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(54) Title: ANTIMICROBIAL SALT SOLUTIONS FOR CHEESE PROCESSING APPLICATIONS

(57) **Abrégé/Abstract:**

Antimicrobial formulations and solutions for food safety and quality applications are disclosed. Because some of these formulations and solutions contain a substantial concentration of salt, they are adaptable to a variety of food-processing applications, such as for chilling brine applications, disinfecting meat baths/rinses, beef injection brines, poultry chill tanks, brines used in cheese manufacture, as a wash to kill salmonella and other bacteria on hard-boiled eggs or egg shells, and as a wash to disinfect produce, which can become contaminated with salmonella and other pathogenic bacteria in the field. These uses of concentrated salt solutions that depress the freezing point of the solution provide a low temperature bath or shower in which food products can be cooled.



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(57) Abstract: Antimicrobial formulations and solutions for food safety and quality applications are disclosed. Because some of these formulations and solutions contain a substantial concentration of salt, they are adaptable to a variety of food-processing applications, such as for chilling brine applications, disinfecting meat baths/rinses, beef injection brines, poultry chill tanks, brines used in cheese manufacture, as a wash to kill salmonella and other bacteria on hard-boiled eggs or egg shells, and as a wash to disinfect produce, which can become contaminated with salmonella and other pathogenic bacteria in the field. These uses of concentrated salt solutions that depress the freezing point of the solution provide a low temperature bath or shower in which food products can be cooled.

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ANTIMICROBIAL SALT SOLUTIONS FOR CHEESE PROCESSING APPLICATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Patent Application No. 11/439,500, filed May 22, 2006, which is incorporated hereby by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to antimicrobial solutions for food safety and quality applications.

BACKGROUND OF THE INVENTION

[0003] The prevention of contamination of food product by pathogenic microorganisms is important to protect public health. The reduction of spoilage microorganisms in food manufacturing facilities can extend product shelf lives and reduce the amount of food that is discarded as waste. There is a need for improved methods of controlling microorganisms in food production plants. Microorganisms can accumulate at a variety of different points in a food manufacturing operation; the more points at which viable microorganisms can be controlled, the lower the chances of food contamination and the safer the manufacturing process.

[0004] The use of acid-anionic surfactants as antibacterial agents is known. These agents have limited utility in environments where operation at low temperature is required, as their effectiveness drops off significantly at lower temperature and, of

course, operation below 0°C is typically prevented by freezing. Their activity is also directly dependent on maintaining a relatively low pH, with activities dropping rapidly above pH 3.

[0005] Other antibacterial agents have been identified, but their use is problematic due to their non-food quality status. For example, a wide variety of chemical disinfecting agents are in use in food plants. However, there are often disadvantages to these chemicals. In some instances they are too toxic to come into direct contact with the food itself, and may present worker safety or environmental waste disposal issues. In other instances they are insufficiently effective to provide adequate kill of microorganisms, especially at low temperatures. Additionally, the relatively high cost of these chemicals adds to the cost of food production and, consequently, increases the cost of the end product itself.

[0006] Salt has been used for thousands of years as a food preservative. Often, however, salt solutions alone are not sufficiently effective as antibacterial agents, as they do not provide a speedy mechanism for killing unwanted bacteria that permits their exclusive use in food processing environments. Also there are certain pathogenic microorganisms that survive very well in salt brines even at cold temperatures, such as *Listeria monocytogenes*.

[0007] Thus, a problem associated with the antimicrobial solutions for food safety applications that precede the present invention is that they do not provide an improved antimicrobial solution for food safety applications having operating parameters adaptable to a multiplicity of applications in the food processing industry.

[0008] Another problem associated with the antimicrobial solutions for food safety applications that precede the present invention is that they do not provide an antimicrobial solution for food safety applications having safe, acceptable ingredients for use in food processing to prevent bacteria from accumulating in food processing operations.

[0009] Yet another problem associated with the antimicrobial solutions for food safety applications that precede the present invention is that they do not provide an antimicrobial solution for food safety applications that can be used at temperatures below room temperature, and preferably below the normal freezing point of water (0°C).

[0010] Still a further problem associated with the antimicrobial solutions for food safety applications that precede the present invention is that they may contain or lead to toxic and/or environmentally undesirable additives. For example, they may contain quaternary ammonium chloride as the anti-bacterial ingredient, or they may form chlorinated or brominated byproducts, or they may contain phosphates.

[0011] Yet another problem associated with the antimicrobial solutions for food safety applications that precede the present invention is that they do not provide an antimicrobial solution for food safety applications that is relatively inexpensive to purchase, use and maintain.

[0012] Yet another problem associated with some of the antimicrobial solutions for food safety applications that precede this invention is that they require low pH for effectiveness, and low pH solutions have detrimental effects on concrete floors and

can contribute to corrosion of equipment. There is a need for antimicrobial solutions which are highly effective at neutral or near neutral pH.

[0013]For the foregoing reasons, there has been defined a long felt and unsolved need for an improved antimicrobial solution for food safety applications.

SUMMARY OF THE INVENTION

[0014]An embodiment of the invention described herein is a food-safe solution or composition for use in solution that may be used in a variety of applications to control microorganisms in food plant operations, including the disinfection of food processing brines. The solution or composition of said embodiment may comprise surfactant and salt. The salt can be selected from inorganic salts such as the sodium, potassium, magnesium, calcium, iron, and ammonium salts of chloride, sulfate, nitrate, phosphate, carbonate and hydroxide or organic salts such as the sodium, potassium, magnesium, calcium and ammonium salts of formate, acetate, gluconate, propionate, and hydroxypropionate. Suitable surfactants may include sodium lauryl sulfate, linear alkylbenzene sulfonates, alcohol sulfates, alkyl sulfates, alkyl sulfonates, sodium alkyl methyltaurines, alpha-olefin sulfonates, alcohol ethoxylates, nonylphenyl ethoxylates, alkylpolyglucosides, fatty alcohols, fatty acids and fatty acid salts, lignosulfonates and lignin derivatives, hydroxypoly(oxyethylene) derivatives, fatty alkanolamides, fatty amine oxides, sodium dioctylsulfosuccinate, dodecylbenzene sulfonic acid and salts thereof, the sodium salt of sulfonated oleic acid, sodium dodecylbenzene sulfonate, lauramine oxide, dodecyldiphenyloxide-disulfonic acid and salts thereof.

[0015] These and other aspects of the present invention are elucidated further in the detailed description.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0016] The following description of the invention is intended to illustrate various embodiments of the invention. As such, the specific modifications discussed are not to be construed as limitations on the scope of the invention. It will be apparent to one skilled in the art that various equivalents, changes, and modifications may be made without departing from the scope of the invention, and it is understood that such equivalent embodiments are to be included herein.

[0017] It has been discovered that salts act synergistically with surfactant ingredients to provide a significant and unexpected increase in antibacterial effectiveness in solution.

[0018] In one embodiment of the present invention, a formulation for food safety applications is provided comprising surfactant and salt, and solutions comprising said formulation. In another embodiment of the present invention, a formulation for food safety applications is provided comprising acid, surfactant and salt, and solutions comprising said formulation.

[0019] Many applications for these and other embodiments according to the present invention are envisioned. One application is for disinfecting a food processing bath or rinse. For example, a solution of an antimicrobial composition according to the present invention could be used in or as a chill brine to minimize the bacterial contamination of the chill brine.

[0020] Further, bacterial contamination during slaughter is typically highest at the surface of the meat, and these solutions may be used as a method to kill bacteria directly on the meat surface in a manner that is food safe and will impart no toxic chemicals to the meat. A solution of the antimicrobial composition could be sprayed or showered on to animal carcasses or the carcasses could be directly immersed in a bath of the solution. The brine could be pre-chilled to provide a simultaneous cooling and disinfection. The antimicrobial brine can also be used to wash animals prior to slaughter, to minimize contamination from the animals' hides, skins or feathers. It can also be used as a disinfection wash/chill step for beef trim and other further processed meat and poultry parts.

[0021] Another application for some embodiments of the present invention is for beef injection brines. Brines are injected into enhanced beef products, and there is concern that the brine may drive bacteria, such as *E. coli* O157:H7, from the surface into internal areas of the meat. Cooking intact cuts of beef to rare or medium rare doneness could then lead to food-borne illness. Another concern is that the brine, which is recycled in the process, will become contaminated. Under the current regulatory environment, it is crucial that beef processors are able to prove lot-to-lot separation. Use of a validated antimicrobial in the injected brine solution could prevent the brine injection system from tying together multiple production lots. Other potential uses in the meat industry include hide curing, offal chilling and natural casing preservation.

[0022] In the poultry industry, contamination of the carcasses by *Salmonella* spp. and *Campylobacter* spp. is a major public health concern. Some embodiments according to the present invention could be used in poultry chill tanks to reduce this

contamination and provide an energy-efficient cooling step, thus improving product shelf life and quality.

[0023] Brines used in cheese manufacture present another application for embodiments of the present invention. Cheese manufacture often involves a prolonged soak in concentrated brine. This step can introduce a significant risk for *L. monocytogenes* contamination. This risk could be minimized through the use of an antimicrobial salt solution in the brine.

[0024] Yet another application is as a wash to kill salmonella on eggs. Also, hard-boiled eggs are often pre-disinfected and shipped in brine. Use of some embodiments of the present invention would permit the disinfection step to be carried out in the storage brine itself. Yet another application is a wash to disinfect produce, which can become contaminated with salmonella and other pathogenic bacteria in the field.

[0025] Further it has been found that the salt/surfactant combination maintains antilisterial activity even in the presence of organic material. As the brine is recirculated in the meat processing facility, organic material (meat juice from leaking packages, meat from broken packages, debris rinsed from the outside of packages, etc.) can inhibit other antimicrobials such as chlorine. The salt/surfactant system maintained good activity despite the presence of this organic material.

[0026] The following examples further illustrate the synergistic and unexpected results from combining surfactant with salt.

