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ABOGADOS

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
División de Nuevas Creaciones
E. S. D.

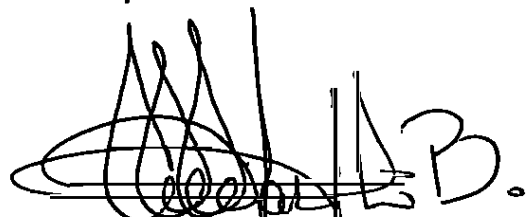
SUPERINDUSTRIA Y COMERCIO
Radicacion : 05019024 00000003 Folios: 32
Fecha (AMD): 2005-06-17 15:34:38
Tramite : 002 PATENTES D 1 REGISTRO/ 329 COMPLENEN
Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Ref: EXPEDIENTE No.05-019024
Solicitud de Patente a nombre de
KRAFT FOODS HOLDINGS, INC

En el asunto de la referencia me permito presentar la copia certificada de la solicitud estadounidense No. 10/792.502 del 3 de Marzo de 2004, de la que se reclamó prioridad.

Observo que su texto coincide con el de la descripción y reivindicaciones en Español presentadas en esta solicitud.

Como el expediente esta completo, pido se ordene la publicación del extracto.
Atentamente,


JERÓNIMO CASTILLO BONILLA
C.C. 79.462.057 Bogotá
T.P. 104167 C.S. Judicatura

PA 128928

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

March 02, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 10/792,502

FILING DATE: March 03, 2004

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS



L. Edelen

L. EDELEN
Certifying Officer

UTILITY PATENT APPLICATION TRANSMITTAL
(for Noncontinuing, Nonprovisional Applications
under 37 C.F.R. § 1.53(b))

Attorney Docket No. 67540

Customer No. 22242

17497 U.S. PTO
10/792502

Mail Stop PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith for filing under 37 C.F.R.
§ 1.53(b) is the nonprovisional, noncontinuing
patent application for:

Title: **ANTIMICROBIAL EFFECT OF
CHITOSAN IN BEVERAGES**

First Named Inventor or Application Identifier:
SOEDJAK et al.

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"Express Mail" Mailing Label Number

EV 447343199 US

Date of Deposit **March 3, 2004**

I hereby certify that this paper or fee is being deposited
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Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Edward Price

(Typed or printed name of person mailing)

(Signature of person mailing)

- ☒ 21 pages of the specification (including claims) are enclosed.
- ☐ _____ sheet(s) of drawings are enclosed. ☐ Formal ☐ Informal
- ☒ An executed Oath or Declaration and Power of Attorney naming the actual inventors is enclosed.
- ☐ The names of persons believed to be the actual inventors are set forth in the enclosed unexecuted Oath or Declaration and Power of Attorney (§ 1.41(a) and § 1.53(b)).
- ☒ An Assignment of the invention to Kraft Foods Holdings, Inc., and cover sheet are enclosed.
- ☐ A check in the amount of \$_____ to cover the fee for recording the assignment is enclosed.
- ☒ The Recordation Form Cover Sheet includes authorization to charge \$40.00 to our Deposit Account for recording the assignment.
- ☐ A 37 C.F.R. § 3.73(b) statement is enclosed where an assignee seeks to take action.

Noncontinuing Utility § 1.53(b)-1-0903

- ☐ An Information Disclosure Statement is enclosed.
- ☐ A Form PTO/SB/08 is enclosed.
- ☐ _____ References (copies) listed on the Form PTO/SB/08 are enclosed.
- ☒ A Return Receipt Postcard is enclosed (MPEP §503).
- ☐ Priority of application number _____ filed on _____ in _____ is claimed under 35 U.S.C. §119.
- ☐ A certified copy of the priority document is enclosed.
- ☐ A Computer Program Listing Appendix is enclosed.
- ☐ A Transmittal Cover Letter for Computer Program Listing Appendix is enclosed.
- ☐ Two (2) Compact Discs are enclosed.
- ☐ A Nucleotide and/or Amino Acid Sequence Submission is enclosed.
- ☐ A Computer Readable Copy is enclosed.
- ☐ A Paper Copy (Identical to Computer Copy) is enclosed.
- ☐ A Statement Verifying Identity of above Copies is enclosed.
- ☒ The filing fee is calculated below:

Fee Calculation For Claims As Filed

Basic Fee						\$ 770.00
Independent Claims	<u>4</u>	-	<u>3</u>	=	<u>1</u> x \$ 86.00	= \$ 86.00
Total Claims	<u>34</u>	-	<u>20</u>	=	<u>14</u> x \$ 18.00	= \$ 252.00
Fee for Multiply Dependent Claims					\$ 290.00	
					Total Filing Fee	\$ 1,108.00

☐ Applicant(s) assert entitlement to Small Entity Status (37 C.F.R. § 1.27), reducing the Filing Fee by half to: \$ 0.00

☐ A check in the amount of \$_____ to cover the filing fee is enclosed.

