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(57) Abstract: The present invention relates to the use of flavouring ingredients and compositions as antifungal agents. The inven-
tion also relates to a method for preserving food products and beverages comprising adding said flavouring ingredients or composi-
tions to the food product or beverage.



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ANTIFUNGAL FLAVOURING INGREDIENTS AND COMPOSITIONS

Technical Field

The present invention relates to the use of flavouring ingredients and compositions as antifungal agents. The invention also relates to a method for preserving food products and beverages comprising adding said flavouring ingredients or compositions to the food product or beverage.

Background and Prior Art

Preservation of food products and beverages is one of the main problems encountered in the food industry, due to the growth of yeast and moulds in such food products and beverages. Preservative materials having antifungal activity and in particular capable of inhibiting the growth of yeast strains and moulds which are mainly responsible for the spoilage of food products and beverages are therefore the object of constant research.

Several yeast and mould species are known to be responsible for the spoilage of food products and beverages. Mainly, yeasts such as *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida crusei*, *Dekkera bruxellensis*, *Pichia anomala*, *Zygosaccharomyces bailii*, *Dekkera naardensis* and *Candida albicans* and moulds such as *Byssochlamys nivea*, *Byssochlamys fulva*, *Neosartorya fischeri*, *Talaromyces harzanium*, *Fusarium solani*, *Aspergillus fumigatis*, *Aspergillus niger*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium roqueforti*, *Fusarium oxysporum*, and *Mucor rouxii* are involved in such food and beverage spoilage.

Due to the multitude of fungal species found to spoil food products and beverages, there is a need for effective non-specific preservative agents, with a broad spectrum of antifungal activity. Such agents will need to be compatible with a number of different preparations, including sweet and savoury food and drinks.

Since most of the food products and beverages are flavoured, it would be desirable that the flavouring ingredients also provide an antifungal effect since this would reduce the amount of additional antifungal ingredients required.

Several ingredients are known as having antifungal activity. Some of these ingredients are also used as flavouring compounds. For instance, WO 2009/133272 teaches that carvacrol and thymol are useful as preservative materials, which are active against microbial spoilage of beverages. Similarly, WO 2005/012210 describes the use of compounds such as perilla acid and geranic acid as preservative materials for food.

It would also be desirable to provide further preservative materials having antifungal activity and especially antifungal flavouring ingredients which are effective at very low dosage since flavouring ingredients cannot normally be used in high amounts without providing an overpowering flavour which is often undesirable to the consumer.

It is an object of the present invention to provide one or more of the above-mentioned benefits and/or to address one or more of the above-mentioned problems.

Summary of the Invention

Accordingly, the present invention provides for the use of at least one flavouring ingredient selected from 2-methylhexanoic acid, 3,4-dimethylphenol and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one or of a flavouring composition comprising at least one of such flavouring ingredients as an antifungal agent.

In another aspect, the invention provides a method for preserving a food product or a beverage comprising adding to such food product or beverage at least one flavouring ingredient selected from 3,4-dimethylphenol, 2-methylhexanoic acid and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one or a flavouring composition comprising at least one of such flavouring ingredients.

In a further aspect, the present invention relates to an antifungal agent in the form of a composition comprising at least one ingredient selected from 2-methylhexanoic acid, 3,4-dimethylphenol and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one.

In still another aspect, the present invention relates to a food product or beverage comprising an antifungal agent of the invention.

Detailed Description of the Invention

3,4-Dimethylphenol, 2-methylhexanoic acid, (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one and flavouring compositions comprising such ingredients
5 surprisingly have antifungal activity and provide efficient activity as preservative for food products and beverages.

In other words, the use of such an ingredient or composition as antifungal agent refers to a method for killing, inhibiting or inactivating at least a proportion of one or more strains of
10 fungus, comprising contacting said fungus with the flavouring ingredient or composition.

Preferably, the contacting step consists in adding the flavouring ingredient or composition to a food product or to a beverage, in which the fungus strain is present or is expected to grow.

15

Antifungal activity of ingredients and compositions

The term “antifungal” is used to mean effective to kill, inhibit or inactivate at least a proportion of one or more strains of fungus, such as yeast and/or mould.

