of substrate) is considered to be about 1 to about 5 ml, more preferably about 1 to about 2 ml, with the most preferred being about 1.5 ml (or about 1% to about 5%, preferably about 1% to about 2% by weight).

Treating the desired substrates with the lysozyme composition of the present invention can be undertaken after bacterial infection, to reduce or maintain the bacterial levels in the sample, or, most advantageously, prior to bacterial infection, to prevent or inhibit bacterial infection from occuring. In so doing, the useful life of those substrates prone to bacterial infection, such as those noted above, is extended by delaying or preventing bacterial infection and, thus, spoilage. As noted above, the lysozyme composition can

also be mixed with animal fodder or feed to prevent bacterial contamination at the source (pasture, water, etc.). The inventive composition can also be used to disinfect and/or decontaminate food processing equipment and also as a preservative for solutions such as pharmaceutical preparations.

Bacteria upon which this composition is known to have a positive (i.e., bactericidal and/or bacteriostatic) effect include those bacteria most often associated with food contamination and/or other forms of spoilage, such as Salmonella typhimurium, Salmonella enteriditis, Escherichia coli (E. coli), Clostridium botulinum, Clostridium perfringens, Campylobacter jejuni, Vibrio parahemolyticus, Psuedomonas fluorescens, Candida geotrichum, Streptococcus salivarius, Bacteroides vulgatus, Pseudomonas aeruginosa, Pseudomonas cepacia, Staphylococcus aureus, Candida albicans, Aspergillus niger, Aspergillus fischeri, Bacillus subtilis, Listeria monocytogenes, yeasts, spores and others.

In another surprising and advantageous embodiment of the present invention, the lysozyme composition can act as both preservative preparation and curding agent on milk and milk products by "overloading", i.e., increasing the concentration of the acid/acid-immunomodulating agent. In this way, the inventive composition can be used to produce curded milk products resembling cottage cheese, white cheese and yogurt, with extended shelf life when compared with milk products such as whole milk, skim milk, etc. they are deriving. These extended shelf life curded milk products also have, in many cases, different and/or improved taste and can also have other advantageous characteristics such as

being vitaminated or becoming therapeutic agents as in the curded products containing aspirin, all depending on the acid used.

In order to produce such improved extended shelf life curded milk products, the lysozyme composition of the present invention is prepared having the formulation described above with respect to the lysozyme and mineral components, and also the pH, but the concentration of the acid/acid-immunomodulating agent is altered. In other words, the lysozyme present in the composition is an amount of about 5 to about 20 mg per liter of milk or milk product, the mineral component is present in an amount of about 0.5 to about 15 mg per liter of milk or milk product and the pH is adjusted to be no greater than about 5 and most preferably about 3.5. The acid/acid-immunomodulating agent, in this case, is increased in this formulation in an amount of about 10 to about 120 mg per milliliter of milk or milk product.

The effects of such "overloaded" acidic component are as follows: when the amount of acid/acidimmuno-modulating agent is about 10 mg per milliliter to about 25 mg per milliliter, the color and physical appearance of milk left at room temperature, even for up to 14 days, remains the same. The taste can change, depending on the particular acid utilized. For instance, when ascorbic acid is used, the taste is more pleasant, slightly acid, with a citro-orange flavor. Untreated milk, when left at room temperature for fourteen days, changes in color, odor and taste (becoming sour), the classic characteristics of spoiled milk. When the amount of acid/acid-immunomodulating agent is about 25 mg per milliliter to about 120 mg per milliliter, curding of the milk or milk product, in the absence of

other milk curding cultures, is observed after about three to seventy-two hours. The taste of the curded product can vary from pleasant and new, when acids such as ascorbic and pantothenic are used, to bitter when acetylsalicylic acid is used, although the bitter taste can be easily overcome by use of sweeteners such as saccharin, aspartame, or sugar, syrup, honey, fruit juices, etc.

By the use of the composition of this invention, a variety of curded milk products such as vitaminated milk products (when acid vitamins such as ascorbic acid, pantothenic acid, folic acid, PABA, benzoic acid, hydrochloride acid, amino acids, etc. are used) and aspirin milk products (when acetylsalicylic acid or other salicylic acid salts are used) would become available for marketing. Such aspirin milk products are extremely advantageous due to their lessening or elimination of aspirin side effects such as stomach burns, gastric and intestinal ulcers and bleeding, especially for individuals forced to take aspirin for extended periods of time such as sufferers of chronic forms of arthritis. Of course also available and advantageous are combination vitaminated/aspirin milk products.

It may be also desired to include other components such as aromas or odorants in the composition of the present invention. Included among aromas which might be used is allyl capronate, allyl cyclo hexil propionate, allyl heptoate, anisyl acetate, benzyl caprilate, benzyl cinnamate, cinnanyl acetate, herbyl propionate, etc., and mixtures thereof. Surprisingly, certain aromas, especially the allyl capronate in concentrations of preferably 5-100 ppm, have an additional synergistic effect on bacterial inhibition by the inventive lysozyme composition.

The following examples further illustrate and explain the present invention by the antibacterial effectiveness of compositions of the present invention.