☒ Charge \$ 1.108.00 to Deposit Account No. 06-1135.

☐ The payment of the Filing Fee is to be deferred until the Declaration is filed. Do not charge our Deposit Account.

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- ☒ A separate written request under 37 C.F.R. §1.136(a)(3), which is a general authorization to treat any concurrent or future reply requiring a petition for an extension of time under 37 C.F.R. §1.136(a) for its timely submission as incorporating a petition for an extension of time for the appropriate length of time, is enclosed.
- ☒ The Director is hereby authorized to charge any additional fees which may be required in this application under 37 C.F.R. §§1.16-1.17 during its entire pendency, or credit any overpayment, to Deposit Account No. 06-1135. Should no proper payment be enclosed herewith, the Director is authorized to charge the unpaid amount to Deposit Account No. 06-1135. This sheet is filed in triplicate.
- ☐ Also enclosed:
- ☒ Address all future communications to CUSTOMER NUMBER 22242.

March 3, 2004
Date

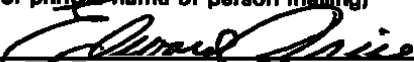


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Noncontinuing Utility §1.53(b)-3-0903

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named	Inventor:	SOEDJAK et al.	Filed:	Herewith	Title:	ANTIMICROBIAL EFFECT OF CHITOSAN IN BEVERAGES	Attorney Docket No.:	87540	Customer No.:	22242
							CERTIFICATE OF MAILING BY "EXPRESS MAIL"			
							"Express Mail" Mailing Label Number			
							EV 447343199 US			
							Date of Deposit March 3, 2004			
							I hereby certify that this paper or fee is being deposited with sufficient postage utilizing the United States Postal Service "Express Mail Post Office to Addressee" Service under 37 CFR §1.10 on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.			
							Edward Price			
							(Typed or printed name of person mailing)			
										
							(Signature of person mailing)			

**GENERAL AUTHORIZATION FOR PETITION FOR
EXTENSION OF TIME UNDER 37 C.F.R. § 1.136(a)(3)**

Mail Stop PATENT APPLICATION
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant(s) hereby request under 37 C.F.R. §1.136(a)(3) by this general authorization that any concurrent or future reply submitted by Applicant(s) to the United States Patent and Trademark Office for the above-identified patent application requiring a petition for an extension of time under §1.136(a) for its timely submission be treated as incorporating therein a petition for an extension of time for the appropriate length of time.

If Applicant(s) do not timely pay for any extension fee(s) pursuant to 37 C.F.R. §1.136(a) which may become due for this application under 37 C.F.R. §1.17 by check, the Director is hereby authorized to charge such fee(s), and any additional fees which may be required in this application under 37 C.F.R. §§1.16-1.17 during its entire pendency, or credit any overpayment, to Deposit Account No. 06-1135.

March 3, 2004

Date _____

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General Authorization 0903

ANTIMICROBIAL EFFECT OF CHITOSAN IN BEVERAGES

Field of the Invention

The present invention is directed to methods of killing microorganisms and/or inhibiting their growth in beverages, particularly beverages containing fruit juice, by adding a first antimicrobial compound (i.e., chitosan, tannic acid, or mixtures thereof) and a second antimicrobial compound (especially benzoate, sorbate, EDTA, and mixtures thereof). In addition, the invention is directed to beverages that contain chitosan alone or in combination with second antimicrobial compound.

Background of the Invention

A major concern in the making of beverages for human consumption is the growth of microorganisms. Fruit juice-containing drinks provide a particularly good environment for the growth of bacteria, mold, and yeast. Unless measures are taken to control such growth, these products rapidly deteriorate. Methods of inhibiting microbial growth include heat pasteurization during packaging (hot packing), and the use of aseptic packing conditions. Although these methods are highly effective in eliminating microorganisms responsible for spoilage, they are expensive, unsuitable for certain beverages, and incompatible with some types of containers.

Preservatives such as sorbates and benzoates may also be included in beverages to reduce microbial growth and are typically present when cold fill packing methods are used. However, a balance must be struck between an effective concentration of preservative and a tendency to adversely affect flavor as concentration increases. As a result, better preservative compositions are constantly being sought.

Attempts to develop more effective preservatives have included: the use of salts of polyphosphates to enhance the potency of sorbate preservatives (U.S. Patent 5,431,940; see also U.S. Patents 6,294,214 and 6,440,482); the use of dialkyl dicarbonates (U.S. Patent 3,979,524);

combining a sorbate preservative with natamycin and a dialkyl dicarbonate (U.S. Patent 6,376,005); and combining sorbate or benzoate salts with ascorbic acid and with dimethyl dicarbonate (U.S. Patent 5,866,182). Ideally, a preservative composition should have a wide spectrum of activity (i.e., killing and/or inhibiting the growth of bacteria, mold, and yeast), be safe for human consumption, be effective at low concentration, be inexpensive, and not adversely effect the flavor of the beverage to which it is added.