[0027] Tests have identified a variety of surfactants which are extremely effective at killing *L. monocytogenes* in salt brines at neutral or near-neutral pH. These

surfactants showed an unexpected and dramatic synergistic effect when used in combination with a salt. Tests were generally run according to the following procedure:

1. Inoculate a separate tube containing approximately 10 ml of Brain Heart Infusion (BHI) broth with the following *L. monocytogenes* strains: H2446 (CDC Global Standard), Scott A (serotype 4b), 12243 (serotype 1/2a), and two strains isolated from the environment of a cooked meat and poultry facility, designated WP1, and WP4. Incubate the tubes at least 5 days at 7-10°C +/- 2°C.
2. Assume the growth to be 10⁹ cfu/ml. Serially dilute each culture in cold (~7°C). Butterfield's Phosphate Buffered Water (PBW) to 10⁸ cfu/ml (1:10). Since five cultures of *L. monocytogenes* are being used as a cocktail, begin the dilution series using 2.20 ml of each culture added to 99 ml of PBW.
3. Plate (-6, -7, -8) the diluted culture to get the starting count of the inoculum on Modified Oxford medium (MOX) using a thin agar overlay (TAL) technique (overlay with Trypticase Soy Agar [TSA]) to revive injured cells.
4. Add 1 ml of the diluted cocktail to 100 ml of cold test solution.
5. Mix the solutions well.
6. Determine the *L. monocytogenes* population at time 0 and 4 hours. Plate -1 (0.1 ml on 1 plate), -2, -3, and -4 dilutions using spread plates on MOX TAL with TSA.
7. Incubate the test solutions at test temperature for the duration of the experiment.
8. Incubate the MOX TAL with TSA plates at 20°C +/- 2°C for 72 +/- 3 hours. Count representative colonies, which are black, and multiply by the dilution factor.

[0028] Table 1 provides a summary of results of these tests on several different surfactants in solution either alone or in combination with 20% sodium chloride, wherein the solutions were incubated at 2°C (+/- 1°C):

Table 1. *L. monocytogenes* (cfu/mL) after 4 Hours in Solutions at 2°C

| Solution Composition | <i>L. mono</i> count (cfu/mL) |
|---|--------------------------------------|
| Water (control) | 7.1×10^4 |
| 20% NaCl (control) | 1.0×10^5 |
| 50 ppm sulfonated oleic acid, Na salt | 1.3×10^5 |
| 50 ppm sulfonated oleic acid, Na salt + 20% NaCl | 7.7×10^3 |
| 50 ppm lauramine oxide | 3.6×10^3 |
| 50 ppm lauramine oxide + 20% NaCl | < 10 |
| 50 ppm fatty alkanolamide | 1.1×10^4 |
| 50 ppm fatty alkanolamide + 20% NaCl | <10 |
| 50 ppm nonylphenol ethoxylate | 1.3×10^5 |
| 50 ppm nonylphenol ethoxylate + 20% NaCl | 40 |
| 50 ppm sodium linear alkylbenzene sulfonate | 5.9×10^3 |
| 50 ppm sodium linear alkylbenzene sulfonate + 20% NaCl | < 10 |
| 50 ppm alkyl polyglucoside | 9.6×10^4 |
| 50 ppm alkyl polyglucoside + 20% NaCl | 10 |

[0029] As shown in Table 1, there is an unexpected and dramatic synergistic effect between sodium chloride and the surfactants in killing *L. monocytogenes*. It can be

seen that *L. monocytogenes* survived in very high concentration in 20.0% NaCl. A solution comprising 50 ppm surfactant alone resulted in only a 0 to 1.3 log reduction in *L. monocytogenes* compared to plain water. However, when the surfactants were combined with 20.0% NaCl brine, the kill of *L. monocytogenes* rose to a > 4 log reduction compared to the solution with only 20.0% NaCl and no surfactant.

[0030] Table 2 shows data from another experiment which was carried out to determine the effect of different salts and different salt concentrations in combination with surfactants on *L. monocytogenes* survival in brines.

Table 2. *L. monocytogenes* (cfu/mL) after 4 Hours in Solutions at 2°C

| Solution Composition | <i>L. mono</i> count (cfu/mL) |
|--|--------------------------------------|
| Water (control) | 5.45×10^5 |
| 20% NaCl (control) | 4.0×10^5 |
| 20% Potassium Acetate | 4.2×10^5 |
| 20% Sodium Acetate | 3.0×10^4 |
| 20% Sodium Formate | 3.6×10^5 |
| 50 ppm lauramine oxide | 5.8×10^4 |
| 50 ppm lauramine oxide + 20% NaCl | < 10 |
| 50 ppm lauramine oxide + 10% NaCl | < 10 |
| 50 ppm lauramine oxide + 5% NaCl | < 10 |
| 50 ppm lauramine oxide + 1% NaCl | 2.1×10^4 |
| 50 ppm lauramine oxide + 20% Potassium Acetate | < 10 |
| 50 ppm lauramine oxide + 20% Sodium Acetate | < 10 |
| 50 ppm lauramine oxide + 20% Sodium Formate | < 10 |

| Solution Composition | L. mono count (cfu/mL) |
|--|------------------------|
| 50 ppm lauramine oxide + 20% MgSO ₄ | < 10 |
| 50 ppm lauramine oxide + 10% MgSO ₄ | < 10 |
| 50 ppm lauramine oxide + 5% MgSO ₄ | < 10 |
| 50 ppm lauramine oxide + 1% MgSO ₄ | 1.5×10^4 |
| 50 ppm C ₁₂ (branched) sodium diphenyl oxide disulfonate | 3.1×10^4 |
| 50 ppm C ₁₂ (branched) sodium diphenyl oxide disulfonate + 20% NaCl | < 10 |
| 50 ppm alcohol ethoxylate | 1.9×10^5 |
| 50 ppm alcohol ethoxylate + 20% NaCl | < 10 |
| 50 ppm sodium olefin sulfonate | 1.8×10^5 |
| 50 ppm sodium olefin sulfonate + 20% NaCl | < 10 |

[0031] Data in Table 2 again shows that while the surfactant alone or sodium chloride alone has little effect on the survival of *L. monocytogenes* in solution, the combination of even low concentrations of surfactant with sodium chloride in solution has a powerful cidal effect on *L. mono*, giving over 4 log kill or higher. The data run on a particular surfactant, in this case a lauramine oxide, shows that it can be "activated" to be highly cidal towards *L. monocytogenes* over a broad range of sodium chloride concentrations. In solutions containing 5% and 20% NaCl, 50 ppm of the surfactant was highly cidal towards *L. monocytogenes*. The data in Table 2 also shows that salts other than sodium chloride are effective. A variety of organic salts, including formates and acetates, as well as magnesium sulfate all showed the same ability to "activate" low concentrations of surfactant to kill *L. monocytogenes* in solution, even though the salts by themselves had little effect on the organisms.

[0032] Because some embodiments of the present invention contain a substantial concentration of salt, these embodiments are ideal for a variety of applications. For instance, they are ideal for chilling brine applications. Chilling brines make use of concentrated salt solutions that depress the freezing point of the solution to provide a low temperature bath or shower in which food products can be efficiently cooled. Bacterial contamination of the chill brine is a food safety hazard, requiring that the brine be frequently disposed and often requiring rigorous cleaning of the equipment to remove bacterial biofilms. Contamination by *L. monocytogenes* is of particular concern in many ready-to-eat meat, poultry, seafood and dairy processing chill brine applications because it is known to survive in high salt concentrations and because many of the currently available disinfectant chemicals are either not suitable for direct food contact or become ineffective at the cold temperatures of the chill bath. Brine chillers are used extensively to cool frankfurters and other sausage products in continuous-cook operations. Dozens of nationwide recalls and at least one large food-borne outbreak have been caused by *L. monocytogenes* contamination of these types of products.

[0033] One useful application for these formulas is in chill brines used in the manufacture of cooked sandwich meats, sausages, and links. U.S. Patent Application Serial No. 10/460,769, filed June 12, 2003, describes embodiments consisting of a surfactant and an acid together which worked synergistically with the salt in food production chill brines to kill *L. monocytogenes*. One drawback of these embodiments was their acidity, which could have detrimental effects on concrete floors and steel equipment. The present embodiment provides certain types of surfactants which are very effective when combined with either inorganic or organic

salts in solution at killing *L. monocytogenes* even in the absence of an acidifying agent.

[0034] Several tests were carried out to determine the effectiveness of embodiments according to the invention in meat processing chill brines. In one experiment, brine was taken at the end of a production week from a brine chiller used in a ready to eat, cooked beef production line. The sodium chloride concentration in this brine was approximately 17%. Samples of the brine with and without added surfactant were inoculated with a cocktail of *L. monocytogenes* as per the procedure described above, incubated at 4°C for four hours, and then plated to determine *L. monocytogenes* survival. Results are summarized in Table 3.

Table 3. *L. monocytogenes* (cfu/mL) after 4 Hours in Beef Plant Brine at 4°C

| Brine Composition | <i>L. mono</i> count (cfu/mL) |
|---|--------------------------------------|
| Brine Control (no additive) | 1.4×10^6 |
| 50 ppm lauramine oxide | < 10 |
| 25 ppm lauramine oxide | < 10 |
| 12.5 ppm lauramine oxide | < 10 |
| 50 ppm sodium linear alkylbenzene | < 10 |
| 25 ppm sodium linear alkylbenzene | < 10 |
| 12.5 ppm sodium linear alkylbenzene sulfonate | < 10 |
| 50 ppm nonylphenol ethoxylate | < 10 |
| 25 ppm nonylphenol ethoxylate | < 10 |
| 12.5 ppm nonylphenol ethoxylate | 20 |
| 50 ppm fatty alkanolamine | < 10 |
| 25 ppm fatty alkanolamine | < 10 |

| Brine Composition | L. mono count (cfu/mL) |
|----------------------------------|------------------------|
| 12.5 ppm fatty alkanolaminde | 40 |
| 50 ppm sodium olefin sulfonate | 10 |
| 25 ppm sodium olefin sulfonate | 60 |
| 12.5 ppm sodium olefin sulfonate | 110 |

[0035] The data in Table 3 indicate that the brine taken from the meat processing plant very easily supported the survival of *L. monocytogenes*, raising the possibility of a food safety hazard should contamination of the brine ever occur. However, addition of even small concentrations of a single surfactant provided >5 log kill of *L. monocytogenes* in the brine. The surfactants are effective at remarkably low concentration when in combination with salt in solution. As little as 12.5 ppm of several of the surfactants in Table 3 killed essentially all of the inoculum. This experiment was also significant because it indicates that the salt/surfactant combination maintains antilisterial activity even in the presence of organic material. As the brine is recirculated in the meat processing facility, organic material (meat juice from leaking packages, meat from broken packages, debris rinsed from the outside of packages, etc.) can inhibit other antimicrobials such as chlorine. The salt/surfactant system maintained good activity despite the presence of this organic material.