20 Preferably, it refers to the ability of an ingredient or composition to effectively inhibit the growth of at least two strains selected from *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida crusei*, *Dekkera bruxellensis*, *Pichia anomala*, *Zygosaccharomyces bailii*, *Dekkera naardensis*, *Candida albicans*, *Byssoschlamys nivea*, *Byssoschlamys fulva*, *Neosartorya fischeri*, *Talaromyces harzanium*,
25 *Fusarium solani*, *Aspergillus fumigatis*, *Aspergillus niger*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium roqueforti*, *Fusarium oxysporum*, and *Mucor rouxii* and more preferably selected from *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida crusei*, *Dekkera bruxellensis*, *Byssoschlamys nivea*, *Byssoschlamys fulva*, *Neosartorya fischeri*, *Talaromyces harzanium*, *Fusarium solani*,
30 *Aspergillus fumigatis*, *Aspergillus niger*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium roqueforti* and *Fusarium oxysporum*.

More preferably the ingredient or composition has antifungal activity against three or more, or even four or more of the above mentioned fungal strains.

In a preferred aspect of the invention, the flavouring ingredient or composition for the purpose of the present invention is effective as antifungal agent at concentrations of 1000ppm or less, preferably of 500ppm or less, more preferably of 250ppm or less, most
5 preferably of 125ppm or less.

The term “effective” when used to describe the capacity of a compound or composition to inhibit growth of a yeast or mould strain is defined as the ability of such ingredient or composition to inhibit growth of the strain in a growth medium consisting of a 105g/L
10 sucrose solution with an initial inoculum of 10^2 - 10^3 cfu/mL of such yeast or mould strain, so that the average optical density of the growth medium, as measured at 380nm for yeast strains and at 500nm for mould strains, is maintained below 0.2 after 21 days of culture at 21 °C or 25°C under aerobic conditions. The growth medium of which the optical density is measured consists of the growth medium as it is at the end of the 21 days growth
15 period. It therefore consists of the growth medium (sucrose solution at 105 g/L), together with the organisms which were able to grow during the 21 days culture period and the flavouring ingredient or composition.

For the purposes of the present invention, growth inhibition activity of a given compound
20 against one of the above mentioned strains is measured as follows.

A 105 g/L solution of sucrose in water is prepared. Preferably, the sucrose solution is at pH 3 ± 0.2 or at pH 5 ± 0.2 . When the sucrose solution pH is set to one of these values, the solution is preferably autoclaved at 121°C for 15 minutes and the pH is then re-checked to
25 ensure that it remains within the required range.

Test samples are prepared in a microtiter plate comprising 100μL of one of the sucrose solutions prepared above, together with an initial inoculum of the fungal strain of which growth is intended to be inhibited, so as to achieve a microbial level comprised between
30 10^2 and 10^3 cfu/mL.

The flavouring ingredient or composition of which the antifungal activity is intended to be evaluated is then added to the microtiter plate. Dilutions of the tested flavouring ingredient or composition in each test sample are prepared in a microtiter plate to afford

final concentrations of 1000ppm, 500ppm, 250ppm and 125ppm. The microtiter plates are then stored in a standard incubator under aerobic conditions at 21°C or at 25°C.

The optical density (OD) of each sample is measured just after addition of the flavouring ingredient or composition (control) and after 21 days of incubation. The OD is measured at 380 nm for yeast strains and at 500 nm for mould strains.

All tests are done in triplicate to ensure statistical relevance of the results obtained.

Growth inhibition of a particular strain by an ingredient at a particular concentration is given if the OD measured after 21 days of culture in these conditions is below 0.2, provided that the same effect is also observed with a higher concentration of the active ingredient.

Flavouring ingredient or flavouring composition having antifungal effect

The flavouring ingredients 3,4-dimethylphenol, 2-methylhexanoic acid and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one were identified as being particularly efficient to inhibit growth of the yeast and mould strains mentioned above. These flavouring ingredients, as well as flavouring compositions comprising them, can therefore advantageously be used as antifungal ingredients for the preservation of food products and beverages.

Flavouring compositions are meant here as carefully balanced mixtures of flavouring ingredients. Preferably, 3,4-dimethylphenol, 2-methylhexanoic acid or (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one is present in an amount of from 1 to 25% by weight, more preferably 7.5 to 25%, most preferably 12.5 to 25% by weight, based on the total weight of the composition.

Such flavouring compositions preferably comprise at least one additional ingredient having antifungal activity, as described above in any particular aspect of the invention. Among such suitable additional ingredients, one can mention salicylic acid, carvacrol, dihydro eugenol, farnesol, a mixture of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol, thymol, 7-methyl-3-octen-2-one, 5-methyl-2-phenyl-2-hexenal, orcinyle

(3-methoxy-5-methylphenol), maniguette seed extract, orange extract (for example Tetrarome[®] orange, origin: Firmenich SA, Geneva, Switzerland), 2-pentylfuran and terpinolene. This list is not exhaustive and the skilled person will, following the protocol detailed above for establishing antifungal activity, readily ascertain whether a flavouring ingredient has the desired antifungal activity. Particularly preferred additional flavouring ingredients include 5-methyl-2-phenyl-2-hexenal, dihydroeugenol and terpinolene.