Summary of the Invention

The present invention is based upon the discovery that chitosan works synergistically with other preservatives (particularly benzoate, sorbate, EDTA, and mixtures thereof) to kill and/or prevent the growth of bacteria, yeast, and mold in ready-to-drink beverages, including juice-containing ready-to-drink beverages. This has at least two important consequences. First, a combination of chitosan with benzoate, sorbate, EDTA, or mixtures thereof is more effective than when these agents are used alone. Second, when used in combination, these antimicrobials are effective at lower concentrations. Another agent that is believed to produce similar results to chitosan is tannic acid.

Generally, the invention is directed to a method of killing and/or inhibiting the growth of microorganisms in a beverage by adding a first antimicrobial compound (chitosan, tannic acid, or mixtures thereof) together with second antimicrobial compound (e.g., benzoate, sorbate, EDTA, or mixtures thereof). The first antimicrobial compound and second antimicrobial compound are added to a concentration sufficient, in their combined action, to reduce and maintain microorganism levels to less than about 1 cfu/ml for at least about 8 weeks at room temperature. Using the present invention, it is not necessary to use heat treatments to inactivate food spoilage microorganism, including yeasts and mold. Thus, cold packing methods can be used. Of course, such heat treatments could be used if desired.

In another embodiment, the invention is directed to a method of killing and/or inhibiting the growth of microorganisms in a beverage by adding a first antimicrobial compound (i.e., chitosan, tannic acid, or mixtures thereof) together with a second antimicrobial compound. The first and second
5 antimicrobial compounds are added to a concentration sufficient, in their combined action, to reduce and maintain microorganism levels to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

Preferably the second antimicrobial compound used in the methods described above is benzoate, sorbate, EDTA (ethylenediamine tetraacetic
10 acid), or mixtures thereof. These may be added in any form compatible for use in a food product (e.g., as sodium or potassium salts).

In one embodiment, this invention provides a method of killing and/or inhibiting the growth of microorganisms in a packaged food product, comprising adding a first antimicrobial compound selected from the group
15 consisting of chitosan, tannic acid, or mixtures thereof and a second antimicrobial compound, wherein the first antimicrobial compound and second antimicrobial compound are added to a concentration sufficient, in their combined action, to reduce and maintain microorganism levels in the packaged food product to less than about 1 cfu/ml for at least about 8 weeks
20 at room temperature.

In another embodiment, the present invention provides a packaged beverage comprising a first antimicrobial compound selected from the group consisting of chitosan, tannic acid, or mixture thereof and a second
25 antimicrobial compound, wherein the first antimicrobial compound and second antimicrobial compound are added at a concentration sufficient, in their combined action, to reduce and maintain microorganism levels in the packaged beverage to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

In another embodiment, the present invention provides a process for
30 providing a cold fill packaged beverage, the process comprising:

(1) preparing a beverage, wherein the prepared beverage comprises a first antimicrobial compound selected from the group consisting of chitosan, tannic acid, or mixture thereof and a second antimicrobial compound selected from the group consisting of benzoate, sorbate, EDTA, and mixtures thereof;

5 (2) placing the prepared beverage in an container;

(3) sealing the container to provide the cold fill packaged beverage, wherein steps (2) and (3) are carried out at a temperature of 0 to about 80°C; and wherein the first antimicrobial compound and second antimicrobial compound are added to the beverage at a concentration sufficient, in their
10 combined action, to reduce and maintain microorganism levels in the cold fill packaged beverage to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

In still another embodiment, the present invention provides a process for providing a cold fill packaged beverage, the process comprising:

15 (1) preparing a beverage, wherein the prepared beverage comprises a chitosan;

(2) placing the prepared beverage in an container;

(3) sealing the container to provide the cold fill packaged beverage, wherein steps (2) and (3) are carried out at a temperature of 0 to about
20 80°C; and wherein the chitosan is added to the beverage at a concentration sufficient, to reduce and maintain microorganism levels in the cold fill packaged beverage to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

Detailed Description of the Invention

25 Chitosan is a polysaccharide typically produced by the deacetylation of chitin in base at high temperature. Although chitin is insoluble in most solvents, chitosan dissolves in dilute solutions of organic acids, including citric acid. Methods for solubilizing chitosan in a variety of liquids are well known in the art (see, e.g., U.S. Patent 5,453,282; U.S. Patent 5,654,001; and U.S.

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Patent 6,323,189). It has been used in a number of biomedical applications and has, in recent years, become a popular dietary supplement.