[0036] Often the effectiveness of antimicrobial additives decreases at lower temperatures. Another test was run to determine the effectiveness of these formulas in an even colder meat processing brine. Five samples of spent chill brine were obtained at different times from a hot dog manufacturing plant, which uses a nearly saturated sodium chloride brine at a temperature of approximately -20°C. The brine

samples were tested with and without addition of 50 ppm of an alcohol ethoxylate surfactant in the same manner as described above, except they were incubated for 4 hours at -20°C before plating. Results are shown in Table 4.

Table 4. *L. monocytogenes* (cfu/mL) after 4 Hours in Hot Dog Plant Chill Brine at -20°C

| Brine Sampling Date | Brine Control | Brine + 50 ppm Alcohol Ethoxylate |
|---------------------|-------------------|-----------------------------------|
| 7-7-05 | 1.5×10^5 | <10 |
| 7-14-05A | 5.3×10^5 | <10 |
| 7-14-05B | 2.8×10^5 | <10 |
| 3-18-05 | 5.7×10^5 | <10 |
| 6-10-05 | 4.9×10^5 | <10 |
| 4-19-05 | 6.5×10^5 | <10 |

[0037] The data in Table 4 indicate that the process brines supported the survival of *L. monocytogenes* very well even at -20°C. However, addition of 50 ppm of alcohol ethoxylate resulted in kill of essentially the entire ~ 5 log inoculum within 4 hours. Tests were subsequently run on even lower concentrations of the alcohol ethoxylate surfactant in the brine. Concentrations of 12.5 ppm were as effective as 50 ppm.

[0038] In addition to being effective against organisms in an aqueous solution, tests indicated that some embodiments of the invention were also effective against organisms in a biofilm. Biofilms can provide a haven for pathogens, increasing their resistance to antimicrobial treatments, and thereby providing another possible source of food contamination. Tests were run to see if some embodiments were effective against a *L. monocytogenes* biofilm. Challenge tests were run according to

the procedure below. Test solutions were prepared from a sample of hot dog plant chill brine which was treated with various levels of the alcohol ethoxylate surfactant. Cooked turkey was added to the test solution before inoculation to simulate a worst case "dirty" brine with a high degree of organic load.

1. Inoculate five cultures, *L. monocytogenes* H2446 (CDC Global Standard), Scott A-serotype 4b, 12243-serotype 1/2a, WP1 and WP4 in 10ml Brain Heart Infusion broth (BHI). Incubate the tubes for 7 days at 10°C +/- 2°C.
2. Aseptically dispense 50 ml of sterile Tryptic Soy Broth (TSB) + 0.6% Yeast Extract (YE) into sterile disposable 50 ml conical shaped plastic tubes. Make enough tubes for each time point.
3. Aseptically drop one coupon into the broth in each tube.
4. Make a cocktail of the five cultures and add 0.1ml into each tube. Incubate the tubes for 7 days at 7°C +/- 2°C.
5. Dispense 40 ml of antimicrobial salt solutions containing sterile phosphate buffer into 50 ml plastic tubes.
6. After biofilm has grown, aseptically remove coupon and rinse each side for 5 seconds with sterile distilled water to remove unattached cells.
7. Aseptically drop each rinsed coupon into the antimicrobial salt solution tube and incubate for appropriate time (1 hour and 24 hour) at -20°C +/- 2°C.
8. Aseptically add 45 ml of sterile phosphate buffer (PBW) to 50 ml conical shaped plastic tubes along with 10 sterile glass beads.

9. After incubate time is complete, aseptically move the coupon from the antimicrobial salt solution to the sterile (PBW) solution containing beads.
10. Shake the tube with glass beads for about 2 minutes to remove attached cells.
11. Plate the cells in the PBW solution on TSA + 0.6% YE using appropriate dilutions and incubate at 20°C for 72 +/- 2 hours.
12. Plate the antimicrobial salt solution on TSA + 0.6% YE using appropriate *dilutions and incubate 20°C for 72 +/- 2 hours. *Please make note: The first dilution should take place in 9 ml DE Neutralizing Buffer. After incubation, count typical colonies and record results to cfu/g.

[0039] Results of this challenge study are given in Table 5.

Table 5. *L. mono* Biofilm Challenge in Hot Dog Plant Chill Brine at -20°C

| Surfactant Concentration | 1 hour (cfu/coupon) | 24 hours (cfu/coupon) |
|---------------------------------|----------------------------|------------------------------|
| Control (0 ppm) | 44,000 | 9700 |
| 15 ppm alcohol ethoxylate | 5,000 | 5600 |
| 25 ppm alcohol ethoxylate | 3500 | 250 |
| 50 ppm alcohol ethoxylate | 10-100 | <10 |

[0040] As shown in Table 5, it appears that even at the near neutral pH of the plant chill brine, low concentrations of surfactant are effective at killing *L. monocytogenes* in a biofilm. In this experiment, higher concentrations of surfactant were required to achieve 4 log kill than was seen in the solution challenge studies. This may be due to the greater resistance of the biofilm, but it also may be due to the brine being

made very "dirty" with high organic loading in this experiment. Even with very "dirty" brine, 50 ppm alcohol ethoxylate showed > 2 log kill of the biofilm within 1 hour and showed > 3 log kill after 24 hours.

[0041] Tests were run to determine the effectiveness of formulas against organisms other than *L. monocytogenes*. Uncharacterized microorganisms were cultured from a sample of raw ground beef and used to challenge 24% sodium chloride brines with and without different surfactants. The test solutions were inoculated with the ground beef organism culture and incubated for 4 hours at -5°C before plating. Results are given in Table 6.

Table 6. Total Plate Count (cfu/mL) after 4 Hours in 24% NaCl Brine at -5°C

| Brine Composition | Total plate count (cfu/mL) |
|---|----------------------------|
| Brine Control (no additive) | 6.3×10^5 |
| 800 ppm nonylphenoethoxylate | 40 |
| 800 ppm sodium salt of sulfonated oleic acid | 5.2×10^3 |
| 800 ppm alcohol ethoxylate | <10 |
| 800 ppm Toximul 3479F | <10 |
| 800 ppm sodium linear alkylbenzene sulfonate | <10 |
| 800 ppm C ₁₂ (branched) sodium diphenyl oxide disulfonate + 20% NaCl | 1.0×10^3 |
| 800 ppm Toximul TA-5 | 20 |
| 800 ppm Toximul 8382 | 7.8×10^3 |
| 800 ppm decyl alcohol ethoxylate, POE-6 | 10 |
| 800 ppm Toximul 3409F | 160 |
| 800 ppm Toximul 3455F | 60 |

[0042] Data in Table 6 indicates that a number of surfactants in combination with brine are also effective in killing the total plate count organisms found in raw ground beef.

[0043] In another embodiment of the present invention, an unexpected synergistic effect has also been found between acid, sodium chloride and sodium lauryl sulfate (SLS) antibacterial additive. Replicate tests were run to determine if this effect was statistically significant. Ten percent by weight solutions were prepared of a formula of 0.6% citric or malic acid, 100 ppm SLS, and 99.4% sodium chloride. Solutions were also prepared containing an identical concentration of acid and SLS but no sodium chloride. A bacterial culture suspension (*Escherichia coli* ATCC 11229) that had been incubated for 24 hours in Brain Heart Infusion (BHI) broth and had an initial inoculum count of about 10^9 CFU/ml was serially diluted in cold Butterfield's Phosphate Buffered Water (BPBW) to 10^5 CFU/ml. A 1.0 ml aliquot of this suspension was added to 100 ml of test solution at room temperature and mixed well, providing an initial inoculum of 10^3 CFU/ml. After 30 minutes, the *E. coli* populations were enumerated by plating on tryptic soy agar (TSA), making serial dilutions as necessary in BPBW. Plates were incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for approximately 24 hours. Colonies were then counted and compared to the initial inoculum counts. Results of these tests run on 16 replicates of each test solution are given in Table 7.

Table 7. Effectiveness of Acid/SLS Solutions with and without Salt on *E. coli*

| Test Solution | Average Concentration of <i>E. Coli</i> (CFU/ml) |
|-----------------------------|--|
| citric acid, SLS, with salt | 540 |

| | |
|--------------------------------|------|
| citric acid, SLS, without salt | 1054 |
| malic acid, SLS, with salt | 141 |
| malic acid, SLS, without salt | 2419 |

[0044] Referring to Table 7, it can be seen that for both the citric acid/SLS and malic acid/SLS additives, the number of bacteria remaining alive after 30 minutes is much lower when salt is present than when there is no salt present. Analysis of the data indicates that there is a statistically significant increase in kill in the presence of salt ($p < 0.05$). In contrast, a 10% solution of pure sodium chloride does not provide any significant kill of the test microorganisms.

[0045] To study chilling brine application of the current embodiment, tests were run on 17% by weight solutions of formulas consisting of between 0.3% and 6.0% citric acid, between 50 and 500 ppm SLS, and between 94% and 99.7% sodium chloride. Test solutions were cooled to -7°C and inoculated with several strains of *L. monocytogenes*. Within 4 hours most solutions showed a 3 log kill of microorganisms and within 24 hours nearly all solutions showed no measurable plate count of the inoculum. A brine solution made up of sodium chloride alone caused less than a 1 log reduction of the *L. monocytogenes* over a 24-hour period.

[0046] An experiment was run to determine if solutions containing sodium chloride, sodium lauryl sulfate, and various acids would kill *L. monocytogenes* at cold temperatures. The following test procedure was used: A bacterial culture suspension (*L. monocytogenes* H2446 [CDC Global Standard]; Scott A-serotype 4b; 12243-serotype 1/2a; and a recent cooked meat and poultry facility isolate, WP4) that had been incubated for at least 5 days in BHI broth and had an initial inoculum count of about 10^9 CFU/ml was serially diluted in cold BPBW to 10^5 CFU/ml. A 1.0

ml aliquot of this suspension was added to 100 ml of cold ($-7^{\circ}\text{C} + 2^{\circ}\text{C}$) test solution and mixed well, providing an initial inoculum of 10^3 CFU/ml. The test solutions were incubated at $-7^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the experiment. At intervals of 0, 4, and 24 hours the *L. monocytogenes* populations in the test solutions were determined on Modified Oxford agar (MOX). MOX plates were incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for approximately 48 hours. Colonies were then counted and compared to the initial inoculum counts.

