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(54) Title: PRESERVATIVE METHOD

(57) Abstract: A method for producing a microbiologically stable and safe food composition is described. The method includes the step of mixing a food composition comprising an anionic polymer with a saturated preservative having an overall positive charge, whereby the saturated preservative is added in the last mixing step, in order to produce a food composition free of spoilage and pathogens.



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PRESERVATIVE METHOD

Field of the Invention

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The present invention is directed to a preservative method. More particularly, the present invention is directed to a method for preserving a food composition comprising an anionic polymer with a preservative system that includes a saturated preservative having an overall positive charge, whereby the saturated preservative is added in the last mixing step, in order to produce a food composition free of spoilage and pathogens, i.e., that is microbiologically safe and stable.

Background of the Invention

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Preservatives, like sorbate, benzoate and organic acids have been used in food products. Such preservatives offer a degree of microbiological inhibition. However, conventional preservative systems, in order to be effective, require the presence of organic acids, low pH values, or both in order to achieve microbiological stability across a wide range of food compositions. While high levels of organic acid and/or low pH values can contribute to the stability of edible products, the use of the same almost invariably results in food compositions having inferior taste, olfactory and visual characteristics.

25

It is of increasing interest to develop a preservative system that may be used across a wide range of food compositions, especially ambient stable and chilled-food compositions that utilize anionic polymeric thickening agents to replace some or all of the oil or fat in the system. This invention, therefore, is directed to a method for preserving a food composition with a preservative system comprising a saturated preservative having an overall positive charge.

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The method of this invention, unexpectedly, results in a microbiologically ambient stable food composition in the absence of organic acids. The method of this invention also, surprisingly, results in microbiologically safe chilled-food compositions, even at elevated pH values. Moreover, the method of this invention does not adversely impact the taste, olfactory and visual characteristics of the food compositions comprising the above-described preservative system.

In International Publication WO 03/094638, preservative and protective systems derived from lauric acid and arginine are described. This reference recognizes the phenomenon of precipitation of anionic hydrocolloids with LAE, a compound derived from lauric acid and arginine, which is an ethyl ester of the lauramide of arginine monohydrochloride. The present invention addresses this undesired interaction when LAE and anionic thickening components are combined and intimately mixed into a food composition.

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Additional Information

Efforts have been disclosed for making preservative systems. US Published Patent Application No. 2006/0177548 describes a method of producing a microbiologically stable and safe food composition.

20

Other efforts have been disclosed for making preservative systems. In International Publication WO 03/013454, preservative systems for cosmetic preparations are described.

25

Even other efforts have been disclosed for making microbiologically stable food compositions. In U.S. Patent No. 6,036,986, cinnamic acid for use in tea-containing beverages is described.

None of the additional information above describes a method for using a saturated preservative having an overall positive charge with an anionic thickening polymer effective for use and co-mixing across a wide range of food compositions to render the same microbiologically stable and safe.

Summary of the Invention

In a first aspect, the present invention is directed to a method for preserving a food composition comprising:

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providing a food composition comprising an anionic polymer;
mixing said food composition with a preservative system comprising:

(a) about 20 ppm to about 200 ppm of said food composition of a cationic
10 saturated preservative having an overall positive charge;

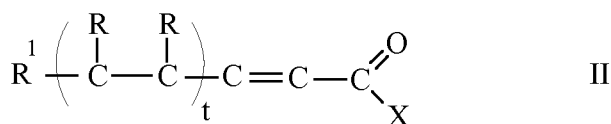
(b) optionally, from about 0.015 percent to about 0.500 percent by weight of
said food composition of a second preservative component;

15 wherein said saturated preservative having an overall positive charge is
added to the food composition in the last mixing step;

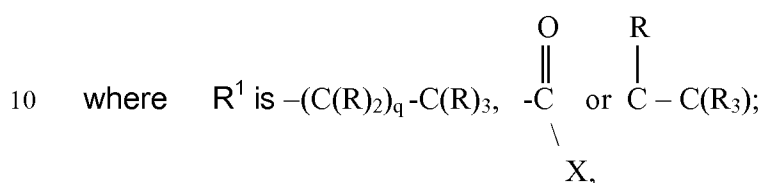
thereby rendering said food composition microbiologically safe and stable. In
particular, the food composition displays no outgrowth of *Lactobacilli*, yeast and
20 mold for at least three (3) months before opening and when kept at a
temperature of 25°C and at a pH of less than 4.2, or for at least (4) weeks before
opening when kept at a pH of less than 6 at a temperature of 5°C, and prevents
the outgrowth of pathogens, and achieves at least a 2 log decline of pathogens
within about a seven (7) day period when kept at a pH from 3.0 to less than 5.0.