5 Tannic acid and chitosan have been experimentally tested and have been found to work synergistically with sorbate/benzoate or sorbate/benzoate/EDTA in ready-to-drink beverages and especially in juice-containing ready-to-drink beverages. Sorbate is a commonly used preservative and has been found to be effective against mold, yeast, and certain types of bacteria. Benzoate has a similar range of activity, although it is generally somewhat less potent than sorbate, and operates best in an
10 acidic environment. EDTA is a common chelating agent that traps metal impurities in foods that would otherwise promote microbial growth and rancidity. All of these agents are available commercially from a variety of sources.

The present methods and compositions are most useful in fruit juice-containing beverages which may be either carbonated or non-carbonated.
15 The juices preferably include citric acid-containing juices such as orange juice, lemon juice, lime juice, grapefruit juice, tangerine juice, and mixtures thereof. Other fruit juices that may be used include apple juice, grape juice, pear juice, nectarine juice, currant juice, raspberry juice, gooseberry juice, blackberry juice, blueberry juice, strawberry juice, pomegranate juice, guava
20 juice, kiwi juice, mango juice, papaya juice, watermelon juice, cantaloupe juice, cherry juice, cranberry juice, pineapple juice, peach juice, apricot juice, plum juice, and the like. Such fruit juices can be natural fruit juices (i.e., obtained directly from the natural fruit), processed fruit juices, reformulated fruit juices, and the like. Although not preferred, the invention is also
25 compatible with beverages containing vegetable juices.

In general, the most preferred beverages are non-carbonated beverages having a pH of about 2.5 to about 4.5, including naturally acidic beverages or acidified beverage. The preservatives are compatible with
30 artificial or natural sweeteners and with other additives typically used in food products, so long as they do not adversely effect the organoleptic properties

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of the beverage. These additives may include, for example, flavorants, colorants, stabilizers, thickeners, nutrients such as vitamins and minerals, emulsifiers, and antioxidants. When using chitosan, it is preferred that low molecular weight (preferably about 6000 g/mol or less) forms be used because of their relatively small effect on viscosity.

The present invention allows the killing and/or inhibiting growth of microbiological contamination, including that which is introduced with the ingredients and that from environmental sources during handling and packaging. The first and second (if used) antimicrobial compounds are added to a concentration sufficient to reduce and maintain microorganism levels to less than about 1 cfu/ml for at least about 8 weeks at room temperature. It has been found that, for beverages contaminated with microbiological contamination of up to about 10^4 cfu/ml, the compositions and methods of the present invention are effective for killing the microorganisms present to levels of below about 1 cfu/ml within about 5 weeks and for maintaining the level below about 1 cfu/ml for at least about 8 weeks at room temperature. It is expected that such levels will be maintained below about 1 cfu/ml for longer periods of time unless subsequent contamination occurs (i.e., via failure of primary package). Of course, efforts should be made to avoid and/or reduce microbiological contamination of food products, including beverages, when using the present invention to provide even greater margins of safety.

The first antimicrobial compound is chitosan, tannic acid, or mixtures thereof; chitosan is the preferred antimicrobial compound for use in this invention. The chitosan may be added to a final concentration of about 0.1 to about 200 ppm (preferably about 1 to about 100 ppm). In other embodiments, tannic acid (preferably added to a final concentration of about 10 to about 100 ppm) may be used in the place of, or together with, chitosan. Examples of the second antimicrobial compound include benzoate, sorbate, EDTA, and mixtures thereof. The benzoate or sorbate, when used, is generally added to a final concentration of about 10 to about 1000 ppm (with

other appropriate ranges being about 50 to about 500 ppm and about 50 to about 150 ppm); EDTA, when used, should be at a final concentration of about 0.5 to about 300 ppm, with preferred and more preferred concentrations being about 1 to about 100 ppm and about 10 to about 50 ppm, respectively. In a preferred embodiment, the first antimicrobial compound is chitosan and the second antimicrobial compound is a mixture of benzoate, sorbate, and EDTA. Generally, the total level of added antimicrobial compounds should be less than about 2000 ppm, and preferably less than about 1000 ppm, in the relevant food product.

The present invention also encompasses beverages made by the methods discussed above. For example, in a preferred embodiment the present invention includes ready-to-drink beverages containing at least 1 percent fruit juice, about 1 to about 200 ppm chitosan, and about 10 to about 1000 ppm benzoate or sorbate. Other agents, such as tannic acid and EDTA, may also be included. Preferred concentrations are those discussed above and would include, for example, a beverage with about 1 to about 100 ppm chitosan, and about 50 to about 500 ppm of either sorbate or benzoate. Tannic acid can also be added to compositions at a preferred concentration of about 10 to about 100 ppm.