Example I

A lysozyme composition is prepared by:

preparing a 0.1 Molar (M) citric acid buffer solution by dissolving 10.5 grams of citric acid in 500 milliliters (ml) of water and a 0.1 M sodium citrate buffer solution by dissolving 14.6 grams of sodium citrate in 500 ml of water, and combining the two solutions;

mixing 131.92 milligrams (mg) of zinc sulfate ($ZnSO_4$), which provides the equivalent of 30 mg zinc, into 20 ml of the combined buffer solution;

100 mg of hen egg white lysozyme and 300 mg of ascorbic acid are then added and the pH adjusted to 3.5 with 6 Normal (N) hydrochloric acid, to form the exemplary lysozyme composition, one cubic centimeter (cc) of which contains 5 mg of lysozyme, 15 mg of ascorbic acid, and 1.5 mg of zinc.

A comparative control composition was also prepared in the same manner except that the pH was buffered at 6.6.

Method of Assay

In order to evaluate the antibacterial capacity of the prepared compositions, serial dilutions are prepared in duplicate in 2x strength nutrient broth (commercially available from Difco Co. of Detroit, Michigan), starting from a 1:1 dilution through 1:2048.

Preparation of Organisms

A mid-log phase culture is prepared of Salmonella typhimurium (ATCC 11331). Cells grown on nutrient agar are harvested in sterile 0.9% saline, washed twice and adjusted to achieve an inoculum concentration of about 10^7 cells/ml as confirmed by plate counts. Ten microliters of the resulting organism suspension is used to inoculate 200 microliters of each medium, to achieve a final organism concentration of 10^5 cells per cavity.

The inoculated micro-titer plates are incubated at 35°C for 24 hours. Plates are subsequently stored at room temperature for the balance of the test period. Subsequent visual readings of turbidity are taken at 48 hours, 72 hours and 1, 2, 3 and 4 weeks.

Minimum inhabitory concentration (MIC) reactions are scored on the basis of the development of turbidity.

In order to obtain the Minimum bacterial concentration (MBC) values, each well showing no signs of turbidity are cultured onto 5% blood agar plates to determine the presence of viable microorganisms. Growth is evaluated after 48 hours of incubation at 35°C.

The results are shown in Table 1 (for the exemplary lysozyme composition, pH - 3.5) and Table 1a (for the comparative composition, pH - 6.6).

Table 1

Cumulative Bacterial Response

Well	Dilution	24 hr	48 hr	72 hr	1 wk	2 wks	3 wks
1	1:1	(-)x	(-)x	(-)x	(-)x	(-)x	(1)x
2	1:2	(-)x	(-)x	(1)x	(-)x	(1)x	(2)x
3	1:4	(-)x	(1)x	(1)x	(1)x	(1)x	(3)x
4	1:8	(-)x	(2)x	(2)x	(1)x	(2)x	(3)x
5	1:16	(-)x	(2)	(2)	(1)x	(2)	(4)
6	1:32	(1)	(4)	(4)	(1)x	(4)	(4)

Key for Tables 1 and 1a:

x - Antibacterial Activity
(-) - No Growth
(1) - Slight Turbidity
(2) - Slight to Moderate
(3) - Moderate
(4) - Heavy Turbidity
* - Viable organisms present

7	1:64	(3)	(4)	(4)	(4)	(4)	(4)
8	1:128	(4)	(4)	(4)	(4)	(4)	(4)
9	1:256	(4)	(4)	(4)	(4)	(4)	(4)
10	1:512	(4)	(4)	(4)	(4)	(4)	(4)

Table 1 (Continued)

Cumulative Bacterial Response

Well	Dilution	24 hr	48 hr	72 hr	1 wk	2 wks	3 wks
11	1:1024	(4)	(4)	(4)	(4)	(4)	(4)
12	1:2048	(4)	(4)	(4)	(4)	(4)	(4)

The (1) and (2) turbidity markings in Wells 1, 2, 3 and 4 are due to protein precipitation/bacterial killing and are not due to bacterial growth. As noted, all tests marked with an "x" indicate bacterial killing/ bactericidal effect.

Table 1a

Cumulative Bacterial Response

Well	Dilution	24 hr	48 hr	72 hr	1 wk	2 wks
1	1:1	(-)*	(-)*	(-)*	(-)x	(-)x	(-)x
2	1:2	(-)*	(1)*	(1)*	(1)	(1)x	(1)x
3	1:4	(-)*	(2)*	(4)*	(2)	(2)	(2)
4	1:8	(3)	(3)	(4)	(4)	(4)	(4)
5	1:16	(3)	(4)	(4)	(4)	(4)	(4)
6	1:32	(4)	(4)	(4)	(4)	(4)	(4)
7	1:64	(4)	(4)	(4)	(4)	(4)	(4)

Table 1a (Continued)

Cumulative Bacterial Response

Well	Dilution	24 hr	48 hr	72 hr	1 wk	2 wks
8	1:128	(4)	(4)	(4)	(4)	(4)	(4)
9	1:256	(4)	(4)	(4)	(4)	(4)	(4)
10	1:512	(4)	(4)	(4)	(4)	(4)	(4)
11	1:1024	(4)	(4)	(4)	(4)	(4)	(4)
12	1:2048	(4)	(4)	(4)	(4)	(4)	(4)

The results of Example I clearly show the strong antibacterial effect of the exemplary lysozyme composition. The difference between the exemplary lysozyme composition (Table 1) and the comparative control composition (Table 1a) in terms of bacteria killing is striking, showing the exemplary lysozyme composition to be very effective whereas the comparative composition is not, and yet the compositions are identical except that the exemplary lysozyme composition is at pH 3.5 and the comparative composition is at pH 6.6.