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The second preservative component second preservative component may
be a polyene macrolide antibiotic; or a compound having the formula II:



5



$X^{(-)}$ is: a monohydrohalide, preferably chloride (Cl^-);

15 R is independently a C_1 - C_4 alkyl or hydrogen;

q is 0 to 12, and t is from 0 to 6, with the proviso that when R^1 forms part of an sp^2 hybridized carbon-carbon bond, t does not equal zero; most preferably sorbic acid. Further, aromatic preservatives suitable for use in this invention include, benzoic acid, coumaric acid, salicylic acid, vanillic acid, caffeic acid, cinnamic
20 acid, ferulic acid, salts thereof, derivatives thereof, mixtures thereof. The second preservative component may also include acetic, propanoic, 2-hydroxypropanoic (lactic), butyric, propionic, phosphoric, valeric, adipic, gluconic, malic, citric, tartaric, ascorbic, carnosic acid or a mixture thereof.

25 Food composition, as used herein, means a composition suitable for consumption by humans, including a filling, dip, soup, sauce, spread, dressing, refrigerated salad, batter or beverage.

Microbiologically stable (i.e., spoilage free) means no outgrowth of
30 spoilage bacteria, yeast and/or mold and no flavor loss for at least about three (3) months, and preferably, for at least about ten (10) months before opening when kept at about $25^\circ C$ and at a pH of less than about 4.2. When chilled,

microbiologically stable means no outgrowth of spoilage bacteria, yeast and/or mold and no flavor loss for at least about four (4) weeks, and preferably, for at least about six (6) weeks before opening when kept at about 5°C and a pH of less than 6.0.

5

Microbiologically safe (for products kept at about 25°C and 5°C) means preventing the outgrowth of pathogens and achieving at least about a 2 log die off of pathogens (like *Listeria monocytogenes*) within a fourteen (14) day period (preferably a seven (7) day period) when kept at a pH from about 3.0 to less than

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6.0.

Cationic Saturated Preservative

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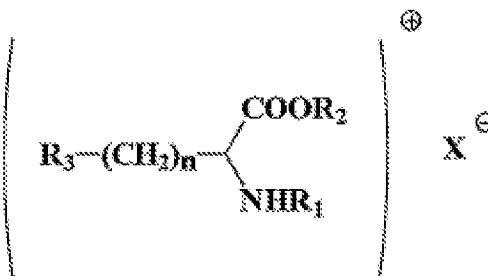
There is no limitation as to the saturated preservative, which includes cationic compounds including but not limited to quaternary compounds. Preferably, the saturated preservative used in this invention is suitable for human consumption, and preferably, has a pK_a of under about 5.0. Saturated cationic preservative is used in the food compositions in amounts of about about 20ppm to about 200 ppm.

20

Illustrative examples of the type of cationic saturated preservatives suitable for use in this invention include those having the formula I:

(I)

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Where:

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R₁ is: a linear or branched alkyl chain from a saturated fatty acid or a saturated fatty hydroxy acid containing 8 to 14 carbon atoms bonded to the alpha-amino acid group through an amidic bond;

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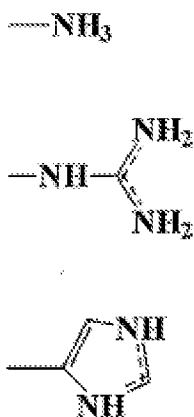
R₂ is: a linear or branched alkyl group containing 1 to 4 carbon atoms;

X⁽⁻⁾ is: a monohydrohalide, preferably chloride (Cl⁻);

R₃ is: a structure of formula Ia

15

(Ia)



n is: from 1 to 4.

In a most preferred embodiment, the cationic saturated preservative is derived from lauric acid and arginine and is an ethyl ester of the lauramide of arginine monohydrochloride (LAE), whereby a more detailed description of the same may be found in U.S. Patent Application No. 2004/0265443 A1.

Anionic Polymer

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An anionic polymer is necessary in the food compositions of the present invention for mouthfeel. These are generally classified as thickening agents or gums. Thickening agents derived from cellulose may also be employed and they include carboxymethylcellulose, sodium carboxymethylcellulose, and mixtures of these polymers. The anionic polymer may have sulphate or, preferably, carboxylate groups. Although not limited thereto, preferably, the anionic polymer is xanthan gum or pectin, more preferably food grade xanthan gum.

15

Typically, anionic polymers make up from about 0.05 to about 1.0%, and preferably, from about 0.1 to about 0.75%, and most preferably, from about 0.125 to about 0.35% by weight of the total weight of the food composition, including all ranges subsumed therein.