The chitosan used in the methods and compositions described above may be in any molecular form compatible with preparation of a beverage for human consumption. However, in general, the chitosan should have a low average molecular weight (i.e., less than about 50,000 g/mol), with an average molecular weight of less than about 6000 g/mol being preferred. The beverages should generally contain at least 1 percent fruit juice, with concentrations in the range of about 5 to about 95 percent and about 5 to about 50 percent being preferred. They should be acidic, with a pH in the range of about 2.0 to about 6.0 and, preferably, in the range of about 2.5 to about 4.5.

One advantage of the present invention is that it allows for the preparation of beverages using a cold fill packing process. Cold fill processes

are well known in the art and, unlike "hot fill" processes, involve the packing of liquids at a temperature of under about 80°C and typically at a temperature of between 0 and about 35°C. The use of the present invention in a cold fill process offers a number of advantages, including, for example, improved robustness with regard to high levels of microbial contamination, reduced formulation costs (i.e., relatively low levels of antimicrobial compounds are effective), improved formula flexibility (i.e., preservative system is effective over wide range of water hardness and water alkalinity conditions), improved product flavor (i.e., due to low levels of antimicrobial compounds which can be used and the ability to avoid heat treatment conditions), increased shelf life at ambient temperature, and the like.

The conditions of such cold fill packing may be varied in accordance with the desires of the producer and any constraints imposed by the particular liquid being packaged. Thus, the present invention encompasses an improvement in a cold fill process for the packing of a beverage that is characterized by the addition of chitosan to a final concentration of between about 1 and about 200 ppm. Preferably, sorbate, benzoate and/or EDTA are included in the process at the concentrations described above. When the first and second antimicrobial compounds are used together, no additional preservatives are required. Nevertheless, the method is compatible with other preservatives, coloring agents, stabilizers, and the like, so long as they do not adversely affect the organoleptic properties of the beverage. The method will be most advantageously used for fruit juice-containing beverages as discussed above at a pH of about 2.0 to about 6.0, and preferably at about 2.5 to about 4.5. Tannic acid may also be used in cold fill processes together with chitosan. The tannic acid may be used in any form compatible with food products and should be added to a final concentration of about 1 to about 500 ppm, with preferred final concentrations being about 10 to about 300 ppm and about 10 to about 100 ppm.

The beverages of the present invention can be prepared using conventional methods well known in the art. Although cold filling is generally

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preferred, the methods and compositions are compatible with hot packing or aseptic packaging operations as well. Methods for making beverage compositions are described, for example, in U.S. Patents 4,737,375 and 6,294,214. These methods, or any others known in the art, may all be used
5 with the methods and compositions described herein.

Unless noted otherwise, all percentages or levels used in the present specification are by weight.

Examples

Example 1. A non-carbonated liquid beverage with a pH of less than
10 4.0 was prepared by blending water, high fructose corn syrup, pear juice concentrate, citric acid, ascorbic acid (vitamin C), and flavor. On a reconstituted basis, the beverage contained 10 percent fruit juice. The beverage was fortified with sufficient ascorbic acid to provide at least 100 percent of the USRDI (U.S. Recommended Daily Intake) of vitamin C. Water
15 of a hardness of about 60 ppm was used to formulate the beverage.

In addition, this beverage was formulated with the following preservatives:

5 ppm of chitosan;
200 ppm of sodium benzoate;
20 200 ppm potassium sorbate; and
30 ppm EDTA.

The beverage was inoculated with a cocktail of mold species at a level of about 2×10^2 cfu/ml. The beverage was sealed and placed in a 76°F chamber. Samples were aseptically extracted and plated to determine the
25 level of mold remaining in the beverage. The table below summarizes the results:

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Time (weeks)	Yeast (cfu/ml)
Initial Inoculation	210
1	3
2	2
3	<1
4	<1
6	<1
8	<1

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Example 2. This example illustrates the effectiveness of using
10 chitosan alone in a cold fill process. A non-carbonated liquid beverage with a
pH of less than 4.0 was prepared by blending water, high fructose corn syrup,
pear juice concentrate, citric acid, ascorbic acid (vitamin C), and flavor. On a
reconstituted basis, the beverage contained 10 percent fruit juice. The
beverage was fortified with sufficient ascorbic acid to provide at least 100
15 percent of the USRDI (U.S. Recommended Daily Intake) of vitamin C. Water
of a hardness of about 60 ppm was used to formulate the beverage.

This beverage was formulated with 20 ppm of chitosan; no sodium
benzoate, potassium sorbate, or EDTA was added.

The beverage was inoculated with a cocktail of yeast species at a level
20 of about 10^3 cfu/ml. The beverage was cold sealed and placed in a 76°F
chamber. Samples were aseptically extracted and plated to determine the
level of yeast remaining in the beverage. The table below summarizes the
results:

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Time (weeks)	Yeast (cfu/ml)
Initial Inoculation	960
1	<1
2	<1
3	<1
4	<1
6	<1
8	<1

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Comparative Example. A similar, but non-inventive, non-carbonated beverage was prepared as in Example 2 except that (1) water with a hardness of about 220 was used and (2) beverage was formulated with a different preservative system. The preservative system provided no chitosan, 250 ppm sodium benzoate, 250 ppm potassium sorbate, and 400 ppm EDTA.