Example II

A lysozyme composition is prepared according to the method described in Example I, except that the concentration of lysozyme is varied as indicated.

Method of Assay

In order to evaluate the antibacterial capacity of the prepared compositions, serial dilutions are prepared in duplicate in 2x strength Trypticase soy broth (commercially available from Difco Co. of Detroit, Michigan), starting from a 1:1 dilution through 1:256.

Preparation of Organisms

A mid-log phase culture is prepared of Listeria monocytogenes (ATCC 7644). Cells grown on Trypticase soy agar with 5% defibrinated sheep blood are harvested in sterile 0.9% saline, washed twice and adjusted to achieve an inoculum concentration of about 10^7 cells/ml as confirmed by plate counts. Ten microliters of the resulting organism suspension is used to inoculate 200 microliters of each medium, to achieve a final organism concentration of 10^5 cells per cavity.

The inoculated micro-titer plates are incubated at 35°C for 24 hours. Plates are subsequently stored at room temperature for the balance of the test period. Subsequent visual readings of turbidity are taken at 24 hours, 48 hours, 72 hours and 1 week.

Minimum inhabitory concentration (MIC) reactions are scored on the basis of the development of turbidity.

In order to obtain the Minimum bacterial concentration (MBC) values, each well showing no signs of turbidity are cultured onto 5% blood agar plates to determine the presence of viable microorganisms. Growth is evaluated after 48 hours of incubation at 35°C.

The results are shown in Table 2.

Table 2

Cumulative Bacterial Response

Well	Dilution	% Conc.	24 hr	48 hr	72 hr	1 week
1	1:1	0.50	(-)x	(-)x	(1)x	(1)x
2	1:2	0.25	(-)x	(-)x	(1)x	(1)x
3	1:4	12.50	(-)*	(-)*	(1)*	(2)*
4	1:8	6.25	(1)*	(2)*	(2)*	(2)*
5	1:16	3.125	(2)*	(2)*	(2)*	(3)*

Key for Table 2:

x - Antibacterial Activity
(-) - No Growth
(1) - Slight Turbidity
(2) - Slight to Moderate
(3) - Moderate
(4) - Heavy Turbidity
* - Viable organisms present

6	1:32	1.56	(4)	(4)	(4)	(4)
7	1:64	0.78	(4)	(4)	(4)	(4)
8	1:128	0.39	(4)	(4)	(4)	(4)
9	1:256	0.20	(4)	(4)	(4)	(4)

Example IIIa

Screening tests are conducted to determine the effectiveness of a lysozyme composition prepared as described in Example I against bacteria and fungi using the method of United States Pharmacopeia (USP), Chapter XIX, p. 587. The microorganisms used are Aspergillus fischeri and Bacillus subtilis. The inoculi are harvested from 24 hour slants for the bacteria and 7 days for the fungi. Initial plate counts are run to determine the level of organisms at the beginning of the test:

Aspergillus fischeri, FDA M1148	15,000/ml
Bacillus subtilis, ATCC 9382	100,000/ml

The concentration of lysozyme and pH, and the results, are set out in Table 3a.

Table 3a

Aspergillus fischeri

Conc.	pH	1 wk	2 wks	3 wks	4 wks
0.2	6.8	<10	<1	<1	<1
0.5	3.5	<1	<1	<1	<1

Bacillus subtilis

0.2	6.8	<10	<1	<1	<1
0.5	3.5	<1	<1	<1	<1

Example IIIb

Screening tests are conducted to determine the effectiveness of a lysozyme composition prepared as described in Example I against bacteria and fungi using the method of United States Pharmacopeia (USP), Chpater XIX, p. 587. The inoculi are harvested from 24 hour slants for the bacteria and 7 days for the fungi. Initial plate counts are run to determine the level of organisms at the beginning of the test:

Candida albicans, ATCC 10231	120,000/ml
Aspergillus niger, ATCC 16404	100,000/ml
Escherichia coli, ATCC 8739	400,000/ml
Pseudomonas aeruginosa, ATCC 9027	170,000/ml
Staphylococcus aureus, ATCC 6538	170,000/ml

The concentration of lysozyme and pH, and the results, are set out in Table 3b.

Table 3b

Conc. %	pH	Organism	1 wk	2 wks	3 wks	4 wks
0.2	3.5	C. albicans	90,000	45,000	30,000	15,000
0.2	3.5	A. niger	20,000	65,000	60,000	50,000
0.2	3.5	E. coli	55,000	9,000	2,900	300
0.2	3.5	P. aerugin.	75,000	22,000	11,000	21,000
0.2	3.5	S. aureus	<1	<1	<1	<1

Table 3b (Continued)

Conc. %	pH	Organism	1 wk	2 wks	3 wks	4 wks
0.5	3.5	C. albicans	<1	<1	<1	<1
0.5	3.5	A. niger	<1	<1	<1	<1
0.5	3.5	E. coli	<1	<1	<1	<1
0.5	3.5	P. aerugin.	20	<1	<1	<1
0.5	3.5	S. aureus	<1	<1	<1	<1

Example IV

In order to evaluate the antibacterial activity of the inventive composition on fresh food, the following test samples are prepared:

Sample 1 - 50.0 grams of raw fresh ground beef ("hamburger") is weighed into a sterile container.