20

Xanthan Gum

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Xanthan (otherwise called xanthan gum) is a microbial exopolysaccharide produced by the naturally occurring bacterium *Xanthomonas campestris*. It is a widely used biopolymer in the food and pharmaceutical industries. It is also used in many other fields such as petroleum production, pipeline cleaning, enhanced

oil recovery, textile printing and dyeing, ceramic glazes, slurry explosives and in cosmetics. It is used for the purposes of thickening, suspending, stabilizing and gelling.

5 Xanthan consists of a pentasaccharide repeating subunit. It consists of two D-glucopyranosyl units, two D-mannopyranosyl units and a D-glucopyranosyluronic acid as determined by methylation analysis and uronic acid degradation. The molecule has a (1 → 4) linked β-D- glucopyranosyl backbone as found in cellulose, with a trisaccharide side-chain attached to the O-
10 3 position on alternate glucosyl units. The side chain is constructed such that the D-glucuronosyl unit is flanked by the two mannosyl units. Approximately half of the terminal D-mannosyl units have a pyruvic acid moiety across the O-4 and O-6 positions. The other D-mannosyl unit is substituted at the O-6 position with an acetal group. Xanthan is available readily as the sodium or potassium salt, or as
15 mixtures of sodium, potassium or calcium salts. Xanthan has been estimated to have a molecular weight between 2-50 X 10⁶. This wide range of values is believed to be due to polymer chain association.

Alginate

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Another anionic polymer may be an alginate. Alginates may be found in and isolated from various organisms, in particular from algae belonging to the order *Phaeophyceae* and soil bacteria such as *Azotobacter vinelandii* and *Azotobacter crococcum* and from several strains of *Pseudomonas* bacteria.
25 Common algal sources of alginates include *Laminaria digitata*, *Ecklonia maxima*, *Macrocystis pyrifera*, *Lessonia nigrescens*, *Ascophyllum nodosum*, *Laminaria japonica*, *Durvillea antartica*, *Durvillea potatorum* and, especially, *Laminaria hyperborea*.

Alginic acid is a linear hetero-polysaccharide comprising units of β -D-mannuronic acid and α -L-guluronic acid. Alginic acid may comprise homopolymeric sequences of mannuronic acid, homopolymeric sequences of guluronic acid, and mixed sequences of mannuronic aid and guluronic acid units.

5

Salts of alginic acid used in the method of the present invention may include alkali metal salts, for example sodium and potassium salts, and ammonium and alkanolamine salts.

10

Preferred are water-swellaable, preferably water soluble, salts of alginic acids. Most preferably they are provided as solutions, substantially without precipitates therein.

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The term "alginates" as used herein includes salts of alginic acid, irrespective of the relative proportion of mannuronic and guluronic units, and is intended to include glycolated or alkoxyated derivatives, especially those derivatised with propylene glycol. However, preferred compounds are not alkoxyated or glycolated. Guluronic acid-rich alginic acid and guluronic acid-rich alginates are of particular interest.

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Insoluble Fibers

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Regarding insoluble fibers suitable for use in this invention, such fibers are found, for example, in fruits, both citrus and non-citrus. Other sources of the insoluble fibers suitable for use in this invention are vegetables like legumes, and grains. Preferred insoluble fibers suitable for use in this invention can be recovered from tomatoes, peaches, pears, apples, plums, lemons, limes, oranges, grapefruits or mixtures thereof. Other preferred insoluble fibers suitable for use in this invention may be recovered from the hull fibers of peas, oats, barley, mustard, soy, or mixtures thereof. Still other fibers which may be

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employed include those that are plant or root-derived as well as those which are wood-derived. Typically, the food compositions, and particularly dressing compositions, of this invention comprise from 0.0 to about 3%, and preferably, from about 0 to about 2% by weight insoluble fibers, based on total weight of the food composition, and including all ranges subsumed therein. Such insoluble fibers are available from suppliers like J. Rettenmaier and Sohne GMBH under the Vitacel name and Herbstreith & Fox under the Herbacel name. These insoluble fibers typically have lengths from about 25 to about 400 microns, and preferably, from about 50 to 185 microns, and most preferably, from about 100 to about 165 microns, including all ranges subsumed therein. The widths of such fibers are typically between about 3.0 to about 20.0 microns, and preferably, from about 5.0 to about 10.0 microns. It is also within the scope of this invention for the insoluble fiber used to be supplied with from about 0 to 15% by weight soluble fiber, based on total weight of insoluble fiber and soluble fiber and including all ranges subsumed therein.

