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[54]	Title:	METHOD FOR STERILIZING DRINKS CONTAMINATED WITH ACETIC ACID BACTERIA		
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[57]	Abstract:	The invention relates to a method and the use of synergistically acting mixtures for sterilizing and subsequent preservation of drinks which have been contaminated with acetic acid bacteria.		

Method for sterilizing drinks contaminated with acetic acid bacteria

The invention relates to a method and the use of synergistically acting mixtures for sterilizing and subsequent preservation of drinks which have been contaminated with acetic acid bacteria.

Drinks may be contaminated by microorganisms, which in the end leads to spoilage and uselessness of the product. Furthermore, microbial contamination of drinks represents a health risk to the user. Survival or growth of microorganisms in the drinks is prevented by using chemical preservatives. Germs are increasingly noticeable in drinks, which germs were identified for the first time as causing drink spoilage a little over ten years ago and appear with increasing frequency. These take the form of bacteria which belong to the family of acetic acid bacteria and especially to the genus *Asaia*, *Neoasaia*, *Acetobacter*, *Gluconobacter*, *Gluconacetobacter* or similar (Yamada, Y. and Yukphan, P., Genera and species in acetic acid bacteria. International Journal of Food Microbiology, 125, 15-24, 2008). It has been increasingly shown that preservatives are not sufficiently potent enough and are therefore used at very high concentrations in order to prevent the bacteria mentioned being able to propagate and thereby accordingly contaminating the drink (Horsakova, I.; et al., *Asaia sp.* as a bacterium decaying the packaged still fruit beverages., Czech. Journal of Food Science, 27, 362-365, 2009). The use of high concentrations of preservatives, however, is undesirable from an economic point of view and is limited by statutory regulations.

A preservative composed of potassium sorbate and/or sodium benzoate and dimethyl carbonate and ascorbic acid is known from WO 96/09774 and is suitable for sterilizing and preserving drinks. A disadvantage of this preservative is also that it does not act efficiently against acetic acid bacteria.

The use of sulphur dioxide in high concentrations to control the growth of acetic acid bacteria is known from Watanabe and Ino, 1984 (Watanabe, M.L. and Ino S.; Studies on bacteria isolated from Japanese wine. Part 2. Growth of the *Acetobacter* sp. A-1 during the fermentation and storage of grape must and red wine. Yamanashi-ken Shokuhin Kogyo Shidosho Kenkyu Hokoku 16:13, 1984) and also from Juven, B.J. and Shomen, I., (Juven, B.J. and Shomen, I., Spoilage of soft drinks caused by bacterial flocculation. Journal of Food Protection, 48, 52, 1985). A disadvantage of this method is also that the efficacy is not efficient enough and so high concentrations of sulphur dioxide must be used which frequently negatively affect the flavour of the drink and can cause allergic reactions.

There was therefore a continuing need for a method for sterilizing drinks in which acetic acid bacteria are efficiently removed and the disadvantages of the prior art are overcome.

It has been found, surprisingly, that the mixture used in accordance with the invention acts against acetic acid bacteria in a synergistic manner and acetic acid bacteria are efficiently controlled.

The invention therefore relates to the use of synergistically acting mixtures comprising

a) sorbic acid or benzoic acid and/or alkali metal salts and/or alkaline earth metal salts thereof and

b) dimethyl dicarbonate and

c) sulphur dioxide and/or sulphur dioxide-releasing substances

for sterilizing drinks which have been contaminated by acetic acid bacteria, preferably for the subsequent preservation thereof.

The alkali metal salts of sorbic acid used are preferably sodium sorbate, potassium sorbate and lithium sorbate or mixtures of said salts. The alkaline earth metal salts of sorbic acid used are preferably calcium sorbate or magnesium sorbate or mixtures of said salts. Particular preference is given to using potassium sorbate.

The alkali metal salts of benzoic acid used are preferably sodium benzoate, potassium benzoate and lithium benzoate or mixtures of said salts. The alkaline earth metal salts of benzoic acid used are preferably calcium benzoate or magnesium benzoate or mixtures of said salts. Particular preference is given to using sodium benzoate.

Sulphur dioxide and/or sulphur dioxide-releasing substances are also used, such as inorganic or organic sulphur compounds. Preference is given to using inorganic sulphur compounds. The sulphur dioxide-releasing substances particularly preferably used are sodium hydrogen sulphite, sodium disulphite, potassium hydrogen sulphite, potassium disulphite, calcium hydrogen sulphite, calcium disulphite, potassium disulphite or mixtures of said salts. Very particular preference is given to using potassium disulphite as the sulphur dioxide-releasing substance.