Sample 2 - 49.0 grams of hamburger is weighed into a sterile container and 1.0 ml of bacteria broth (comprising a 1:1 mixture of Staphylococcus aureus (ATCC 25923) broth and Salmonella enteritidis (ATCC 13076) broth, total bacterial count of at least 200,000 per ml) is added and mixed therein.

Sample 3 - 49.0 grams of hamburger is weighed into a sterile container and 0.5 ml of an exemplary lysozyme composition prepared in accordance with Example I, and 1.0 ml of bacterial broth are added and mixed therein.

Sample 4 - 49.0 grams of hamburger is weighed into a sterile container and 1.5 ml of exemplary lysozyme composition, and 1.0 ml of bacterial broth are added and mixed therein.

Sample 5 - 49.0 grams of hamburger is weighed into a sterile container and 0.5 ml of exemplary lysozyme composition added and mixed therein.

Each of the samples is stored for fourteen days at 42°F (5.6°C). An initial sample extract of 1.0 grams is taken as well as sample extracts at 24 hours, 7 days and 14 days. Each of the withdrawn 1.0 grams extracts is extracted with 100 ml of water and 0.25 to 1.0 ml plated on agar (Mannitol Salt Agar, Bismuth Sulfite Agar, MacConkey Agar and/or Brilliant Green Agar). The plates are incubated for 18 to 36 hours at 36°C and the counts recorded.

The results are interpreted by plotting all counts per cc for the five sample versus the storge times and determining the quantitative effect of the exemplary lysozyme composition. The results are set out in Tables 4a and 4b.

Table 4a

Salmonella Counts

			Days		
Sample	Components	Initial	1	7	14
1	Hamburger	4400	TNTC	TNTC	TNTC
2	Hamburger Broth	6800	TNTC	TNTC	TNTC

Table 4a (Continued)

Salmonella Counts

Sample	Components	Initial	Days 1	7	14
3	Hamburger Broth 0.5 ml Lysozyme Comp.	1400	1300	600	600
4	Hamburger Broth 1.5 ml Lysozyme Comp.	400	200	50	N
5	Hamburger 0.5 ml Lysozyme Comp.	3000	TNTC	800	800

Key for Tables 4a and 4b:
N - Not Detected
TNTC - Too Numerous to Count

Table 4b

Staphylococcus Counts

Sample	Components	Initial	Days 1	7	14
1	Hamburger	N	N	N	N
2	Hamburger Broth	6000	TNTC	TNTC	TNTC

Table 4b (Continued)

Staphylococcus Counts

Sample	Components	Initial	Days 1	7	14
3	Hamburger Broth 0.5 ml Lysozyme Comp.	3200	N	N	N
4	Hamburger Broth 1.5 ml Lysozyme Comp.	N	N	N	N
5	Hamburger 0.5 ml Lysozyme Comp.	N	N	N	N

The results of Example IV clearly show the bacteriostatic and bactericidal activity of the composition of the present invention in both concentrations tested.

Example V

The procedure of Example V is repeated except that instead of hamburger, mayonnaise is used as the bacterial substrate. The results are set out in Tables 5a and 5b.

Table 5a

Salmonella Counts

Sample	Components	Days Initial	1	7	14
1	Mayonnaise	N	N	N	N
2	Mayonnaise Broth	3500	TNTC	TNTC	TNTC
3	Mayonnaise Broth 0.5 ml Lysozyme Comp.	N	N	N	N
4	Mayonnaise Broth 1.5 ml Lysozyme Comp.	N	N	N	N
5	Mayonnaise 0.5 ml Lysozyme Comp.	N	N	N	N

Key for Tables 5a and 5b:
N - Not Detected
TNTC - Too Numerous to Count

Table 5b

Staphylococcus Counts

Sample	Components	Days Initial	1	7	14
1	Mayonnaise	N	N	N	N
2	Mayonnaise Broth	4400	TNTC	TNTC	TNTC
3	Mayonnaise Broth 0.5 ml Lysozyme Comp.	N	N	N	N
4	Mayonnaise Broth 1.5 ml Lysozyme Comp.	N	N	N	N
5	Mayonnaise 0.5 ml Lysozyme Comp.	N	N	N	N

Example VI

A lysozyme composition is prepared by:

preparing an iodine stock solution (450 ug/ml) by dissolving 45 mg of iodine in 75 ml of distilled water in a 100 ml flask, with sonication. Once dissolved, water is added to provide 100 ml of solution;

6.5 grams of sodium caprylate and 2.5 grams of lysozyme chloride are each transfered to an

Example VI (Continued)

80 ml beaker and mixed;

dissolving the mixed sodium caprylate and lysozyme chloride in approximately 35-40 mls of iodine solution (450 ug/ml) and water is added to provide 100 ml of solution.