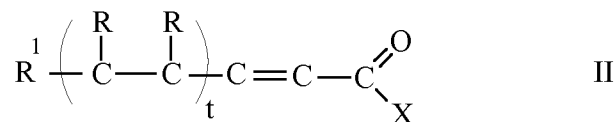
Optional Preservatives

As to the optional (but often preferred) second preservative component,
 5 the same is limited only in that it may be employed in food compositions suitable
 for human consumption, and preferably, has a pK_a of under about 5.5. The
 second preservative component is used in the food compositions in amounts of
 about 0.0 % to about 0.500 %, preferably about 0.015 to about 0.200, more
 preferably about 0.100 to about 0.200 % by weight of the food composition.

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Illustrative examples of unsaturated preservatives suitable for use in this
 invention as a second preservative component include those classified as a
 polyene macrolide antibiotic, as well as those having the formula:

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where R¹ is $-(C(R)_2)_q - C(R)_3$, $-\begin{array}{c} O \\ // \\ C \\ \backslash \\ X \end{array}$ or $\begin{array}{c} R \\ | \\ C - C(R)_3 \end{array}$, and R and X are as previously defined,

20

R is independently a C₁-C₄ alkyl or hydrogen, preferably hydrogen, q is 0 to
 about 12, and t is from 0 to about 6, with the proviso that when R¹ forms part of
 an sp² hybridized carbon-carbon bond, t does not equal zero. In a most
 25 preferred embodiment, the unsaturated preservative is a polyene macrolide
 antibiotic like natamycin (or pimaricin), a compound represented by II, like sorbic
 acid, propenoic acid, 2-hexenoic acid, fumaric acid, or a mixture thereof.

Regarding further optional (but often preferred) second preservative components, aromatic preservative preferably has a pK_a of under about 5.0 and is water soluble. Illustrative and non-limiting examples of the aromatic preservatives suitable for use in this invention include, benzoic acid, coumaric acid, salicylic acid, vanillic acid, caffeic acid, cinnamic acid, ferulic acid, salts thereof, derivatives thereof, mixtures thereof. Normally, in order to exert an antimicrobial effect in the absence of other antimicrobial agents, at least about about 0.050 to about 0.200% by weight aromatic preservative is used as an additive.

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The second preservative component may also include acetic, propanoic, 2-hydroxypropanoic (lactic), butyric, propionic, phosphoric, valeric, adipic, gluconic, malic, citric, tartaric, ascorbic, carnosic acid or a mixture thereof.

15

The total weight of preservative system employed in the food composition of this invention is limited only to the extent that the resulting food composition is microbiologically stable and safe as defined herein. Typically, however, the food compositions made via the method of this invention have from about 0.002 to about 1.5, and preferably, from about 0.005 to about 0.4, and most preferably, from about 0.01 to about 0.30 percent by weight preservative system (as pure preservative), based on total weight of food composition and including all ranges subsumed therein.

20

Method

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Applicants have discovered an optimized method of preparing reduced oil food formulations in order to achieve maximum anti-microbial effect from the saturated preservative having an overall positive charge. Note, reduced oil food formulations require the use of thickening agents. In the process according to the present invention, the saturated preservative having an overall positive

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charge is added last to the formulation. In other words, the formulation including anionic polymeric thickening agents (e.g. gums) is mixed first, followed by a last step of addition of the saturated preservative having an overall positive charge.

5 Without wishing to be bound by theory, Applicants believe that reserving cationic saturated preservative at the end permits the anionic sites on the anionic polymer, i.e. that would bind and/or precipitate the cationic preservative making it ineffective, to be taken up by other cations present in the system, including by not limited to hydrogen, sodium, potassium, calcium, and magnesium.

10

 When conducting the method of this invention, components of the preservative system other than the saturated preservative can be combined with ingredients to make a food composition or combined with a food composition having already been prepared whereby combined is meant to optionally include marinating. Surprisingly, and again, when conducting the method of this
15 invention, a food composition, like a filling, dip, sauce, spread, dressing, beverage or the like, is rendered microbiologically safe and stable in the absence of additional preservatives and at elevated pH values.

20 The food compositions made via the method of this invention, unexpectedly, are not sour even when the same are formulated to have a pH below 4.20. Such food compositions can comprise meat, fish, crustaceans, poultry products, bread crumbs, vegetables (including chunks and puree), protein, wheat, sweeteners (including sugar and artificial sweeteners), oil,
25 emulsions, fruit (including chunks and puree), cheese, nuts, mixtures thereof or the like.

 Illustrative and non-limiting examples of preferred food compositions prepared via the method of this invention include pourable dressings, fruit based

compositions and mayonnaise comprising salads like coleslaw, tuna, macaroni, and chicken salad.

Most preferred compositions according to the present invention are
5 pourable dressings and mayonnaise type dressings with reduced oil levels of about 65 % or less. The relatively low oil content of such dressings requires use of thickening agents in the formulation. Most effective thickening agents are comprised of molecules having an overall anionic charge, such as soluble fibers, insoluble fibers and gums. Preferred among these are xanthan gum and citrus
10 fibers.