Bacteria from the family of acetic acid bacteria are generally gram negative, aerobic bacilli and are known to a person skilled in the art. The type strain of these bacteria is *Acetobacter aceti* and have been assigned meanwhile to novel bacteria of the family of the acetic acid bacteria, mainly in the group of *Asaia sp.* Acetic acid bacteria are

preferably from the mixture removed from the drinks selected from the following group of Asaia sp., Acetobacter sp. Gluconobacter sp., Gluconacetobacter sp., Saccharibacter sp., Swaminanthia sp., Acidomonas sp., Kozakia sp., Neoasaia sp., Granulibacter sp., Acidocella sp., Acidiphilium sp., Roseococcus sp., Acidosphaera sp. and Rhodopila sp. The mixture particularly preferably has an effect on Asaia bogorensis, Asaia lannaensis, Gluconobacter oxydans and Gluconacetobacter liquefaciens.

Drinks are preferably understood to mean refreshing drinks, such as and with preference soft drinks, fruit juices and fruit juice-containing drinks, flavored refreshing drinks such as lemonades, teas (so-called ready-to-drink teas), such as and with preference iced tea, mixed drinks of a tea/fruit juice-containing refreshing drink, but also corresponding concentrates and also wine coolers and non-alcoholic wines. Particular preference is given to iced tea drinks.

The sorbic acid and/or alkali metal and/or alkaline earth metal salts thereof are generally used in an amount of 50 ppm to 1000 ppm, based on the amount of drink to be sterilized, but can also be used in smaller or larger amounts. The amount of sorbic acid and/or alkali metal and/or alkaline earth metal salts thereof used is preferably between 100 ppm and 350 ppm, based on the amount of drink to be sterilized. Benzoic acid and/or alkali metal and/or alkaline earth metal salts thereof are generally used in an amount of 50 ppm to 1000 ppm, based on the amount of drink to be sterilized, but can also be used in smaller or larger amounts. The amount of benzoic acid and/or alkali metal and/or alkaline earth metal salts thereof used is preferably between 100 ppm and 350 ppm, based on the amount of drink to be sterilized. DMDC is generally used in an amount of 50 ppm to 1000 ppm, based on the amount of drink to be sterilized, but can also be used in smaller or larger amounts. The amount of DMDC used is preferably between 75 ppm and 250 ppm, based on the amount of drink to be sterilized.

The sulphur dioxide and/or the sulphur dioxide-releasing substances are generally used in an amount of up to 500 ppm, but can likewise be used in higher amounts. The sulphur dioxide and/or the sulphur dioxide-releasing substances are preferably used in an amount of 5 ppm to 100 ppm, based on the amount of drink to be treated. The sulphur dioxide and/or the sulphur dioxide-releasing substances are more preferably used in an amount of 5 ppm to 20 ppm, based on the amount of drink to be treated.

Very particular preference is given to using a mixture of potassium sorbate and/or sodium benzoate and dimethyl dicarbonate and potassium disulphite. This mixture acts particularly synergistically. Very particular preference is given to using potassium sorbate and/or sodium benzoate in an amount of 100 ppm to 350 ppm and dimethyl dicarbonate in

an amount of 100 ppm to 250 ppm and potassium disulphite in an amount of 5 ppm to 100 ppm, based on the amount of drink to be sterilized.

Contamination by acetic acid bacteria is understood to mean in accordance with the invention that the detection methods known from the prior art to those skilled in the art give rise to a significant level of detection of acetic acid bacteria in the drinks. The detection of a bacterium carried out according to these methods is already a contamination in the context of the invention.

Since the use according to the invention also relates to the incorporation of the compounds in the drinks to be sterilized, the invention likewise relates to a method for sterilizing drinks which have been contaminated by acetic acid bacteria, in which

a) sorbic acid or benzoic acid and/or alkali metal salts and/or alkaline earth metal salts thereof and

b) dimethyl dicarbonate and

c) sulphur dioxide and/or sulphur dioxide-releasing substances

are incorporated into the drinks.

The incorporation may be effected for example by separate addition of the compounds a), b) and c) in any sequence or by addition of these compounds in a mixture. This mixture may be diluted by further solvent or also by other additives such as emulsifiers or other suitable food additives. Hydrophilic, organic and water-miscible solvents may be used as solvent. Preferably, no additional solvent is added. The compounds are preferably incorporated separately into the drinks.

Preservation by the preservatives added preferably occurs after the sterilization of the drinks.

The mixture used in accordance with the invention acts in a synergistic manner against acetic acid bacteria. Drinks can be efficiently and cost-effectively sterilized and preserved in this manner.

Examples

A drink was contaminated with a particular amount of a mixture of microorganisms and a mixture of preservatives was investigated for efficacy compared to the individual substances. The mixture of bacteria is composed of *Asaia bogorensis, Asaia lannaensis, Gluconobacter oxydans and Gluconacetobacter liquefaciens.* After one week, a portion of the drink was investigated for the presence of microorganisms.