Preparation of Organisms

A sample of dry yeast is transferred to Sabouraud dextrose broth (Difco) and incubated for 24-48 hours at 26°C. An aliquot of the active yeast culture is then subcultured onto Sabouraud dextrose agar, reincubated for an additional 24 hours and checked for purity. Several colonies are picked, suspended in 100 ml of sterile 0.9% saline and adjusted to achieve a suspension corresponding to 85% light transmission at 530 nm. A sample of this calibrated suspension is diluted 1:1000 in sterile saline. This represents a concentration of 10^4 cells/ml.

Two milliliters of the resulting suspension is used to inoculate 300 ml quantities of test material (lysozyme composition plus grape juice) and control (grape juice alone.)

Preparation of Test Samples

One milliter of the Lysozyme Composition is introduced into 300 ml of grape juice (Welch's 100% all natural, Lot 41800 21700). The preserved juice and one unpreserved control are then immediately inoculated with the standardized organism suspension.

Counts are performed by the pour plate method using Sabouraud dextrose agar at T=0, T=24, T=48, T=72 and T=144 hours. Results are expressed in terms of colony forming units, (CFU)/ml.

Assay Validation

All plates showing no growth after 48 hours of incubation are reinoculated with ten microliters of $Nx10^3$ cells/ml and reevaluated for growth promotion.

All reinoculated plates demonstrated adequate growth. The results are set out in Table 6.

Table 6

Sample	Components	Initial	Hours 24	48	72	144
1	Grape Juice	600 CFU/ml	$9.3x10^4$	$9.6x10^6$	$2.6x10^7$	$2.8x10^8$
2	Grape Juice Lysozyme Comp.	N	N	N	N	N

Key for Table 6:

N - Not Detected

TNTC - Too Numerous to Count

EXAMPLE VII

In order to evaluate the anti-bacterial ability of the inventive composition on fresh food, the following test samples are prepared:

Sample 1 - 100.0 grams of finely ground scrod fish ("scrod") is weighed into a sterile container.

EXAMPLE VII (Continued)

Sample 2 - 100.0 grams of scrod is weighed into a sterile container and thoroughly mixed with 2.0 ml of the lysozyme composition (0.4 ml/1.60 ml saline).

Sample 3 - 25.0 grams of Sample 2 is weighed into a sterile container, to which is added a Closteridia perfringens (ATCC 13124) broth (0.05 ml) and the sample mixed thoroughly.

Sample 4 - 25.0 grams of Sample 1 is weighed into a sterile container to which is added Closteridia broth (0.05 ml) as before.

The samples are stored at 42°F (5.6°C). An initial sample extract of 1.0 grams is taken as well as sample extracts at 2, 4 and 9 days. From 0.05 to 0.20 ml of each of these extracts is plated on anaerobic blood agar and incubated for 24 to 48 hours at 36°C. The counts are then recorded. The results are shown in Table 7.

Table 7

Sample	Components	Days Initial	2	4	9
1	Scrod	N	N	N	N
2	Scrod Lysozyme Comp.	N	N	N	N
3	Scrod Lysozyme Comp. 0.05 ml Clostridia Broth	N	N*	N	N
4	Scrod 0.05 ml Clostridia Broth	23000	29000	31000	16000**

Table 7 (Continued)

Sample	Components	Initial	Days 2	4	9

Key for Table 7:

N - Not detected
Counts are in CFU/s
* = One colony isolated and Clostridia not confirmed
** = Decrease possible due to decrease in anerobic conditions

EXAMPLE VIII

Two lysozyme compositions are prepared in accordance with the method of Example VI except that Lysozyme Composition I differs from Lysozyme Composition II in that iodine is replaced by zinc. The anti bacterial activity of these compositions is evaluated on fresh foods by preparing the following test samples:

Sample 1 - 100.0 grams of finely ground chicken ("chicken") is weighed into a sterile container

Sample 2 - 100.0 grams of chicken is weighed into a sterile container and thoroughly mixed with 1.0 ml of Lysozyme Composition I broth (0.20 ml/0.80 ml saline).

Sample 3 - 100.0 grams of chicken is weighed into a sterile container and thoroughly mixed with 1.0 ml of Lysozyme Composition II broth (0.20 ml/0.80 ml saline).

Sample 4 - 25.0 grams of Sample 2 is weighed into a sterile container to which is added Salmonella typhimirium (ATCC 14028) broth (0.05 ml) and mixed thoroughly.

EXAMPLE VIII (Continued)

Sample 5 - 25.0 grams of Sample 3 is weighed into a sterile container to which is added Salmonella broth (0.05 ml) as before and mixed thoroughly.

Sample 6 - 25.0 grams of Sample 1 is weighed into a sterile container and thoroughly mixed with Salmonella broth (0.05 ml) as before.

The samples are stored at 42°F (5.6°C). An initial sample extract of 1.0 grams is taken as well as sample extracts at 2, 4, 7 and 14 days. From 0.05 to 0.2 ml of each of these samples is plated on bismuth sulfite agar and incubated for 18 to 36 hours at 35°C and the count is recorded. The results are set out in Table 8.