Preferred food compositions can also comprise starches, cellulose, vitamins, chelators, buffers, antioxidants, colorants, acidulants (including inorganic acids), emulsifiers, alcohol, water, spices (including salt), syrups, milk,
15 food grade dispersants or stabilizers (like propylene glycol alginate), solubilizing agents (like propylene glycol), milk powder or mixtures thereof.

The packaging suitable for use with the food compositions made according to this invention is often a glass jar, food grade sachet, a plastic tub or
20 squeezable plastic bottle. Sachets are preferred for food service applications, a tub is preferred for spreads and a squeezable plastic bottle is often preferred for non-spreads and domestic use.

The following examples are provided to illustrate an understanding of the
25 present invention. The examples are not intended to limit the scope of the claims.

Example 1

Avocado-based compositions were made by mixing the following ingredients:

TABLE 1

	A. <u>Ingredient-Oil Phase</u>	<u>Weight Percent of Formula</u>
5	Soybean oil	18.6
	Polysorbate 60	0.3
10	B. <u>Ingredient-Fiber Phase</u>	<u>Weight Percent of Formula</u>
	Water	43.1
	Sorbic Acid	0.10
	Citrus fiber	2.60
	Potato starch	1.00
15	Milk powder	0.75
	Gums	0.21
	Corn syrup	11.13
	EDTA	0.007
	Color	0.075
20	Sugar	1.00
	Salt	1.02
	C. <u>Ingredient-Intermediate Mix</u>	<u>Weight Percent of Formula</u>
25	Fiber phase	61.0
	Oil phase	18.9
	Avocado flesh	19.7
	Hydrochloric acid	0.34
	Propylene glycol	0.045
30	Natamycin	<u>0.0004</u>
	D. <u>Ingredient-Final Mix</u>	<u>Weight Percent of Formula</u>
	LAE	<u>0.005</u>
35		100.0

Ingredients of the oil and fiber/intermediate phases were combined and mixed under moderate shear at atmospheric pressure and ambient temperature in a conventional mixer to produce a coarse emulsion. The coarse emulsion was then subjected to a homogenizer (e.g., APV Gaulin Homogenizer) pressurized to

about 250 bar. The resulting emulsion was combined with the ingredients in the final mix to produce an avocado-based composition. The same was then subjected to a votator for about three (3) minutes at 75°C resulting in an avocado-based composition having a pH of about 3.5.

5

Example 1A

Avocado-based compositions (pH ~ 3.5) were made in a manner similar to the one described in Example 1 except that LAE was added in the intermediate
10 mix in lieu of the final mix.

Example 1B

Avocado-based compositions (pH ~ 3.5) were made in a manner similar to
15 the one described in Example 1 except that 0.0005% by weight of nisin was used in lieu of LAE.

Table 2

	APRY ⁱ	LBL ⁱⁱ	LBH ⁱⁱⁱ
Example 1	N	N	N
Example 1A	Y	N	Y
Example 1B	Y	N	N

i = Acid preservative resistant yeast; initial inoculation about 100 cfu/g

20 ii = Lactobaccilli low; initial inoculation about 100 cfu/g

iii = Lactobaccilli high; initial inoculation about 1000 cfu/g

N = no growth; Y = growth

Cfu = colony forming unit

25 Table 2 shows the results of a stability/spoilage challenge study for the avocado-based compositions made in Examples 1, 1A, and 1B. The avocado-based composition of Example 1 was made in a manner consistent with the

invention described herein. Surprisingly, no outgrowth of spoilage yeast and bacteria was observed for at least 3 months at the identified inoculation levels. Example 1A, an avocado-based composition with LAE added together with the fiber, shows the growth of yeast and bacteria within a three month period.

5 Example 1B, an avocado-based composition with sorbic acid, nisin and natamycin, shows yeast growth within three months notwithstanding the presence of natamycin as an antifungal agent. The results show that food compositions are unexpectedly microbiologically stable and safe when subjected to the method of this invention.

10

Example 2

A blue cheese dressing having a pH of about 3.8 was made by mixing the following ingredients, with LAE being mixed last:

15

TABLE 3

	<u>Ingredient</u>	<u>Weight Percent of Formula</u>
	Water	Balance
	Soybean Oil	43.0
20	Vinegar (10%)	6.01
	NaCl	2.00
	Lactic acid (88%)	0.372
	Flavor	0.44
	Polysorbate 60	0.22
25	Vitamin	0.005
	Cheese crumbs	12.0
	Sucrose	1.96
	Dispersant	0.174
	Potassium sorbate	0.10
30	Garlic Powder	0.10
	EDTA	0.007
	Xanthan Gum	0.70
	Propylene glycol	0.045
35	LAE	0.005

Example 2A (Comparative)

The blue cheese dressing of this Example was made in a manner similar to the one described in Example 2, above, except that LAE was added together with all
5 the ingredients, rather than at the end.