[0047] Results are given in Table 8. Each test solution was a 17% by weight solution of the listed formula prepared in soft water.

Table 8. Effect of Solutions of NaCl, SLS and various acids on *L. monocytogenes*

| Sample | Time 0 CFU/ml | 4 hr CFU/ml | 24 hr CFU/ ml | pH | Water Activity |
|--|------------------|----------------|---------------------|------|-------------------|
| 100% NaCl | 1550 | 1250 | 1170 | 7.88 | 0.88 |
| 2.0% Malic Acid 500 ppm SLS 98.0% NaCl | 0 | 0 | 0 | 1.21 | ND |
| Water Control | 1270 | 400 | 0 | 9.34 | 0.999 |
| 0.3% Malic Acid 100 ppm SLS, 99.7% NaCl | 480 | 5 | 0 | 4.1 | ND |
| 0.5% Malic Acid, 100 ppm SLS, 99.5% NaCl | 176 | 0 | 0 | 3.31 | ND |
| 0.7% Malic Acid, 100 ppm SLS, 99.3% NaCl | 117 | 0 | 0 | 2.99 | 0.88 |
| 0.3% Citric Acid, 500 ppm SLS, 99.7% NaCl | 5 | 0 | 0 | 4.14 | ND |
| 0.5% Citric Acid, 500 ppm SLS, 99.5% NaCl | 0 | 0 | 0 | 3.37 | ND |

| Sample | Time 0 CFU/ml | 4 hr CFU/ml | 24 hr CFU/ ml | pH | Water Activity |
|---|------------------|----------------|---------------------|------|-------------------|
| 0.7% Citric Acid, 500 ppm SLS, 99.3% NaCl | 0 | 0 | 0 | 2.98 | 0.88 |
| 0.3% Malic Acid, 500 ppm SLS, 99.7% NaCl | 11 | 0 | 0 | 4.15 | ND |
| 0.5% Malic Acid, 500 ppm SLS, 99.5% NaCl | 3 | 0 | 0 | 3.39 | ND |
| 0.7% Malic Acid, 500 ppm SLS, 99.3% NaCl | 0 | 0 | 0 | 3.06 | 0.879 |
| 1.0% Citric Acid, 500 ppm SLS, 99.0% NaCl | 0 | 0 | 0 | 2.69 | ND |
| 1.0% Malic Acid, 500 ppm SLS, 99.0% NaCl | 0 | 0 | 0 | 2.81 | ND |
| 2.0% Lactic Acid, 500 ppm SLS, 98.0% NaCl | 0 | 0 | 0 | 2.65 | 0.885 |
| 2.0% Phosphoric Acid (75%), 500 ppm SLS, 98.0% NaCl | 0 | 0 | 0 | 1.52 | 0.884 |
| 1.0% Benzoic Acid, 500 ppm SLS, NaCl | 0 | 0 | 0 | 3.93 | 0.879 |
| 2.0% Citric Acid, 500 ppm SLS, 98.0% NaCl | 0 | 0 | 0 | 2.3 | 0.884 |
| 2.0% Malic Acid, 500 ppm SLS, 98.0% NaCl | 0 | 0 | 0 | 2.46 | 0.882 |

[0048] In another experiment, 17% by weight solutions of formulas containing various levels of sodium chloride, citric acid, and sodium lauryl sulfate were tested for effectiveness in killing *L. monocytogenes* at cold temperatures. The same test procedure was used as described above, except test solutions were plated on MOX with a Thin Agar Overlay of TSA (to aid in the recovery of injured cells). Results are given in Table 9. The data indicate that the relative amounts of acid and surfactant can be varied to suit different applications. As shown in table 9, in pH sensitive

applications, the acid may be decreased without losing effectiveness. Similarly, in applications where a lower level of surfactant is desired, the performance can be maintained by raising the concentration of acid.

Table 9. Effect of Solutions of NaCl, SLS, and Citric Acid on *L. monocytogenes* at -6.7°C

| Sample | Time 0 CFU/ml | 4 hr CFU/ml | 24 hr CFU/ ml | pH |
|---|------------------|----------------|---------------------|------|
| 100% NaCl | ~7000 | ~6250 | 2290 | 7.66 |
| 0.3% Citric Acid, 50 ppm SLS, 99.7% NaCl | ~4940 | 163 | 0 | 4.19 |
| 0.3% Citric Acid, 100 ppm SLS, 99.7% NaCl | 2230 | 97 | 0 | 4.27 |
| 0.3% Citric Acid, 150 ppm SLS, 99.7% NaCl | 3080 | 105 | 0 | 4.3 |
| 0.3% Citric Acid, 200 ppm SLS, 99.7% NaCl | 1970 | 42 | 0 | 4.28 |
| 0.3% Citric Acid, 300 ppm SLS, 99.7% NaCl | 1490 | 20 | 0 | 4.3 |
| 0.3% Citric Acid, 400 ppm SLS, 99.7% NaCl | 221 | 1 | 0 | 4.29 |
| 0.3% Citric Acid, 500 ppm SLS, 99.7% NaCl | 99 | 0 | 0 | 4.32 |
| 0.5% Citric Acid, 50 ppm SLS, 99.5% NaCl | 3360 | 0 | 0 | 3.54 |
| 0.5% Citric Acid, 100 ppm SLS, 99.5% NaCl | 3180 | 1 | 0 | 3.54 |
| 0.7% Citric Acid, 50 ppm SLS, 99.3% NaCl | 3710 | 0 | 0 | 3.14 |
| 0.7% Citric Acid, 100 ppm SLS, 99.3% NaCl | 1020 | 0 | 0 | 3.13 |
| 1.0% Citric Acid, 50 ppm SLS, 99.0% NaCl | 1840 | 0 | 0 | 2.82 |
| 1.0% Citric Acid, 100 ppm SLS, 99.0% NaCl | 970 | 0 | 0 | 2.82 |
| 2.0% Citric Acid, 50 ppm SLS, 98.0% NaCl | 114 | 0 | 0 | 2.41 |
| 2.0% Citric Acid, 100 ppm SLS, 96.0% NaCl | 479 | 0 | 0 | 2.41 |
| 4.0% Citric Acid, 50 ppm SLS, 96.0% NaCl | 6 | 0 | 0 | 2.12 |
| 4.0% Citric Acid, 100 ppm SLS, 96.0% NaCl | 1 | 0 | 0 | 2.12 |
| 6.0% Citric Acid, 50 ppm SLS, 94.0% NaCl | 1 | 0 | 0 | 1.99 |

[0049] In another experiment, two sets of solutions were tested. The first set (samples 1-12 in Table 10 below) was prepared in hard tap water and contained about 17.0% by mass of the formulation. These samples were inoculated with 10^3 CFU/ml *L. monocytogenes* by the same procedure as described above. A second set of samples was prepared from brine taken from a ready-to-eat meat processing operation. The recirculated brine had been used to chill packaged meat for one week. After a week of use the brine typically contains various types of aerobic psychrotrophic and mesophilic bacteria. This experiment was done in order to determine if the additives would kill the microorganisms naturally occurring in actual process brine from a plant. Since the spent chill brine samples already contained NaCl citric acid and/or SLS was added to provide an effective concentration of additive. One set of these samples (samples 13-17) were inoculated with 10^3 *L. monocytogenes* and the other set (samples 18-22) contained only the naturally occurring organisms in the spent chill brine. Results are given in Table 10 below. The data indicate that at lower acid levels, the SLS increases the effectiveness of the mixture, but at higher acid levels, the SLS is not necessary. The results show the formulations are effective in hard water (27 gpg hardness). Other antimicrobials, such as quaternary ammonium compounds can lose significant activity in hard water, often necessitating further additives, such as EDTA as a chelating agent, to maintain antimicrobial activity. The results also demonstrate that the formulations effectively kill *L. monocytogenes* as well as the naturally occurring microorganisms in spent chill brine from an actual meat processing plant.

Table 10. Effects of Antimicrobial Salt Formulas in Hard Water and in Spent Chill Brine

| Sample | Time 0 cfu/ml | 2 hr cfu/ml | 24 cfu/ml |
|---|--------------------------|------------------------|----------------------|
| 100% NaCl | 760 | 1100 | 1100 |
| 0.3% Citric Acid, 100 ppm SLS, 99.7% NaCl | 730 | 670 | 29 |
| 0.3% Citric Acid, 99.7% NaCl | 1460 | 1330 | 830 |
| 0.5% Citric Acid, 100 ppm SLS, 99.5% NaCl | 890 | 240 | 0 |
| 0.5% Citric Acid, 99.5% NaCl | 1060 | 1170 | 330 |
| 0.7% Citric Acid, 100 ppm SLS, 99.3% NaCl | 1010 | 14 | 0 |
| 0.7% Citric Acid, 99.3% NaCl | 1040 | 1030 | 3 |
| 1.0% Citric Acid, 100 ppm SLS, 99.0% NaCl | 840 | 0 | 0 |
| 1.0% Citric Acid, 99.0% NaCl | 990 | 340 | 0 |
| 2.0% Citric Acid, 98.0% NaCl | 910 | 0 | 0 |
| 4.0% Citric Acid, 96.0% NaCl | 1110 | 0 | 0 |
| 6.0% Citric Acid, 94.0% NaCl | 950 | 0 | 0 |
| Brine Control with L. mono | 1260 | 1290 | 600 |
| 1% Citric Acid in Brine with L. mono | 1050 | 0 | 0 |
| 2% Citric Acid in Brine with L. mono | 1140 | 0 | 0 |
| 1% Citric Acid + 50 ppm SLS in Brine with L. mono | 1090 | 0 | 0 |
| 2% Citric Acid + 50 ppm SLS in Brine with L. mono | 1070 | 0 | 0 |
| Brine Control | 6000 | 3100 | 2000 |
| 1% Citric Acid in Brine | 2490 | 190 | 4 |
| 2% Citric Acid in Brine | 1670 | 6 | 0 |
| 1% Citric Acid + 50 ppm SLS in Brine | 2520 | 122 | 0 |
| 2% Citric Acid + 50 ppm SLS in Brine | 1480 | 6 | 0 |

[0050] A test was run to determine if salts other than sodium chloride would show a synergistic antimicrobial effect with an acid and sodium lauryl sulfate. Solutions containing 0.6409 grams malic acid and 0.0107 grams sodium lauryl sulfate per liter were prepared with and without 107.0 grams of various salts (added on an anhydrous basis). Solutions were inoculated with *E. coli* described above and the amount of bacterial kill was measured to determine if the added salt caused an increase in the effectiveness of the acid/surfactant active ingredients. Results are shown in Table 11.