Table 8

Sample	Components	Day Initial	2	4	7	14
1	Chicken	N	N	N	N	N
2	Chicken Comp. I	N	2300*	N	N	N
3	Chicken Comp. II	N	N	N	NA	NA
4.	Chicken Comp. I 0.05 ml Salmonella Broth	2300	N	N	N	N
5.	Chicken Comp. II 0.05 ml Salmonella Broth	5400	1500	1500	NA	NA
6.	Chicken 0.5 ml Salmonella Broth	7700	3100	4600	14000	23000

Table 8 (Continued)

Sample	Components	Initial	Day 2	4	7	14

Key to Table 8:

N - Not detected
NA - Not analyzed
Counts are in CFU/g

Example IX

The lysozyme compositions of Example VIII are again evaluated on fresh food by preparing the following test samples:

Sample 1 - 100.0 grams of finely ground cabbage ("cabbage") is weighed into a sterile container.

Sample 2 - 100.0 grams of cabbage is weighed into a sterile container and thoroughly mixed with 1.0 mls of Lysozyme Composition I broth (0.20 mls/0.80 mls saline).

Sample 3 - 100.0 grams of cabbage is weighed into a sterile container and thoroughly mixed with 1.0 mls of Lysozyme Composition II broth (0.20 mls/0.80 mls saline).

Sample 4 - 25.0 grams of Sample 2 is weighed into a sterile container to which is added Listeria monocytogenes (ATCC 7644) broth (0.05 ml) and mixed thoroughly.

Sample 5 - 25.0 grams of Sample 3 is weighed into a sterile container to which is added Listeria broth (0.05 ml) as above and mixed thoroughly.

Example IX (Continued)

Sample 6 - 25.0 grams of Sample 1 is weighed into a sterile container to which is added Listeria broth (0.05 ml) as above and mixed thoroughly.

The samples are stored at 42°F (5.6°C). An initial sample extract of 1.0 grams is taken as well as sample extracts at 2, 4, 7 and 14 days. From 0.05 to 0.20 mls of each of these extracts is plated on tryptic soy agar and incubated for 18 to 36 hours at 35°C and the counts recorded. The results are set out in Table 9.

Table 9

Sample	Components	Initial	Days 2	4	7	14
1.	Cabbage	1100	560	190	560	740
2.	Cabbage Comp. I	N	560	190	190	190
3.	Cabbage Comp. II	N	N	N	NA	NA
4.	Cabbage Comp. I 0.05ml Listeria Broth	3900	N	N	N	N
5.	Cabbage Comp. II 0.05ml Listeria Broth	1900	770	1400	NA	NA
6.	25 g. Sample I 0.05ml Listeria Broth	92000	27000	31000	39000	54000

Key to Table 9:

N - Not detected
NA - Not analyzed
Counts are in CFU/g

Example X

Three denture cleaners, Kleenite (Richardson-Vicks, Lot No. 7660-0849); Polydent, Super Strength (Block Drug, Lot No. 10158-05306); and Efferdent, Professional Strength (Warner-Lambert, Lot No. D-02029U) are reconstituted with 100 mls of sterile distilled water. Aliquots of 100 microliters of each sample are then transferred into a ten-millimeter (mm) well punched into Sabouraud dextrose agar seeded with Candida albicans (ATCC 10231). In addition, 100 microliter aliquots of each of the above samples supplemented with 0.1 ml of the Lysozyme composition of Example VI are also transferred into a ten millimiter well. One well was spotted in its center with 0.1 ml of the Lysozyme composition of Example VI alone.

After incubation, the wells containing samples having the inventive lysozyme composition showed a dramatic degree of anti-Candida albicans activity without any evidence of diminished activity in the presence of the commercial denture cleaners. In those wells which did not contain the inventive lysozyme composition, no anti-Candida albicans activity was found.

Example XI

Three mouthwashes, Listerine Antiseptic (Warner-Lambert, Lot No. 0273L); Scope (Procter & Gamble Co., Lot No. 18DEC89); and Plax (Oral Research Labs, Lot No. 8641400040) are reconstituted with 100 mls of sterile distilled water. Aliquots of 100 microliters of each sample are then transferred into a ten millimeter (mm) well punched into Sabouraud dextrose agar seeded with

Example XI (Continued)

Candida albicans (ATCC 10231). In addition, 100 microliter aliquots of each of the above samples supplemented with 0.1 ml of the Lysozyme composition of Example VI are also transferred into a ten millimiter well. One well is spotted with 0.1 mls of the Lysozyme composition of Example VI alone.

After incubation, the wells containing samples having the inventive lysozyme composition showed a dramatic degree of anti-Candida albicans activity without any evidence of diminished activity in the presence of the commercial mouthwashes. In those wells which did not contain the inventive lysozyme composition, no anti-Candida albicans activity was found.