A spoilage study was conducted on the blue cheese dressings of Examples 2 and 2A. The dressing composition of Example 2, made in a manner consistent with this invention, showed no outgrowth of acid preservative resistant yeast and
10 *Lactobacilli* at low and high initial inoculation levels (i.e., about 50 cfu/g and 5,000 cfu/g, respectively). The dressing composition of Example 2A displayed growth of spoilage yeast and *Lactobacilli* bacteria within one (1) week.

Example 3

15

Compositions were made by mixing the ingredients in Table 1 above, except that LAE and sorbic acid amounts were varied.

LAE was added at 0.001 weight percent of the formula and xanthan gum
20 at 0.21 %, and sorbic acid level was varied, as well as pH. Water was added as a BALANCE so that all the ingredients in the formulation add to 100.0%. This example explores the order of addition of LAE with and without presence of xanthan gum, and at different pH.

25 Ingredients of the oil and fiber/intermediate phases were combined and mixed under moderate shear at atmospheric pressure and ambient temperature in a conventional mixer to produce a coarse emulsion. The coarse emulsion was then subjected to a homogenizer (e.g., APV Gaulin Homogenizer) pressurized to about 250 bar. The resulting emulsion was combined with the ingredients in the
30 final mix to produce an avocado-based composition. The same was then

subjected to a votator for about three (3) minutes at 75°C resulting in a guacamole composition.

5 When LAE was mixed along with the other ingredients, either with or without xanthan gum, rather than as the final mix, the composition was microbiologically unstable. Sorbic acid was at 0.10 % and pH was about 3.6. Specifically, lactobacilli and APRY yeast levels became unacceptably high.

10 When LAE was mixed along with the other ingredients, without xanthan gum, rather than as the final mix, with pH of about 3.4 and sorbic acid at 0.19 %, the composition was microbiologically stable. Specifically, lactobacilli and APRY yeast levels were acceptable over a period of 8 weeks, i.e., no spoilage.

15 When LAE was mixed in last, with xanthan gum added earlier in the composition, with pH of about 3.47 and sorbic acid at about 0.15%, the composition was microbiologically stable. Specifically, lactobacilli and APRY yeast levels were acceptable over a period of 7 weeks, i.e., no spoilage. Moreover, no spoilage was seen when LAE amount was reduced to 0.00075 and
20 sorbic acid level was reduced to 0.10 % at about the same pH, thereby showing the favorable effect of mixing in LAE as a last step.

Example 4

25 Chicken salad compositions (pH ~ 4.7) were made by combining the following ingredients, with LAE added as a last mixing step:

TABLE 4

	<u>Ingredient</u>	<u>Weight Percent of formula</u>
	Water	Balance
	LAE	0.015
5	Propylene glycol	0.135
	Potassium sorbate	0.100
	Sodium benzoate	0.100
	Onion	6.00
	Celery	14.50
10	Salt	0.120
	Sugar	2.20
	Black Pepper	0.10
	Xanthan Gum	0.20
	Bread Crumbs	3.00
15	HELLMANN'S brand Mayonnaise	24.4
	Phosphoric acid	0.79
	Chicken	48.00

Storage studies of the same indicated no yeast or bacteria outgrowth for at
 20 least seven (7) weeks, even at temperatures of about 7°C. Safety studies also
 indicated at least a 2 log decline in pathogenic (*Listeria monocytogenes*) levels in
 about seven (7) days at 5°C, 7°C and 10°C. In the control, in which LAE was
 omitted, lactic acid bacteria and yeast spoilage took place at between two (2) and
 four (4) weeks at 10°C and 7°C, respectively. There was no decline in *Listeria*
 25 *monocytogenes* counts at 5°C and 7°C, and outgrowth took place at between
 four and five weeks at 10 °C.

Example 5

The following is the guacamole formula and ingredient order of addition
 30 that were used for this example, which studies the effect of pH and order of LAE
 addition on microbial stability:

Water, corn syrup, dry ingredients (includes citrus fiber and xanthan gum), oil
 phase (soybean, trans free cookie bake, polysorbate), salt. The base is
 35 homogenized and the following ingredients are added: acidified avocado,

tomatillos, garlic puree, lime juice, green note flavor, cilantro, and HCl (to adjust the pH to about 3.4). This mixture goes through the votator at 175 F. The following ingredients are added after the votator: salsa, green chilies, cumin. LAE is added last.