Table 11. Effect of Different Salts on the Antimicrobial Action of Malic Acid/SLS

| Solution (salt added) | % Kill of <i>E. Coli</i> |
|------------------------------|---------------------------------|
| No salt addition | 4.4% |
| Sodium sulfate | 87% |
| Magnesium chloride | 56% |
| Potassium chloride | 18% |
| Sodium chloride | 78% |
| Potassium sulfate | 34% |
| Calcium chloride | 55% |
| Magnesium sulfate | 93% |

[0051] Tests run on solutions containing only the salt and no other ingredient indicate that sodium sulfate, potassium chloride and potassium sulfate provide no bacterial kill. Magnesium chloride solution provided 61% kill, calcium chloride provided 26% kill, and magnesium sulfate provided 10% kill. Thus, based on the data developed thus far, sodium sulfate, sodium chloride, and magnesium sulfate appear to

significantly increase the effectiveness of the acid and/or surfactant antimicrobial agent, even though the salts provide little kill on their own.

[0052] The effectiveness of antimicrobial salt formulas was tested against *L. monocytogenes* in a biofilm. Stainless steel coupons (2 x 5 cm, type 302 stainless steel, 2B finish) were cleaned in acetone followed by an alkaline detergent and distilled water and then dried in an autoclave at 121°C for 15 minutes. A culture of *L. monocytogenes* (Scott A - serotype 4b) was prepared by inoculating 10 mL of TSA and incubating overnight at 35°C. 50 mL of sterile TSA + 0.6% yeast extract (YE) was aseptically dispensed into sterile disposable conical shaped plastic tubes and one drop of overnight grown *L. mono* culture was added to each tube. Inoculated tubes were incubated at 25°C for approximately 48 hours. After the biofilm had formed on the coupons, a coupon was aseptically removed from the tube and gently rinsed with distilled water to remove unattached cells. Coupons were then immersed in cold antimicrobial test solution (-6.7°C) and incubated over different time intervals (1 hour, 24 hours, and 5 days). After incubation period, the coupon was shaken in a tube containing 40 mL of sterile PBW and 10 sterile glass beads (4 mm) for 2 minutes to remove the cells attached to the coupon biofilm. The cells were plated in the PBW on TSA + 0.6% YE using appropriate dilutions and incubated at 35°C for 48 hours.

[0053] Results on triplicate samples of antimicrobial test solutions are given in Table 12 below. Each solution contained 17% by weight of a formula consisting of the percentages of citric acid and SLS listed in Table 12 with the balance of the formula being NaCl in each case. The data indicate that not only are the antimicrobial salt

solutions effective at killing bacteria suspended in solution, they are also effective at killing bacteria within a biofilm.

Table 12. Log Concentration of *L. monocytogenes* in Antimicrobial Salt Solutions

| Sample | 1 Hour | 24 Hours | 5 days |
|-------------------------------|--------|----------|--------|
| 0.3% citric acid, 100 ppm SLS | ~5.08 | 4.59 | 1.38 |
| 0.3% citric acid, 100 ppm SLS | ~4.90 | 3.85 | 1.79 |
| 0.3% citric acid, 100 ppm SLS | ~4.81 | 3.48 | 1.92 |
| 0.3% citric acid, 500 ppm SLS | 4.81 | 4.76 | 2.23 |
| 0.3% citric acid, 500 ppm SLS | 4.90 | 3.48 | 2.18 |
| 0.3% citric acid, 500 ppm SLS | ~5.18 | 3.48 | 2.36 |
| 0.7% citric acid, 100 ppm SLS | 1.88 | 0 | 0 |
| 0.7% citric acid, 100 ppm SLS | 2.02 | 0 | 0 |
| 0.7% citric acid, 100 ppm SLS | 1.28 | 0 | 0 |
| 0.7% citric acid, 500 ppm SLS | 0.70 | 1.00 | 0.90 |
| 0.7% citric acid, 500 ppm SLS | 0.90 | 0.70 | 0.30 |
| 0.7% citric acid, 500 ppm SLS | 0.85 | 0 | 0 |
| 2.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 2.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 2.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 2.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 2.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 2.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 6.0% citric acid, 100 ppm SLS | 0 | 0 | ND |

| Sample | 1 Hour | 24 Hours | 5 days |
|--------------------------------|--------|----------|--------|
| 6.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 6.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 6.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 6.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 6.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 12.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 12.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 12.0% citric acid, 100 ppm SLS | 0 | 0 | ND |
| 12.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 12.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| 12.0% citric acid, 500 ppm SLS | 0 | 0 | ND |
| Salt Control A | ~5.04 | ~7.15 | 7.65 |
| Salt Control B | ~5.48 | ~7.15 | 7.42 |
| Salt Control C | -5.48 | -7.11 | 7.65 |
| Water Control | -5.18 | -7.18 | 7.54 |

[0054] Another set of experiments was done in order to determine the effectiveness of different acids and different types of surfactants in the antimicrobial salt formulations. In one experiment, test solutions containing ~17% by weight of formulas containing various levels of sodium chloride, 100 ppm sodium lauryl sulfate, and various levels of different acids were tested for effectiveness in killing *L. monocytogenes* at cold temperatures. The same test procedure was used as described above (test solutions were plated on MOX TAL (Modified Oxford Medium with a Thin Agar Layer) with TSA). Results are given in Table 13. The controls were a solution of pure NaCl, a solution of a blend of 100 ppm SLS in NaCl, and a

solution of a blend of 0.5% citric acid, 100 ppm SLS, and 99.5% NaCl. The subsequent test solutions were a 17% solution of a blend of NaCl and 100 ppm SLS with enough of the listed acid added to provide the same pH (~ 3.6) as the 0.5% citric acid control.

Table 13. Effect of Different Acids on the Antimicrobial Action of NaCl/Acid/SLS

| Sample Solution Composition | Time 0 (CFU/ml) | Time 4 Hours (CFU/ml) |
|------------------------------------|----------------------------|--------------------------------------|
| Salt control | 850 | 1380 |
| Salt + SLS control | 980 | 890 |
| Salt + SLS+ citric acid control | 1230 | 18 |
| Salt + SLS + succinic acid | 1070 | 69 |
| Salt + SLS + isoascorbic acid | 1140 | 59 |
| Salt + SLS + adipic acid | 900 | 4 |
| Salt + SLS + sorbic acid | 820 | 500 |
| Salt + SLS + acetic acid | 1070 | 230 |
| Salt + SLS + propionic acid | 1440 | 6 |
| Salt + SLS + lactic acid | 1050 | 220 |
| Salt + SLS + ascorbic acid | 1230 | 54 |
| Salt + SLS + formic acid | 1930 | 38 |
| Salt + SLS + phosphoric acid | 1050 | 17 |
| Salt + SLS + hydrochloric acid | 1100 | 44 |
| Salt + SLS + tartaric acid | 1180 | 410 |
| Salt + SLS + glutaric acid | 610 | 180 |
| Salt + SLS + benzoic acid | 1020 | 17 |

| Sample Solution Composition | Time 0 (CFU/ml) | Time 4 Hours (CFU/ml) |
|-----------------------------|--------------------|-----------------------------|
| Salt + SLS + salicylic acid | 1100 | 5 |
| Salt + SLS + sulfuric acid | 830 | 0 |

[0055] In another experiment, test solutions containing 17% by weight of formulas containing 99.7% sodium chloride, 0.3% citric acid, and 500 ppm of various types of surfactants were tested for effectiveness in killing *L. monocytogenes* at cold temperatures. The same test procedure was used as described (test solutions were plated on MOX TAL (Modified Oxford Medium with a Thin Agar Layer) with TSA). Results are given in Table 14.

Table 14. Effect of Different Acids on the Antimicrobial Action of NaCl/Acid/SLS Surfactant Tested

| Surfactant Tested | Time 0 (CFU/ml) | Time 4 Hours (CFU/ml) |
|--|--------------------|--------------------------|
| Salt Control (no additive) | 880 | 610 |
| polyoxyethylene-polyoxypropylene block polymer | 820 | 610 |
| sodium salt of sulfonated oleic acid | 240 | 0 |
| sodium xylene sulfonate | 910 | 820 |
| dodecyl diphenyl oxide disulfonate | 0 | 0 |
| sodium linear alkyl-benzene sulfonate | 490 | 0 |
| alpha-olefin sulfonate | 370 | 0 |
| alkylpolyglucoside | 280 | 0 |
| nonylphenol ethoxylate | 460 | 0 |
| fatty alkanolamide | 470 | 0 |
| alcohol ethoxylate | 1080 | 1 |

| Surfactant Tested | Time 0 (CFU/ml) | Time 4 Hours (CFU/ml) |
|-------------------|--------------------|--------------------------|
| lauramine oxide | 2 | 0 |

[0056] One or more embodiments of the present invention can be operated under various sets of conditions. In one, a chilling brine maintained at a temperature of about -1.9°C is employed. The chilling brine comprises, on a dry basis, between about 0.3% and about 1.0% citric acid. The citric acid concentration may be increased to as high as about 2.0%. Between about 100 and about 500 ppm SLS is utilized. The balance of the brine formulation is NaCl, and the formulation is mixed with water to a solution of about 9% to about 12%. In another chilling brine application, a chilling brine is maintained at a temperature of about -6.7°C . The chilling brine comprises between about 0.3% and about 1.0% citric acid. Again, the citric acid concentration may be increased to as high as about 2.0%. Between about 100 and about 500 ppm SLS is utilized. The balance of the brine formula is NaCl, and the formulation is mixed with water to a solution of about 15% to about 17%.

[0057] In accordance with another embodiment of the present invention, tests were conducted to determine the antimicrobial efficacy of a salt formulation containing a surfactant but no added acid. The effect of an aqueous solution comprising about 20 wt.% of various salt and salt/surfactant formulations on *L. monocytogenes* were tested in a manner directly analogous to that set forth above in connection with the data in Table 8. Table 15 sets forth the compositions and the *L. monocytogenes* population (stated as the log of the concentration of the bacteria) found after 4 hours of incubation. [Note that the compositions in Table 15, below, state the concentration in the solution, not in the salt concentrate. Since the

solutions are 20 wt. % of the salt formulation, the concentration of surfactant in the salt formulation would be about five times the stated concentration in the solution.]