Example XIA

The thermal resistance of the inventive composition is tested in the following manner:

a) The lysozyme composition of Example VI is exposed to heat at 80°C for periods of 15, 30, 60, 90 and 120 minutes. After these time intervals, test samples are removed and assayed against N x 10^4 - 10^5 cells/ml of Salmonella typhimurium (ATCC 11331) on blood agar plates (pH=7.2) and nutrient agar plates (pH=6.8). The samples 6re also assayed against baker's yeast on Sabouraud dextrose agar (pH=5.6). In each case, a 12 mm well is punched into the agar media and filled with 0.2 ml of test solution. Heat treatment at 80°C for intervals ranging from 15 to 120 minutes had no effect on the activity of the inventive lysozyme

Example XIA (Continued)

composition against either Salmonella typhimurium or baker's yeast.

b) Lysozyme hydrochloride, when heat treated in 0.05 molar KH_2PO_4 buffer showed reduced activity at 15 minutes and no activity at 30 minutes.

Heat treatments indicate that lysozyme alone does not have a high degree of thermal resistance but that in the inventive composition, thermal resistance is provided.

Example XII

A lysozyme composition is prepared according to the method described in Example VI, except that an aroma (allyl capronate, commercially available as pineapple aroma) is added.

The volume of aroma added varied from 10 to 100 microliters per ml of solution as described in Example X, where zones of inhibition were formed between the composition, in the center well, and candida albicans, in the surrounding agar medium.

The inhibition of growth was significantly greater than that observed in Example X.

The above description is for the purpose of teaching the person of ordinary skill in the art how to practice the present invention, and it is not intended to detail all of those obvious modifications and variations of it which will become apparent to the skilled worker upon

reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention which is defined by the following claims.

Claims

1. A bactericidal and bacteriostatic composition capable of exerting its bactericidal and bacteriostatic effect on a number of bacteria comprising lysozyme, a mineral component, an acid or acid-immunomodulating agent and, further, wherein the pH of the composition is no greater than about 5.0 for the thermolabile formulation and greater than about 8.0 for the thermostable formulation.

2. The composition of claim 1 wherein said lysozyme comprises hen egg white lysozyme.

3. The composition of claim 2 wherein said hen egg white lysozyme is present in an amount of about 0.5 ppm to about 500 ppm.

4. The composition of claim 1 wherein said mineral component is selected from the group consisting of iodine, zinc, germanium, beryllium, chromium, tungsten, selenium, iron, copper, manganese, superoxide dismutase, magnesium and strontium, and mixtures thereof.

5. The composition of claim 4 wherein said mineral component comprises iodine or zinc.

6. The composition of claim 5 wherein zinc is present in an amount of between about 2 ppm and about 25 ppm.

7. The composition of claim 5 wherein iodine is present in an amount of between about 0.125 ppm and about 5 ppm.

8. The composition of claim 1 wherein said acid/acid-immunomodulating agent comprises an acid selected from

the group consisting of caprylic acid, ascorbic acid, pantothenic acid, folic acid, benzoic acid, tartaric acid, para-amino benzoic acid, linoleic acid, gammalinoleic acid, acetylsalicylic acid, caprylic acid, amino acids, and saturated and unsaturated fatty acids, and mixtures thereof.

9. The composition of claim 8 wherein said acid/acid-immunomodulating agent comprises caprylic acid.

10. The composition of claim 9 wherein caprylic acid is present in an amount of about 6 ppm to about 500 ppm.

11. The composition of claim 1 wherein the pH is no greater than about 4.0 for the thermolabile composition, and greater than about 8.0 for the thermostable composition.

12. The composition of claim 11 which further comprises an acidifying agent.

13. A method for reducing or inhibiting the bacteriological activity in a substrate comprising the addition of a bacteriostatic or bactericidal amount of a composition capable of exerting its bactericidal and bacteriostatic effect on a number of bacteria comprising lysozyme, a mineral component, an acid/acidimmuno-modulating agent and, further, wherein the pH of the composition is no greater than about 5.0 for the thermolabile composition and greater than about 8.0 for the thermostable composition.

14. The method of claim 13 wherein said lysozyme comprises hen egg white lysozyme.

15. The method of claim 14 wherein said hen egg white lysozyme is present in an amount of about 0.5 ppm to about 500 ppm.

16. The method of claim 15 wherein said mineral component is selected from the group consisting of iodine, zinc, germanium, beryllium, chromium, tungsten, selenium, iron, copper, manganese, superoxide dismutase, magnesium and strontium, and mixtures thereof.

17. The method of claim 16 wherein said mineral component comprises iodine or zinc.

18. The method of claim 17 wherein zinc is present in an amount of between about 0.5 ppm and about 50 ppm.

19. The method of claim 17 wherein iodine is present in an amount of between about 0.125 ppm and about 5 ppm.

20. The method of claim 13 wherein said acid/acid-immunomodulating agent comprises an acid selected from the group consisting of ascorbic acid, pantothenic acid, folic acid, benzoic acid, tartaric acid, para-amino benzoic acid, linoleic acid, gammalinoleic acid, acetylsalicylic acid, caprylic acid, amino acids, and saturated and unsaturated fatty acids, and mixtures thereof.

21. The method of claim 20 wherein said acid/acid-immunomodulating agent comprises caprylic acid.

22. The method of claim 21 wherein ascorbic acid is present in an amount of about 6 ppm to about 500 ppm.

23. The method of claim 13 wherein the pH is no greater

than about 4.0 for the thermolabile composition and greater than about 8.0 for the thermostable composition.