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The samples were inoculated with a cocktail of yeast species at a level of about 10^3 cfu/ml. Inoculated samples were then treated and evaluated as in Example 2. After 1 week of storage, the growth of yeast was so excessive that counting was not possible; after 2 weeks of storage, the sample had fermented.

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Example 3. A non-carbonated liquid beverage with a pH of less than 4.0 was prepared by blending water, high fructose corn syrup, orange, pineapple, pear and red grape juice concentrate, citric acid, ascorbic acid (vitamin C), and flavor. On a reconstituted basis, the beverage contained 10 percent fruit juice. The beverage was fortified with sufficient ascorbic acid to provide at least 100 percent of the USRDI (U.S. Recommended Daily Intake) of vitamin C. Water of a hardness of about 130 ppm was used to formulate the beverage.

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In addition, this beverage was formulated with the following preservatives:

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- 20 ppm of Chitosan;
- 400 ppm sodium benzoate;
- 200 ppm potassium sorbate; and

30 ppm EDTA.

The beverage was inoculated with a cocktail of mold species at a level of about 2.5×10^2 cfu/ml and yeast species at a level of about 1.3×10^3 cfu/ml. The beverage was sealed and placed in a 76°F chamber. Samples were aseptically extracted and plated to determine the level of mold and yeast remaining in the beverage. The table below summarizes the results.

Time (weeks)	Mold (cfu/ml)	Yeast (cfu/ml)
Initial Inoculation	250	1280
1	18	57
2	<1	2
5	<1	<1
8	<1	<1

Example 4. A non-carbonated liquid beverage with a pH of less than 4.0 was prepared by blending water, high fructose corn syrup, pear juice concentrate, citric acid, ascorbic acid (vitamin C), and flavor. On a reconstituted basis, the beverage contained 10 percent fruit juice. The beverage was fortified with sufficient ascorbic acid to provide at least 100 percent of the USRDI (U.S. Recommended Daily Intake) of vitamin C. Water of a hardness of about 220 ppm was used to formulate the beverage.

In addition, this beverage was formulated with the following preservatives:

- 10 ppm of chitosan;
- 400 ppm of sodium benzoate;
- 200 ppm potassium sorbate; and
- 30 ppm EDTA.

Separate samples of the beverage were inoculated with a cocktail of either yeast species or mold species at a level of about 1.7×10^2 and 44 cfu/ml, respectively. The samples was sealed and placed in a 76°F chamber. Samples were aseptically extracted and plated to determine the level of mold or yeast remaining in the beverage. The table below summarizes the results.

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Time (weeks)	Mold (cfu/ml)	Yeast (cfu/ml)
Initial Inoculation	170	44
1	<1	<1
2	1	<1
3	<1	<1
4	<1	<1
6	<1	<1
8	<1	<1

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All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by those of skill in the art that the invention may be performed within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.



CLAIMS

What is Claimed is:

1. A method of killing microorganisms or inhibiting the growth of microorganisms in a packaged food product, comprising adding a first antimicrobial compound selected from the group consisting of chitosan, tannic acid, or mixtures thereof and a second antimicrobial compound, wherein the first antimicrobial compound and second antimicrobial compound are added to a concentration sufficient, in their combined action, to reduce and maintain microorganism levels in the packaged food product to less than about 1 cfu/ml for at least about 8 weeks at room temperature.
2. The method of claim 1, wherein the packaged food product is a packaged beverage.
3. The method of claim 2, wherein the packaged food product is a cold fill packaged beverage.
4. The method of claim 2, wherein the second antimicrobial compound is selected from the group consisting of benzoate, sorbate, EDTA, and mixtures thereof.
5. The method of claim 3, wherein the second antimicrobial compound is selected from the group consisting of benzoate, sorbate, EDTA, and mixtures thereof.
6. The method of claim 2, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the packaged beverage.

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7. The method of claim 3, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the packaged beverage.

8. The method of claim 4, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the packaged beverage.

9. The method of claim 5, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the packaged beverage.

10. The method of claim 4, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 200 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 10 to about 1000 ppm, or, if EDTA, is added to a final concentration of about 0.5 to about 300 ppm.

11. The method of claim 8, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 200 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 10 to about 1000 ppm, or, if EDTA, is added to a final concentration of about 0.5 to about 300 ppm.

12. The method of claim 10, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 100 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 50 to about 500 ppm, or, if EDTA, is added to a final concentration of about 1 to about 200 ppm.

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13. The method of claim 11, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 100 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 50 to about 500 ppm, or, if EDTA, is added to a final concentration of about 1 to about 200 ppm.