Table 15. Effect of Solutions of NaCl and SLS on *L. monocytogenes*

| Solution Composition | Population at 4 hours (log cfu/mL) |
|-----------------------------|---|
| Water Control | 4.61 |
| 20% NaCl (control) | 4.32 |
| 50 ppm SLS (no NaCl) | 3.90* |
| 50 ppm SLS, 20% NaCl | 0.85** |

* The 3.90 value is an average of two runs that yielded values of 3.84 and 3.95.

**The 0.85 value is an average of two runs that yielded values of 0.70 and 1.00.

[0058] Further tests were run on a variety of different surfactants, demonstrating that a variety of different types of surfactants show a strong synergistic effect in combination with salt: sodium lauryl sulfate, linear alkylbenzene sulfonates, alcohol sulfates, alkyl sulfates, alkyl sulfonates, sodium alkyl methyltaurines, alpha-olefin sulfonates, alcohol ethoxylates, nonylphenyl ethoxylates, alkylpolyglucosides, fatty alcohols, fatty acids and fatty acid salts, lignosulfonates and lignin derivatives, hydroxypoly(oxyethylene) derivatives, fatty alkanolamides, fatty amine oxides, sodium dioctylsulfosuccinate, dodecylbenzene sulfonic acid and salts thereof, the sodium salt of sulfonated oleic acid, sodium dodecylbenzene sulfonate, lauramine oxide, dodecyldiphenyloxide-disulfonic acid and salts thereof.

[0059] Further examples of surfactants that may be used in some embodiments of the present invention include alkyl (C8-C24) benzenesulfonic acid and its ammonium, calcium, magnesium, potassium, sodium, and zinc salts; alkyl (C8-C18) sulfate and its ammonium, calcium, isopropylamine, magnesium, potassium, sodium,

and zinc salts; diethylene glycol abietate, lauryl alcohol, lignosulfonate and its ammonium, calcium, magnesium, potassium, sodium, and zinc salts; nonyl, decyl, and undecyl glycoside mixture with a mixture of nonyl, decyl, and undecyl oligosaccharides and related reaction products (primarily decanol and undecanol) produced as an aqueous based liquid (50 to 65% solids) from the reaction of primary alcohols (containing 15 to 20% secondary alcohol isomers) in a ratio of 20% C9, 40% C10, and 40% C11 with carbohydrates (average glucose to alkyl chain ratio 1.3 to 1.8); α -(o,p-dinonylphenyl)- ω -hydroxypoly (oxyethylene) mixture of dihydrogen phosphate and monohydrogen phosphate esters and the corresponding ammonium, calcium, magnesium, monethanolamine, potassium, sodium, and zinc salts of the phosphate esters; the poly (oxyethylene) content averages 4-14 moles; α -(p-nonylphenyl)- ω -hydroxypoly (oxyethylene) mixture of dihydrogen phosphate and monohydrogen phosphate esters and the corresponding ammonium, calcium, magnesium, monethanolamine, potassium, sodium, and zinc salts of the phosphate esters, the poly (oxyethylene) content averages 4-14 moles or 30 moles; α -(p-nonylphenyl)- ω -hydroxypoly (oxyethylene) produced by the condensation of 1 mole nonylphenol with an average of 4-14 moles or 30-90 moles ethylene oxide; α -(p-nonylphenyl)- ω -hydroxypoly (oxyethylene) sulfate, ammonium, calcium, magnesium, potassium, sodium, and zinc salts; octyl and decyl glucosides mixture with a mixture of octyl and decyl oligosaccharides and related reaction products (primarily n-decanol) produced as an aqueous based liquid (68-72% solids) from the reactions of straight chain alcohols (C8 (45%), C10 (55%)) with anhydrous glucose; oxidized pine lignin and its salts thereof; β -pinene polymers; polyethylene glycol (α -hydro- ω -hydroxypoly(oxyethylene)); mean molecular weight of 194 to 9500 amu; α -(p-tert-Butylphenyl)- ω -hydroxypoly (oxyethylene) mixture of dihydrogen phosphate and

monohydrogen phosphate esters and the corresponding ammonium, calcium, magnesium, monethanolamine, potassium, sodium, and zinc salts of the phosphate esters; the poly (oxyethylene) content averages 4-12 moles; α -(o,p -dinonylphenyl)- ω -hydroxypoly (oxyethylene) produced by the condensation of 1 mole of dinonylphenol with an average of 4-14 or 140-160 moles of ethylene oxide; sodium or potassium salts of fatty acids; sodium α -olefinsulfonate (sodium C14-C16) (Olefin sulfonate); sodium diisobutyl naphthalene sulfonate and/or sodium .isopropylisohexyl naphthalene sulfonate; sodium dodecylphenoxybenzenedisulfonate; sodium lauryl glyceryl ether sulfonate; sodium oleyl sulfate; sodium N-lauroyl-N-methyltaurine, sodium N-palmitoyl-N-methyltaurine and/or sodium N-oleoyl-N-methyltaurine; sodium monoalkyl and dialkyl (C8-C16) phenoxybenzenedisulfonate mixtures containing not less than 70% of the monoalkylated products; 2,4,7,9-tetramethyl-5-decyn-4,7-diol; and/or nonylphenol ethoxylates with average moles of ethoxylation between 4 and 30.

[0060] Further, in other embodiments the surfactant may be one or more of the following alcohol ethoxylates: α -Alkyl (C9-C18- ω -hydroxypoly(oxyethylene) with polyoxyethylene content of 2-30 moles; α -(p-alkylphenyl)- ω -hydroxypoly(oxyethylene) produced by the condensation of 1 mole of alkylphenol (alkyl is a mixture of propylene tetramer and pentamer isomers and averages C13) with 6 moles ethylene oxide; α -Alkyl (C6-C14- ω -hydroxypoly(oxypropylene) block copolymer with polyoxyethylene; polyoxypropylene content is 1-3 moles; polyoxyethylene content is 4-12 moles; average molecular weight is approximately 635 amu; α -Alkyl (C12-C15- ω -hydroxypoly(oxypropylene) poly (oxyethylene) copolymers (where the poly(oxypropylene) content is 3-60 moles and the poly (oxyethylene) content is 5-80 moles; α -(p-Dodecylphenyl)- ω -

hydroxypoly(oxyethylene) produced by the condensation of 1 mole of dodecylphenol with an average of 4-14 or 30-70 moles ethylene oxide; ethylene oxide adducts of 2,4,7,9-tetramethyl-5-decynediol, the ethylene oxide content averages 3.5, 10, or 30 moles; α -Lauryl- ω -hydroxypoly(oxyethylene), sodium salt; the poly(oxyethylene) content is 3-4 moles; secondary alkyl (C11-C15) poly(oxyethylene) acetate salts; ethylene oxide content averages 5 moles; α -[p-1,1,3,3-tetramethylbutyl)phenyl-]- ω -hydroxypoly(oxyethylene) produced by the condensation of 1 mole of p-1,1,3,3-tetramethylbutylphenol with a range of 1-14 or 30-70 moles ethylene oxide; tridecylpoly(oxyethylene) acetate salts where the ethylene oxide content averages 6-7 moles; poly(oxy-1,2-ethanediyl), α -(carboxymethyl)- ω -(nonylphenoxy) produced by the condensation of 1 mole nonylphenol with an average of 4-14 or 30-90 moles ethylene oxide with a molecular weight in the ranges 454-894 and 1598-4238; and/or α -Stearoyl- ω -hydroxy(polyoxyethylene), polyoxyethylene content averages either 8, 9, or 40 moles.

[0061] In yet other embodiments, the surfactant may be selected from the group having the formula: $\text{CH}_3(\text{CH}_2)_{10}\text{-O}(\text{CH}_2\text{CH}_2\text{O})_y\text{H}$, where y = average moles of ethoxylation and is in the range of about 3-9.

[0062] In yet another embodiment of the present invention, it has been discovered that low concentrations of octanoic (caprylic) acid and its salts (such as sodium octanoate) are very effective at killing potential enteric bacterial pathogens and spoilage organisms (such as mold, yeast, and heterofermentative bacteria) in brines. Tests indicate that octanoic acid and its octanoate salts appear to have a surprising synergistic effect in combination with salt at killing microorganisms.

[0063] The present embodiment is particularly advantageous as it is an environmentally friendly antimicrobial solution, which has a variety of potential applications. In contrast, many of the most widely used antimicrobial chemicals such as quaternary ammonium compounds and chlorine derivatives, among others, are potentially harmful to the environment. Octanoic acid is a fatty acid which already has generally recognized as safe ("GRAS") status confirmed for several direct food applications, has very low toxicity, and would be readily biodegraded in the environment.

[0064] A method of use for this embodiment is an improved microbial control for brines used in the manufacture of certain cheese products. The manufacture of certain types of cheese (such as Gouda, Edam, Swiss, provolone, string cheese, Parmesan, Romano, Asiago, brie, camembert, brick cheese, muenster, and feta) involves soaking the cheese in a sodium chloride brine for a prolonged period. These brines often contain high organic loads and can support the survival of a variety of spoilage and pathogenic organisms. The presence of organisms in the brine can result in contamination of the cheese, leading to more rapid spoilage and potential pathogen contamination. There is not a good chemical antimicrobial treatment for cheese brines at the moment. Currently available chemicals are either too toxic to be used in contact with food or have undesirable side effects such as imparting off flavors to the cheese.

[0065] In one example according to the present embodiment, it is demonstrated that concentrations of about 250 ppm octanoic acid in blue cheese brine, gave ~ 3 log kill of coliform, yeast, and mold cultured from a cheese plant brine. At this concentration there was no detrimental effects on cheese flavor.

[0066] In the present example, cultures of various microorganisms native to actual process brines used in cheese manufacture were obtained from a cheese plant. The cultures of yeast, mold, and coliforms were grown and used to test the efficacy of various antimicrobial additives in blue cheese brines. Organism cultures included four different types of yeast (three distinct white yeast cultures and a pink yeast), a black mold culture, a coliform culture, a culture of mixed green mold and yeast that were not separated, and a culture that was identified by the cheese plant as heterofermentative lactic acid bacteria, although no actual identification of the strain was carried out.