24. The method of claim 23 wherein said composition further comprises an acidifying agent.

25. A method for the extension of the shelf life of fresh foods comprising adding a shelf life-extending amount of a composition comprising lysozyme, a mineral component, an acid/acidimmunomodulating agent and further wherein the pH of the composition is no greater than about 5 for the thermolabile composition, and greater than 8.0 for the thermostable composition.

26. The method of claim 25 wherein said lysozyme comprises hen egg white lysozyme.

27. The method of claim 26 wherein said hen egg white lysozyme is present in an amount of about 0.5 ppm to about 500 ppm.

28. The method of claim 25 wherein said mineral component is selected from the group consisting of iodine, zinc, germanium, beryllium, chromium, tungsten, selenium, iron, copper, manganese, superoxide dismutase, magnesium and strontium, and mixtures thereof.

29. The method of claim 28 wherein said mineral component comprises iodine or zinc.

30. The method of claim 29 wherein zinc is present in an amount of between about 0.5 ppm and about 50 ppm.

31. The method of claim 29 wherein iodine is present in an amount of about 0.125 ppm and about 5 ppm.

32. The method of claim 25 wherein said acid/acid-immunomodulating agent comprises an acid selected from the group consisting of ascorbic acid, pantothenic acid, folic acid, benzoic acid, tartaric acid, para-amino benzoic acid, linoleic acid, gammalinoleic acid, acetylsalicylic acid, caprylic acid, amino acids, and saturated and unsaturated fatty acids, and mixtures thereof.

33. The method of claim 32 wherein said acid/acid-immunomodulating agent comprises caprylic acid.

34. The method of claim 33 wherein caprylic acid is present in an amount of about 6 ppm to about 500 ppm.

35. The method of claim 25 wherein the pH is no greater than about 4.

36. The method of claim 35 wherein said composition further comprises an acidifying agent.

37. The method of claim 25 wherein the fresh food whose shelf life is extended is selected from the group consisting of ground beef, mayonnaise, egg nog, low cholesterol foods using modified egg yolk, hot dogs, oils, cheese, milk, yogurt, soft drinks and fish products.

38. A method for extending the shelf life of milk and milk products comprising adding to milk or a milk product a composition comprising about 5 to about 20 milligrams of lysozyme per liter of milk or milk product, about 1.5 to about 5 milligrams of a mineral component per liter of milk or milk product, about 10 to about 120 milligrams of an acid/immunomodulating agent

per milliliter of milk or milk product and further wherein the pH of the composition is no greater than about 5.

39. The method of claim 38 wherein said lysozyme comprises hen egg white lysozyme.

40. The method of claim 38 wherein said mineral component is selected from the group consisting of iodine, zinc, germanium, beryllium, chromium, tungsten, selenium, iron, copper, manganese, superoxide dismutase, magnesium and strontium, and mixtures thereof.

41. The method of claim 38 wherein said acid/ immunomodulating agent comprises an acid selected from the group consisting of caprylic acid, pantothenic acid, folic acid, benzoic acid, tartaric acid, para-amino benzoic acid, linoleic acid, gammalinoleic acid, acetylsalicylic acid, caprylic acid, amino acids, and saturated and unsaturated fatty acids, and mixtures thereof.

42. The method of claim 41 wherein said acid/ immunomodulating agent comprises caprylic acid.

43. The method of claim 41 wherein said acid/ immunomodulating agent comprises acetylsalicylic acid.

44. A method for inducing the curding of milk and milk products while extending the shelf life thereof, comprising adding to milk or a milk product the composition of claim 35 wherein said acid/immunomodulating agent is present in an amount of about 25 to about 120 milligrams per milliliter of milk or milk product.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/04576

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A23B 4/20,4/22,4/24,5/14,5/16,5/18 A23C 3/08 C12N 9/36
U.S.Cl.: 435/206, 426/322,335,330,532

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/94.61,638,639,641,646,650,655,667,682,702 426/61,321,322,330,330.1,330.2,330.3,330.4,330.5,335,532 514/561, 435/206, 426/39

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

CAS, BIOSIS, APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y,P	US, A, 4,810,508 (DELL'ACQUA), 7 March 1989, see column 4.	1-44
Y	US, A, 4,743,454 (TOMES), 10 May 1988, see columns 2-3.	1-24
Y,P	US, A, 4,849,236 (KAKIMOTO), 18 July 1989, see column 2.	1-44
Y	US, A, 4,057,649 (BENSALEM), 8 November 1977, see column 2.	1-44
Y	US, A, 3,404,987 (KOOISTRA), 8 October 1968, see columns 2-3.	1-44
Y	Chemical Abstracts, Volume 80, No. 15, issued 15 April 1974 (Columbus, Ohio, USA), Y. Hidaka et al., "Food Preservation with Enzymes", Ref. No. 81106r, Japan Kiokai 73,88,225, 19 November 1973, see the entire document.	1-44

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17 January 1990	26 JAN 1990
International Searching Authority	**Signature of Authorized Officer**
ISA/US	JEAN C. WITZ

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	GB, A, 357,688 OLIGODYN A.G., 14 April 1931, see pages 1-3.	1-44
Y	US,A, 4,352,826 (PEARLINE), 5 October 1982, see columns 1-2.	44