14. A packaged beverage comprising a first antimicrobial compound selected from the group consisting of chitosan, tannic acid, or mixture thereof and a second antimicrobial compound, wherein the first antimicrobial compound and second antimicrobial compound are added at a concentration sufficient, in their combined action, to reduce and maintain microorganism levels in the packaged beverage to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

15. The packaged beverage of claim 14, wherein the packaged beverage is a cold fill packaged beverage.

16. The packaged beverage of claim 14, wherein the second antimicrobial compound is selected from the group consisting of benzoate, sorbate, EDTA, and mixtures thereof.

17. The packaged beverage of claim 15, wherein the second antimicrobial compound is selected from the group consisting of benzoate, sorbate, EDTA, and mixtures thereof.

18. The packaged beverage of claim 16, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the packaged beverage.

18. The packaged beverage of claim 17, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the packaged beverage.

19. The packaged beverage of claim 16, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 200 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 10 to about 1000 ppm, or, if EDTA, is added to a final concentration of about 0.5 to about 300 ppm.

20. The packaged beverage of claim 17, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 200 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 10 to about 1000 ppm, or, if EDTA, is added to a final concentration of about 0.5 to about 300 ppm.

21. The packaged beverage of claim 19, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 100 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 50 to about 500 ppm, or, if EDTA, is added to a final concentration of about 1 to about 200 ppm.

22. The packaged beverage of claim 20, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 100 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 50 to about 500 ppm, or, if EDTA, is added to a final concentration of about 1 to about 200 ppm.

23. A process for providing a cold fill packaged beverage, the process comprising:

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(1) preparing a beverage, wherein the prepared beverage comprises a first antimicrobial compound selected from the group consisting of chitosan, tannic acid, or mixture thereof and a second antimicrobial compound selected from the group consisting of benzoate, sorbate, EDTA, and mixtures thereof;

(2) placing the prepared beverage in an container;

(3) sealing the container to provide the cold fill packaged beverage, wherein steps (2) and (3) are carried out at a temperature of 0 to about 80°C; and wherein the first antimicrobial compound and second antimicrobial compound are added to the beverage at a concentration sufficient, in their combined action, to reduce and maintain microorganism levels in the cold fill packaged beverage to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

24. The process of claim 23, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 200 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 10 to about 1000 ppm, or, if EDTA, is added to a final concentration of about 0.5 to about 300 ppm.

25. The process of claim 24, wherein the first antimicrobial compound is chitosan at a final concentration of about 1 to about 100 ppm and the second antimicrobial compound, if benzoate or sorbate, is added to a final concentration of about 50 to about 500 ppm, or, if EDTA, is added to a final concentration of about 1 to about 200 ppm.

26. The process of claim 23, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the cold fill packaged beverage.

27. The process of claim 24, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the cold fill packaged beverage.

28. The process of claim 25, wherein the first and the second antimicrobial compounds are the only antimicrobial compounds in the cold fill packaged beverage.

29. A process for providing a cold fill packaged beverage, the process comprising:

(1) preparing a beverage, wherein the prepared beverage comprises a chitosan;

(2) placing the prepared beverage in an container;

(3) sealing the container to provide the cold fill packaged beverage, wherein steps (2) and (3) are carried out at a temperature of 0 to about 80°C; and wherein the chitosan is added to the beverage at a concentration sufficient, to reduce and maintain microorganism levels in the cold fill packaged beverage to less than about 1 cfu/ml for at least about 8 weeks at room temperature.

30. The process of claim 29, wherein chitosan is the only preservative in the cold fill packaged beverage.

31. The process of claim 29, wherein the concentration of chitosan in the cold fill packaged beverage is about 1 to about 100 ppm.

32. The process of claim 30, wherein the concentration of chitosan in the cold fill packaged beverage is about 1 to about 100 ppm.

33. The process of claim 31, wherein the concentration of chitosan in the cold fill packaged beverage is about 10 to about 50 ppm.

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34. The process of claim 32, wherein the concentration of chitosan in the cold fill packaged beverage is about 10 to about 50 ppm.

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ANTIMICROBIAL EFFECT OF CHITOSAN IN BEVERAGES

Abstract

The present invention is directed to compositions and methods which utilize a first antimicrobial compound (i.e., chitosan, tannic acid, or mixtures thereof) in combination with a second antimicrobial compound (preferably benzoate, sorbate, EDTA, or mixtures thereof) to inhibit the growth of microorganisms in food products and especially in beverages. The present invention is also directed to cold fill compositions and cold fill methods using chitosan alone or in combination with the second antimicrobial compound (preferably benzoate, sorbate, EDTA, or mixtures thereof), to kill microorganisms and/or inhibit the growth of microorganisms in beverages.