[0067] Tests were performed by slightly different methods, depending on the organism. The test methods used for each type of organism are outlined below:

[0068] Coliforms procedure:

1. Inoculate a separate tube containing 10 ml of Brain Heart Infusion (BHI) broth for each wild type coliforms 1 and 2. Incubate for 24 +/- 2 hours at 35°C +/- 2°C.
2. Assume growth to be 10⁹ cfu/ml. Since two cultures are being used, combine 5.5 ml of each culture added to 99 ml of cold Butterfield's Phosphate Buffered Water (PBW) to obtain 10⁸ cfu/ml.
3. Plate serial ten-fold dilutions of the culture to get the starting count of the inoculum on Violet Red Bile Agar (VRBA).
4. Add 1 ml of the diluted cocktail to 100 ml of cold test solution.
5. Determine the population at time 0 and 4 hours by plating serial dilutions to VRBA spread plates.

6. Incubate the test solutions at $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the experiment.
7. Incubate the plates at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 72 \pm 2 hours.
8. Count representative colonies and multiply by the dilution factor.

Note: Each test solution should contain $\sim 10^6$ cfu/ml.

[0069] "Lactic bacteria" procedure:

1. Inoculate a separate tube containing 10 ml of Brain Heart Infusion (BHI) broth for each wild type lactics 1 and 2. Incubate for 72 \pm 2 hours at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
2. Assume growth to be 10^9 cfu/ml. Since two cultures are being used, combine 5.5 ml of each culture added to 99 ml of cold Butterfield's Phosphate Buffered Water (PBW) to obtain 10^8 cfu/ml.
3. Plate serial dilutions of the culture to get the starting count of the inoculum on Lactobacilli MRS Agar (MRSA).
4. Add 1 ml of the diluted cocktail to 100 ml of cold test solution.
5. Determine the population at time 0 and 4 hours. Plate serial dilutions using MRSA spread plates.
6. Incubate the test solutions at $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the experiment.
7. Incubate the plates at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 72 \pm 2 hours.
8. Count representative colonies and multiply by the dilution factor.

Note: Each test solution should contain $\sim 10^6$ cfu/ml.

[0070] "Mold" procedure:

1. From refrigerated stock mold cultures, take 1 ml from black mold dilution bottle added to 99 ml of cold Butterfield's Phosphate Buffered Water (PBW) to obtain 10^5 cfu/ml.
2. Take 1 ml from green mold/white yeast mixture dilution bottle added to 99 ml of cold Butterfield's Phosphate Buffered Water (PBW) to obtain 10^6 cfu/ml.
3. Plate serial dilutions of the cultures to get the starting count of the inoculum on Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC).
4. Add 1 ml of the diluted mold culture to 100 ml of cold test solution (do separately for each of the two mold culturers).

5. Determine the population at time 0 and 4 hours. Spread plate serial dilutions to DRBC agar.
6. Incubate the test solutions at $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the experiment.
7. Incubate the plates at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 5 days \pm 2 hours.
8. Count representative colonies and multiply by the dilution factor.

Note: For black mold, each test solution should contain $\sim 10^3$ cfu/ml. For green mold/white yeast mixture, each test solution should contain $\sim 10^4$ cfu/ml.

[0071]"Yeast" procedure:

1. Inoculate a separate tube containing 10 ml of Brain Heart Infusion (BHI) broth for each wild type (Raised, Rough and Flat white yeast along with a single pink yeast). Incubate for 5 days at $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$.
2. Assume growth to be 10^7 cfu/ml. Since 4 cultures are being used, combine 2.75 ml of each culture added to 99 ml of cold Butterfield's Phosphate Buffered Water (PBW) to obtain 10^6 cfu/ml of yeast cocktail.
3. Plate serial dilutions of the culture to get the starting count of the inoculum on Dichloran-Rose Bengal-Chloramphenicol Agar (DRBC).
4. Add 1 ml of the diluted cocktail to 100 ml of cold test solution.
5. Determine the population at time 0 and 4 hours. Spread plateserial dilutions to DRBC agar.
6. Incubate the test solutions at $10^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for the duration of the experiment.
7. Incubate the plates at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 5 days \pm 2 hours.
8. Count representative colonies and multiply by the dilution factor.

Note: Each test solution should contain $\sim 10^4$ cfu/ml.

[0072] Screening tests identified octanoic acid as a promising antimicrobial additive for salt brines. Table 16 gives results of a challenge test run on actual blue cheese and mozzarella process brine samples sent from a cheese plant. The brines were treated with different concentrations of octanoic acid and then challenged with a yeast cocktail (which included the pink and 3 different white mold types), the green mold + yeast combination, and coliforms. Table 16 shows organism concentrations

in the brines after 4 hours at 10⁰C. The plate counts listed parenthetically as (est) were too numerous to count and were estimated.

Table 16. Microorganisms Counts (cfu/mL) After 4 Hours in Blue Cheese Brine

| Solution | Yeast + Mold | Yeast Cocktail | Coliform |
|-----------------------|-----------------------|-----------------------|-----------------------------|
| Control (no additive) | 1.1 x 10 ⁶ | 1.3 x 10 ⁵ | 5.2 x 10 ⁶ (est) |
| 125 ppm octanoic acid | 20 | <10 | 10 |
| 125 ppm octanoic acid | 20 | <10 | 10 |
| 250 ppm octanoic acid | <10 | <10 | <10 |
| 500 ppm octanoic acid | <10 | <10 | <10 |

[0073] The data in Table 16 shows that 125 ppm octanoic acid in the brine results in killing almost the entire 5-6 log inoculum of yeast, mold, and coliforms in the brines. At 250 ppm octanoic acid the plate counts were consistently below the detection limit of the experiment. Contrast this with results of these same formulas prepared in a sample of plant mozzarella brine, shown in Table 17:

Table 17. Microorganisms Counts (cfu/mL) After 4 Hours in Mozz. Cheese Brine

| Solution | Yeast + Mold | Yeast Cocktail | Coliform |
|-----------------------|-----------------------|-----------------------|-----------------------------|
| Control (no additive) | 7.3 x 10 ⁵ | 5.8 x 10 ⁴ | 3.2 x 10 ⁶ (est) |
| 125 ppm octanoic acid | 4.1 x 10 ⁵ | 6.0 x 10 ³ | 3.0 x 10 ⁶ (est) |
| 250 ppm octanoic acid | 6.1 x 10 ⁴ | 200 | 1.7 x 10 ⁶ |

| | | | |
|-----------------------|-------------------|-----|-------------------|
| 500 ppm octanoic acid | 5.6×10^3 | <10 | 3.5×10^4 |
|-----------------------|-------------------|-----|-------------------|

[0074] The octanoic acid was less effective in mozzarella brine than in the blue cheese brine. It did appear to be quite effective against one of the yeast cultures, giving > 3.76 log kill at 250 ppm. However, at 500 ppm octanoic acid there was only a 2 log reduction in the yeast + mold and coliform counts. It is possible that the difference in effectiveness is due to the higher pH of the mozzarella brine.

[0075] In order to determine if the effect of the additive was repeatable, more challenge tests were run on samples of process brine. Table 18 shows results of another challenge study of plant blue cheese brine containing different levels of octanoic acid. In this test the solutions were challenged with two distinct yeast (a white and a pink) types and coliforms from the samples sent by the cheese plant.

Table 18. Microorganisms Counts (cfu/mL) After 4 Hours in Blue Cheese Brine

| Solution | White Yeast | Pink Yeast | Coliform |
|-----------------------|-------------------|-------------------|-------------------|
| Control (no additive) | 4.1×10^3 | 1.1×10^3 | 3.7×10^5 |
| 125 ppm octanoic acid | <10 | <10 | <10 |
| 250 ppm octanoic acid | <10 | <10 | <10 |
| 500 ppm octanoic acid | <10 | <10 | <10 |

[0076] In this experiment also the octanoic acid at levels of 125-250 ppm reduced the levels of yeast and coliform below the detection limit of the experiment. Contrast

again with results on formulas prepared in mozzarella brine which are given in Table 19:

Table 19. Microorganisms Counts (cfu/mL) After 4 Hours in Mozz Cheese Brine

| Solution | White Yeast | Pink Yeast | Coliform |
|------------------------|--------------------|-------------------|-------------------|
| Control (no additive) | 1.2×10^4 | 8.8×10^3 | 3.3×10^5 |
| 125 ppm octanoic acid | 60 | <10 | 1.6×10^4 |
| 250 ppm octanoic acid | <10 | <10 | 3.8×10^3 |
| 500 ppm octanoic acid | <10 | 20 | 3.8×10^3 |
| 1250 ppm octanoic acid | <10 | <10 | 1.2×10^3 |

[0077] This experiment shows the same basic behavior seen in the experiment summarized in Table 17. The octanoic acid appears to be effective at killing yeast in the brine, but is less effective in killing coliforms, giving again about a 2 log kill.

[0078] Another challenge test carried out in blue cheese brine is summarized in Table 20. In this experiment solutions were challenged with cultures of the black mold, the green mold/yeast combination, and the bacteria thought to be "lactic acid" bacteria. In this experiment, a brine control with no additive was neglected, but the data still indicate the strong effectiveness of the octanoic acid against yeast and mold.

Table 20. Microorganisms Counts (cfu/mL) After 4 Hours in Blue Cheese Brine

| Solution | Green Mold + Yeast | Black Mold | "Lactics" |
|------------------------|---------------------------|-------------------------|-------------------------|
| Innoculum | 5.8×10^6 (est) | 2.5×10^4 (est) | 1.2×10^7 (est) |
| 62.5 ppm octanoic acid | 1.0×10^4 | <10 | 1.5×10^5 |
| 125 ppm octanoic acid | <10 | <10 | 2.0×10^3 |
| 250 ppm octanoic acid | <10 | <10 | <10 |
| 500 ppm octanoic acid | <10 | <10 | <1 |

[0079] The data in Table 20 again show that octanoic acid is highly effective in blue cheese brine at killing mold, yeast, and the "lactic acid bacteria."