Individual test substances: potassium sorbate, dimethyl dicarbonate, sodium benzoate, potassium disulphite

Test bacteria: mixture of various acetic acid bacteria isolated from drink filling lines

Substrate: iced tea

Bacterial seeding per mL of substrate: 200 cfu/mL per bacterium in the mixture

Table 1:

Individual active ingredient	**MIC ppm after one week**	
Potassium sorbate	750 mg/L	
Dimethyl dicarbonate	300 mg/L	
Potassium disulphite	60 mg/L	
Active ingredient mixture	**MIC ppm after one week**	**Synergy Index**
Potassium sorbate Dimethyl dicarbonate Potassium disulphite	150 mg/L 150 mg/L 10 mg/L	SI= 0.87
Individual active ingredient	**MIC ppm after one week**	
Sodium benzoate	550 mg/L	
Dimethyl dicarbonate	300 mg/L	
Potassium disulphite	60 mg/L	
Active ingredient mixture	**MIC ppm after one week**	**Synergy Index**
Sodium benzoate Dimethyl dicarbonate Potassium disulphite	125 mg/L 175 mg/L 7.5 mg/L	SI= 0.92

The synergism was determined using the method described by Kull et al. (F.C. Kull, P.C. Eismann, H.D. Sylvestrowicz, R.L. Mayer, Applied Microbiology 9, 538 to 541, 1961). The following relationships apply:

QA/Qa + QB/Qb + QC/Qc = SI

Qa = concentration of substance A which is the MIC

Qb = concentration of substance B which is the MIC

Qc = concentration of substance C which is the MIC

QA = concentration of substance A in the concentration of A/B/C at which microbial growth is suppressed

QB = concentration of substance B in the concentration of A/B/C at which microbial growth is suppressed

QC = concentration of substance C in the concentration of A/B/C at which microbial growth is suppressed

SI = Synergy Index

SI = 1 means additivity

SI > 1 means antagonism

SI < 1 means synergism

CLAIMS

1. Use of synergistically acting mixtures comprising

   a) sobic acid or benzioc acid and/or alkali salts and/or alkaline earth metal salts thereof and

   b) dimethyl dicarbonate and

   c) sulphur dioxide and/or sulphur dioxide-releasing substances for sterilizing drinks which have contaminated by acetic acid bacteria, preferably for the subsequent preservation thereof.

2. Use according to Claim 1, characterized in that the alkali metal and/or alkaline earth metal salt of sorbic acid used are sodium sorbate, potassium sorbate and lithium sorbate or calcium sorbate or mixtures of said salts.

3. Use according to Claim 1, characterized in that the alkali metal and/or alkaline earth metal salt of benzoic acid use are sodium benzoate, potassium benzoate and lithium benzoate or calcium benzoate or mixtures of said salts.

4. Use according to Claim 1, characterized in that the sulphur dioxide-releasing substances used are sodium hydrogen sulphite, sodium disulphite, potassium hydrogen sulphite, potassium disulphite , calcium hydrogen sulphite, calcium disulphite or mixtures of said salts.

5. Use according to Claim 1, characterized in that in Claim 1 a mixture comprising potassium sorbate and/or sodium benzoate, dimethyl carbonate and potassium disulphite is used for sterilizing drinks which have been contaminnated by acetic acid and the subsequent preservation thereof.

6. Use according to Claim 1, characterized in that the sorbic acid and/or alkali metal and/or alkaline earth metal salts thereof are used in an amount of 100 ppm to 350 ppm or the benzoic acid and/or alkali metal and/or alkaline earth metal salts thereof are used in an amount of 100 ppm to 350 ppm and the

DMDC is used in an amount of 75 ppm to 250 ppm and the sulphur dioxide and/or the sulphur dioxide-releasing substances are used in an amount of 5 ppm to 100 ppm, based on the amount of drink to be treated.

7. Use according to Claim 1, characterized in that the drinks treated are refresing drinks, such as in particular iced teas,sports drinks, fruit juices and fruit juice-containing drinks.

8. Use according to Claim 1, characterized in that the mixtures has an effect on acetic acid bacteria selected from the following group of Asia sp., Acetobacter sp. Gluconobacter sp., Gluconacetobacter sp., Saccharibacter sp.,Swaminanthia sp., Acidomonas sp., Kozakia sp., Neoasaia sp., Granulibacter sp., Acidocella sp., Acidiphilium sp., Roseococcus sp., Acidosphaera sp., and Rhodopila sp.,

9. Method for sterilizing drinks which have been contaminated by acetic acid bacteria, characterized in that

   a) sorbic acid or benzoic acid/or alkali metal salts and/or alkaline earth metal salts thereof and

   b) dimethyl dicarbonate and

   c) sulphur dioxide and/or sulphur dioxide-releasing substances

are incorporate into the drinks.

10. Method according to Claim 9, characterized in that preservation of the drinks by the preservatives added occurs after the sterilization of the drinks.