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**DECLARATION
FOR UTILITY OR DESIGN
PATENT APPLICATION**

☒ Declaration Submitted With
Initial Filing

☒ Declaration Submitted After
Initial Filing

) Attorney Docket No.: 1410/67540

) Customer No.: 22242

) First Named Inventor: SOEDJAK et al.

) Application Number: N/A

) Filing Date: HEREWITH

) Art Unit: N/A

) Examiner Name: Not yet assigned

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ANTIMICROBIAL EFFECT OF CHITOSAN IN BEVERAGES

(Title of Invention)

the specification of which:

☒ is attached hereto, or

☐ was filed by an authorized person on my behalf on _____ as United States Application Number _____ or PCT International Application Number _____, and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below, and I have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application, on this invention filed by me or my legal representatives or assigns and having a filing date before that of the application on which priority is claimed:

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<u>Prior Foreign Application Number(s)</u>	<u>Country</u>	<u>Foreign Filing Date</u>	<u>Priority Not Claimed</u>
			☐
			☐
			☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet attached hereto.

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

<u>Provisional Application Number(s)</u>	<u>Provisional Application Filing Date</u>
--	--

☐ Additional provisional application numbers are listed on a supplemental priority data sheet attached hereto.

I hereby claim the benefit under Title 35, United States Code, § 120, of any prior United States application(s), or under § 365(c) of any PCT international application(s) designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known by me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>Prior U.S. Application Number</u>	<u>Prior PCT International Application Number</u>	<u>Filing Date of U.S. or PCT International Application</u>	<u>Patent Number (if applicable)</u>
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☐ Additional U.S. or PCT international application numbers are listed on a supplemental priority data sheet attached hereto.

As a named inventor, I hereby appoint Debbie K. Wright, Registration No. 33,817; Thomas A. Marcoux, Registration No. 29,826; Valerie Calloway, Registration No. 40,546; and the practitioners associated with Customer Number 22242, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith, and request that all correspondence and

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telephone calls in respect to this application be directed to FITCH, EVEN, TABIN & FLANNERY, Suite 1600, 120 South LaSalle Street, Chicago, Illinois 60603-3406, Telephone No. (312) 577-7000, Facsimile No. (312) 577-7007, CUSTOMER NUMBER 22242.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity or enforceability of the application or any patent issued thereon.

Full name of sole or one joint inventor: Helena Setvarini Soediak
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Date: 2/27/04
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Citizenship: USA

Full name of one joint inventor: Gedory Aaron Wiseman
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Inventor's signature: *G. Wiseman*
Date: Feb 20, 2004
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Post Office Address: 225 East 57th Street, Apartment 12G
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Citizenship: USA

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2° EXAMEN DE FORMA, PATENTE DE INVENCION

Radicación N° **05-19024**

Folio 69

Concepto Técnico N° **812**

Clasificación Internacional de Patentes: **A23L 2/44**

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486, se determinó que esta solicitud de **Patente**:

- SI ☒ NO ☐ Contiene un resumen de la invención (Art. 26-e)
- SI ☒ NO ☐ Contiene el título o nombre de la invención (Art. 27-d)
- SI ☒ NO ☐ Contiene la descripción de la invención (Art. 26-b)
- SI ☐ NO ☐ NO ES APLICABLE ☒ Contiene los dibujos necesarios para comprender la invención (Art. 26-d)
- SI ☒ NO ☐ Contiene una o más reivindicaciones (Art. 26-c)
- SI ☐ NO ☐ NO ES APLICABLE ☒ Han sido desarrollados los productos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen (Art. 26-h)
- SI ☐ NO ☐ NO ES APLICABLE ☒ Los productos y o procedimientos han sido obtenidos o desarrollados a partir a partir de los conocimientos tradicionales de las comunidades indígenas, afro americanas, o locales de los Países Miembros, de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes (Art. 26-i)
- SI ☐ NO ☒ La invención se refiere a un producto relativo a un material biológico (Art. 26-j)
- SI ☒ NO ☐ NO ES APLICABLE ☐ La prioridad coincide con la materia reivindicada.
- SI ☒ NO ☐ **CUMPLE LOS REQUISITOS TÉCNICOS**

OBSERVACIONES Y OTROS: SI ☐ NO ☐ Ver hoja(s) anexa(s)

EXAMINADOR TÉCNICO: 202051

Bogotá D C, octubre 21 de 2005.

2020
Bogotá D C

Doctor(a):
JERÓNIMO CASTILLO BONILLA.
Apoderado

REFERENCIA:	Radicación N°	05-19024
	Trámite	002
	Evento	001
	Actuación	416
	Oficio N°	1202
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486, de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la oficina de comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la Decisión 486, a **partir del 01 de marzo de 2006**, por indicación del solicitante.

Cúmplase

Dado en Bogotá D C,

24 OCT. 2005


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe División de Nuevas Creaciones.

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