TABLE 5. Guacamole Base Formula

	Base Wt.
<u>OIL PHASE</u>	%
POLYSORBATE 60	0.1950
VEGETABLE OIL	18.5441
SUB-TOTAL	18.7391
<u>FIBER PHASE:</u>	
WATER	21.7589
CITRUS FIBER	1.7964
XANTHAN GUM	0.1678
SUGAR	0.1540
CORN SYRUP	9.2384
SALT	1.6680
EDTA	0.0072
SORBIC ACID	0.1000
COLOR	<u>0.0616</u>
SUB-TOTAL	34.9523
<u>FINAL MIX</u>	%
BASE	53.6914
AVOCADO PUREE	14.2455
TOMATILLO (green tomato)	13.9860
DEHYDRATED ONION	0.7617
FROZEN GARLIC PUREE	0.3608
CONCENTRATED LIME JUICE	0.0113
SPICES and FLAVORINGS	1.5033
GREEN CHILI PEPPER	4.9306
10% LAE in propylene glycol	0.1000
SALSA	9.4095
RED PEPPER, DEHYDRATED	1.0000
TOTAL	<u>100.0000</u>

Examples 5A and 5B

This example shows the combined effect of pH, acid levels, LAE levels, as well as order of addition.

5

Studies "550-551" and "571-574" are summarized in the tables below. Here, xanthan gum is seen as a "quenching agent". Also studied were the impact of pH (~3.3 vs 3.5) and a sorbic acid increase (from 0.1 to approximately 0.2%), i.e. file "550-551" where xanthan gum was omitted from both variables, and then "order-of-addition" and variations in LAE and sorbic acid concentration, i.e. studies "571-574" (where LAE was added at the end of the batching process).

10

TABLE 6

Formula	pH	Sorbic %	LAE (ppm)	Xanthan	Acid	Post dose acid
#551	3.54	0.1944	100	No	Phosphoric	Yes
#550	3.36	0.1944	100	No	Phosphoric	Yes
#571	3.40	0.1500	75	Yes	HCl	No
#572	3.40	0.1000	75	Yes	HCl	No
#573	3.40	0.1500	100	Yes	HCl	No
#574	3.40	0.1000	100	Yes	HCl	No

TABLE 7. 75 vs 100 ppm LAE; 0.1 vs 0.15% Sorbic Acid at pH 3.4

Initiated on Day 0

#571

Guacamole Formula 2.1.6 75 ppm LAE Sorbic Acid 0.15% pH 3.46	Days	0	7	14	28	42	56	70
	Calculated Inoculum	0.0	1.0	2.0	4.0	6.0	8.0	10.0
	Lactics Hi	3,420	9,000	38,600	99	9	9	9
	Lactics Lo	34	10	9	900	9	81	10
	APRY Hi	15,000	1,060	340	21,200	26,800	36,000	29,000
	APRY Lo	150	10	10	9	9	150	15,120

#572

Guacamole Formula 2.1.7 75 ppm LAE Sorbic Acid 0.10% pH 3.48	Days	0	7	14	28	42	56	70
	Calculated Inoculum	0.0	1.0	2.0	4.0	6.0	8.0	10.0
	Lactics Hi	3,420	6,700	9	900	65,800	59,000	57,000
	Lactics Lo	34	90	9	9	9	9	9
	APRY Hi	15,000	13,000	110,000	370,000	360,000	92,000	122,000
	APRY Lo	150	90	2,560	134,400	97,000	88,000	73,000

#573

Guacamole Formula 2.1.8 100 ppm LAE Sorbic Acid 0.15% pH 3.4	Days	0	7	14	28	42	56	70
	Calculated Inoculum	0.0	1.0	2.0	4.0	6.0	8.0	10.0
	Lactics Hi	3,420	7,800	9	60	9	9	9
	Lactics Lo	34	10	9	60	9	9	9
	APRY Hi	15,000	10,300	680	9	33	2,460	86,000

		150	100	40	9	9	9	9	9	9
APRY Lo										
#574 Guacamole Formula 2.1.9 100 ppm LAE Sorbic Acid 0.10% pH 3.4	Calculated Inoculum		0	7	14	28	42	56	70	
	Days		0.0	1.0	2.0	4.0	6.0	8.0	10.0	
	Weeks		3,500	1,440	24,080	9	9	9	9	9
	Lactics Hi	3,420								
	Lactics Lo	34	30	70	9	9	9	9	9	9
	APRY Hi	15,000	12,600	9,900	179,200	136,000	284,000	208,000	2,340,000	
APRY Lo	150	100	830	115,920	74,000	122,000	123,200	81,000		

In samples 550 and 551, where xanthan gum was omitted, there was no significant increase in lactic acid bacteria or APRY yeast after twelve (12) weeks. A significant increase would be an increase of equal to or greater than 2 logs.