[0080] While the tests above focus on octanoic acid, tests have also shown that salts of octanoic acid such as sodium octanoate are also very effective antimicrobial additives in the presence of salt. Table 21 shows data from an experiment in which blue cheese process brine containing different levels of sodium octanoate was challenged with different organisms:

Table 21. Microorganisms Counts (cfu/mL) After 4 Hours in Blue Cheese Brine

| Solution | PinkYeast | White Yeast | Coliforms |
|----------------------------------|-------------------|--------------------|-------------------|
| Control (0 ppm sodium octanoate) | 1.1×10^3 | 4.1×10^3 | 3.7×10^5 |
| 62.5 ppm octanoic acid | < 10 | 90 | 3.8×10^4 |
| 125 ppm octanoic acid | < 10 | < 10 | < 10 |

| | | | |
|-----------------------|------|------|------|
| 250 ppm octanoic acid | < 10 | < 10 | < 10 |
| 500 ppm octanoic acid | < 10 | < 10 | < 10 |

[0081] The data in Table 21 demonstrate that sodium octanoate is also a very effective antimicrobial agent, even at low concentrations, in the presence of sodium chloride brine.

[0082] Test of Octanoic Acid Brine Formulas With Cheese

[0083] Once it had been determined that octanoic acid was consistently effective at killing microorganisms in the process brines used to produce blue cheese (and to a lesser extent also mozzarella cheese), it was next considered how the additives would perform under conditions more closely simulating actual cheese production conditions in the plant. Of particular interest was whether cheese soaked in the brine would absorb enough octanoic acid to cause any undesirable flavors and whether the presence of actual cheese in the brine would in any way affect the antimicrobial action of the octanoic acid.

[0084] The cheese plant carried out some small scale tests in which wheels of blue cheese were brined in 5 gallon samples of brine containing different levels of octanoic acid. Samples of brine taken before and after brining the cheese wheels were sent to Cargill for microbial challenge testing. The cheese wheels were cured for about a month by the usual plant production procedures and then taste tested. No off flavors were noted in cheese brined in solutions containing up to 250 ppm octanoic acid.

[0085] Brine samples taken before and after cheese brining were challenged with yeast, mold, and coliform cultures sent by the cheese plant. Results are given in Table 22.

Table 22. Microorganisms Counts (cfu/mL) After 4 Hours in Blue Cheese Brine

| Solution | Yeast Cocktail | Black Mold | Coliform |
|---------------------------------------|-------------------|-------------------|-------------------|
| Control | 3.5×10^3 | 1.0×10^3 | 3.4×10^4 |
| 250 ppm octanoic acid (before cheese) | <10 | <10 | <10 |
| 250 ppm octanoic acid (after cheese) | <10 | <10 | <10 |

[0086] The challenge test showed no significant decrease in the antimicrobial effectiveness of the brine against yeast, mold, and coliform after the cheese had soaked in it for 48 hours.

[0087] Synergistic Interaction of Sodium Octanoate and Salt

[0088] Challenge tests were run on solutions containing salt (sodium chloride) alone, sodium octanoate alone, and salt and sodium octanoate in combination. Brine used to make blue cheese often has a pH of approximately 4.7. In order to simulate the antimicrobial effectiveness at this pH, test solutions were prepared in a buffer of 375 ppm acetic acid and 525 ppm sodium acetate, which had a measured pH of 4.74.

Table 23. Microorganisms Counts (cfu/mL) After 4 Hours in Buffered Test Solutions

| Solution | Yeast Cocktail | Black Mold | Coliform |
|----------------|----------------|------------|----------|
| Buffer Control | 8,000 | 590 | 4,700 |
| 24% NaCl | 6,600 | 660 | 11,000 |

| | | | |
|---------------------------------|--------|------|--------|
| 35 ppm Na Octanoate | 9,000 | 710 | 7,000 |
| 35 ppm Na Octanoate + 24% NaCl | 1,300 | < 10 | < 10 |
| 70 ppm Na Octanoate | 7,500 | 510 | 12,000 |
| 70 ppm Na Octanoate + 24% NaCl | < 10 | < 10 | < 10 |
| 140 ppm Na Octanoate | 10,000 | 630 | 7,700 |
| 140 ppm Na Octanoate + 24% NaCl | < 10 | < 10 | < 10 |

[0089] The data in Table 23 indicate a strong synergistic effect between the sodium chloride and the octanoate in antimicrobial effectiveness. A low concentration (35 ppm) of sodium octanoate in the buffer solution alone has no apparent effect on the survival of the yeast, mold, and coliforms cultured from the cheese plant brine. The presence of 24% NaCl by itself in the buffer also has little effect on organism survival. Surprisingly, when the low concentration of octanoate is paired with NaCl in solution, a strong antimicrobial effect results. At a concentration as low 70 ppm, sodium octanoate in the presence of NaCl brine effectively reduced the inocula of yeast, mold, and coliform below the detection limit.

[0090] In some embodiments of the invention the formulation may comprise an inorganic salt and surfactant such that when in solution the solution comprises surfactant in a concentration of: at least about 5 ppm, about 5-5000 ppm, about 5-

500 ppm, about 10-25000 ppm, about 10-100 ppm, about 10-50 ppm, about 25-500 ppm, or about 500-1500 ppm.

[0091] Further, in other embodiments of the present invention, the ratio by weight of salt to surfactant may be greater than 29:1, greater than 1880:1, or greater than 1980:1.

[0092] In other embodiments of the current invention, solutions may comprise at least 2% of the dry composition, at least 5% of the dry composition, up to about 26% of the dry composition, between about 5% and 25% of the dry composition, between about 9% and 17% of the dry composition, or between about 12% and 15% of the dry composition.

[0093] Thus, the data indicate that embodiments of the present invention including solutions of salt and acid and/or surfactant provide efficient kill of bacteria even at temperatures below the freezing point of water. Salts such as sodium sulfate, sodium chloride, and magnesium sulfate act synergistically with the surfactant and/or acid to enhance the antimicrobial effectiveness. The formulations are shown to be effective in killing pathogenic bacteria such as *L. monocytogenes*. The formulas were shown to be effective both in freshly prepared brines and in actual spent process chill brine from a ready-to-eat meat plant. The levels of acid and/or surfactant may be varied to suit the particular application. In addition to effectively killing bacteria suspended in solutions, the some embodiments of the present invention are also shown to be effective at killing bacteria within a biofilm.

[0094] While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set

forth for purpose of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein can be varied considerably without departing from the basic principles of the invention.

CLAIMS

We claim:

1. An antimicrobial composition for use in solution comprising, in combination:

between about 25 ppm and about 100,000 ppm by weight surfactant wherein the surfactant comprises octanoic acid and salts thereof; and

between about 72.5% and 99.99% salt selected from the group consisting of sodium, potassium, magnesium, calcium, iron, and ammonium salts of chloride, sulfate, nitrate, phosphate, carbonate, acetate, formate, propionate, hydroxypropionate, and hydroxide.
2. The antimicrobial composition of claim 1 wherein the salt is sodium chloride.
3. The antimicrobial composition of claim 1 wherein the composition comprises, in combination:

between about 50 and about 10,000 ppm by weight octanoic acid; and

between about 90.0% and about 99.99% by weight sodium chloride.
4. The antimicrobial composition of claim 3 further comprising:

between about 50 and about 2,000 ppm by weight octanoic acid; and

between about 99.80% and about 99.99% by weight sodium chloride.
5. A method for food processing comprising:

formulating a food product solution containing a combination of a surfactant and a salt in an amount effective to kill microorganisms in the solution;

and immersing a food product to be processed in the solution;

the surfactant comprising octanoic acid or a salt thereof, and the salt being selected from the group consisting of sodium, potassium, magnesium, calcium, iron, and ammonium salts of chloride, sulfate, nitrate, phosphate, carbonate, acetate, formate, propionate, hydroxypropionate, and hydroxide.

6. The method for food processing as described in claim 5, wherein the surfactant is octanoic acid or a salt thereof, and the salt is sodium chloride.
7. An antimicrobial solution comprising on a dry basis:
between about 25 ppm and about 100,000 ppm by weight surfactant; and
between about 72.5% and 99.99% by weight salt,
wherein the surfactant is octanoic acid and salts thereof, and the salt is sodium chloride.
8. The antimicrobial solution of claim 7 wherein the surfactant and salt in combination comprise at least about 2% by weight of the solution.
9. The antimicrobial solution of claim 7 wherein the surfactant and salt in combination comprise about 9% to 26% by weight of the solution.
10. The antimicrobial solution of claim 7 wherein the solution comprises
between about 50 ppm and about 1000 ppm by weight surfactant; and
between about 94% and about 99.9% by weight sodium chloride.
11. An antimicrobial solution comprising:
a surfactant comprising octanoic acid or a salt thereof; and
a second salt,

wherein the ratio of the second salt to the surfactant is greater than about 29:1 by weight.

12. The antimicrobial solution of claim 11 wherein the second salt comprises sodium chloride.
13. The antimicrobial solution of claim 12 further comprising at least 50 ppm octanoate acid by weight.
14. The antimicrobial solution of claim 12 further comprising at least 125 ppm octanoate acid by weight.
15. The antimicrobial solution of claim 12 further comprising at least 250 ppm octanoic acid by weight.
16. The solution of claim 12 wherein the ratio of the second salt to the surfactant is greater than about 1880:1 by weight.
17. The solution of claim 12 wherein the surfactant and second salt in combination comprise at least about 2% by weight of the solution.
18. The solution of claim 12 wherein the surfactant and second salt in combination comprise at least about 5% by weight of the solution.
19. A method for food processing comprising:
contacting a food product to be processed with a solution wherein the solution comprises a combination of a surfactant comprising octanoic acid or a salt thereof and sodium chloride sufficient to substantially reduce a presence of microorganisms in the solution.

20. The method of claim 19 where the food product to be processed is cheese or a cheese product.
21. The method of claim 19 wherein the ratio of the sodium chloride to the surfactant of the solution is greater than about 29:1 by weight.
22. The method of claim 19 wherein the ratio of the sodium chloride to the surfactant of the solution is greater than about 1880:1 by weight.
23. The method of claim 19 wherein the surfactant and sodium chloride in combination comprise at least about 2% by weight of the chilling solution.
24. The method of claim 19 wherein the surfactant and sodium chloride in combination comprise at least about 5% by weight of the solution.
25. A method for food processing comprising:
contacting a food product to be processed with a solution,
wherein the solution comprises on a dry basis between about 25 ppm and about 100,000 ppm by weight octanoic acid or a salt thereof and between about 90.0% and 99.99% by weight sodium chloride, and wherein the food product to be processed is cheese or a cheese product.
26. The method of claim 25 wherein the surfactant and sodium chloride in combination comprise at least about 2% by weight of the solution.
27. The method of claim 25 wherein the surfactant and sodium chloride in combination comprise at least about 5% by weight of the solution.