5

In samples 571 and 572, at LAE usage level of 75 ppm, there was a significant increase in APRY yeast levels and lactic acid bacteria levels, whether or not the sorbic acid level was 0.15 and 0.10 %.

10

In samples 573 and 574, at LAE usage level of 100 ppm, the product was stabilized at the high and low lactic inoculum levels and at low APRY levels. (The low inoculum levels are expected at good GMP plants.)

Claims

1. A method for preserving a food composition comprising:
providing a food composition comprising an anionic polymer;
mixing said food composition with a preservative system comprising:

- (a) 20 ppm to 200 ppm of said food composition of a cationic saturated preservative having an overall positive charge;
- (b) optionally, from 0.015 percent to 0.500 percent by weight of said food composition of a second preservative component;

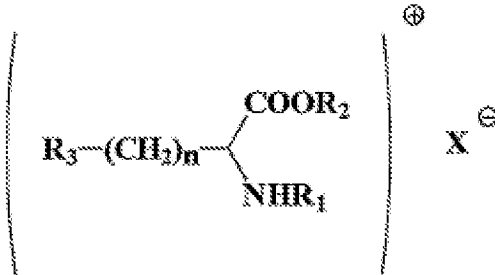
wherein said saturated preservative having an overall positive charge is added to the food composition in the last mixing step;

thereby rendering said food composition microbiologically safe and stable.

2. The method of claim 2, wherein the food composition displays no outgrowth of *Lactobacilli*, acid preservative resistant yeast and mold for at least three (3) months before opening and when kept at a temperature of 25°C and at a pH of less than 4.2, or for at least (4) weeks before opening when kept at a pH of less than 6 at a temperature of 5°C, and prevents the outgrowth of pathogens, and achieves at least a 2 log decline of pathogens within a seven (7) day period when kept at a pH from 3.0 to less than 5.0.

3. The method of claim 1 wherein the food composition is a filling, dip, sauce, spread, dressing, refrigerated salad, batter or beverage.

4. The method of claim 1 wherein the cationic saturated preservatives suitable for use in this invention include those having the formula I: (I)



Where:

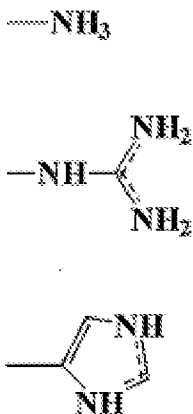
R₁ is: a linear or branched alkyl chain from a saturated fatty acid or a saturated fatty hydroxy acid containing 8 to 14 carbon atoms bonded to the alpha-amino acid group through an amidic bond;

R₂ is: a linear or branched alkyl group containing 1 to 4 carbon atoms;

X⁽⁻⁾ is: a monohydrohalide, preferably chloride (Cl⁻);

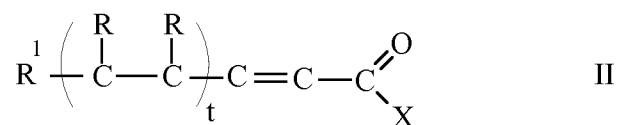
R₃ is: a structure of formula Ia

(Ia)



n is: from 1 to 4.

5. The method of claim 1 wherein the saturated preservative is LAE.
6. The method of claim 1 wherein said second preservative component has a pK_a of less than 5.5.
7. The method of claim 1 wherein said second preservative component is a polyene macrolide antibiotic or a compound having the formula:



where R^1 is $-(C(R)_2)_q-C(R)_3$, $\begin{array}{c} O \\ || \\ -C \\ \backslash \\ X \end{array}$ or $\begin{array}{c} R \\ | \\ C-C(R)_3 \end{array}$;

$X^{(-)}$ is: a monohydrohalide, preferably chloride (Cl^-);

R is independently a C_1 - C_4 alkyl or hydrogen;

q is 0 to 12, and t is from 0 to 6, with the proviso that when R^1 forms part of an sp^2 hybridized carbon-carbon bond, t does not equal zero.

8. The method of claim 1 wherein said second preservative component is benzoic acid, coumaric acid, salicylic acid, vanillic acid, caffeic acid, cinnamic acid, ferulic acid, salts thereof, derivatives thereof or a mixture thereof.

9. The method of claim 1 wherein the food composition or ingredients of the food composition are marinated with said saturated preservative and said second preservative component.

10. The method for preserving a food composition according to claim 1 wherein said second preservative component is selected from the group consisting of acetic, propanoic, 2-hydroxypropanoic, butyric, propionic, phosphoric, valeric, adipic, gluconic, malic, citric, tartaric, ascorbic, carnosic acid or a mixture thereof.

11. The method for preserving a food composition according to claim 1 wherein said food composition is acidified to a pH of less than 3.6.