More preferably, the flavouring composition used in the present invention comprises at least two of such additional ingredients.

Whilst the choice of ingredients will be dictated by their antifungal activity as described above, they should either be capable of providing a flavouring effect in the composition or be flavour neutral so that they can easily be incorporated into the end products mentioned herein. Such ingredients therefore preferably do not confer any off-note to the composition.

It is preferred, in the context of the present invention, to use a flavouring composition rather than a flavouring ingredient as such. More preferably the composition comprises at least three ingredients, since it is preferable that the composition not only provides antifungal benefits but also has a balanced flavour profile. When only one ingredient is used, then the flavour may be unbalanced whereas when two or more flavours are present, less of each ingredient is required and the inherent risk of unbalanced, overpowering flavours is diminished.

In a preferred aspect of the invention each additional ingredient is used in an amount of between 1 and 25% by weight, preferably between 7.5 and 25% by weight and more preferably between 12.5 and 25% by weight, relative to the total weight of the flavouring composition.

Since the flavour composition is typically incorporated into the final product at a level of 0.03 to 1% by weight, more preferably 0.05 to 0.5% by weight, most preferably 0.05 to 0.3% by weight, the flavouring ingredient or composition can surprisingly be present in very small amounts and yet still remain effective. For instance, the antifungal ingredient

or composition may be present in an amount of from 0.004 to 0.5%, more preferably more preferably 0.0075 to 0.05% by weight, based on the total weight of the final food product or beverage.

- 5 The flavouring ingredients or composition used in the present invention may be used to replace, in whole or in part, conventional preservative materials used in food products or beverages, without diminishing preservation of said food product or beverage. By “conventional preservative materials”, it is meant preservative antifungal materials that are not associated with any flavouring characteristics and often have an undesirable
10 aroma or taste, which must then be masked using known flavour ingredients. The presence of such preservative agent may further cause throat burn. Artificial antifungal agents also require appropriate labelling, which leads to a commercial drawback.

The antifungal flavouring ingredient of compositions used in the invention can be
15 combined with conventional preservative agents, in which case this has been found to provide a synergistic effect reinforcing the antifungal character of such ingredients.

By its antifungal and flavouring properties, a flavouring ingredient or composition used according to the invention is equally suitable for any type of food product and/or
20 beverage.

The flavouring compositions used in the invention may contain other constituents which have a positive or synergistic effect on the antifungal activity of the ingredients.

- 25 The flavouring ingredients, the flavouring composition or any ingredient of the flavouring composition can be incorporated in a delivery system or in a liquid emulsion or may be encapsulated following standard procedures.

Encapsulation of the flavouring ingredient or composition can be used in food products
30 and beverages and is advantageous because it enables controlled release of the ingredient composition and is desirable where delivery of a concentrated dose is required. When the whole composition is encapsulated, it also enables simultaneous release of all ingredients. It is believed that this may further improve the antifungal efficiency of the ingredients and

compositions. Encapsulation of the active ingredients has also an advantageous protective effect. The use of encapsulated antifungal agents is particularly advantageous in food products such as chewing-gum, candies and tablets.

- 5 The person skilled in the art is well aware of a variety of encapsulation systems which are suitable for this purpose, the following being preferred for their properties of providing very good barriers to oxidation.

10 A first preferred encapsulating system is a glassy matrix within which the flavouring composition or ingredient is held. More preferably the encapsulation system is a glassy carbohydrate matrix. The carbohydrate matrix ingredient preferably comprises a sugar derivative, more preferably maltodextrin.

15 Particularly preferred maltodextrins are those with a DE of from 10 to 30, more preferably from 15 to 25, most preferably from 17 to 19.

20 Typically, the flavouring ingredient or composition is admixed with a carbohydrate matrix material and an appropriate amount of a plasticizer, such as water, the mixture is heated within a screw extruder to a temperature above the glass transition temperature of the matrix material so as to form a molten mass capable of being extruded through a die and then the molten mass is extruded using established processes, such as described in the prior art. See, for instance, patent application WO 00/25606, published May 11, 2002 or WO 01/17372, published March 15, 2001, and the documents cited therein, the contents of which are hereby included by reference.

25 If desired, further carbohydrate matrix components may be present to further improve the antioxidant barrier properties.

30 Other suitable encapsulation systems are described in, for example, US 4,610, 890 or US 4,707,367, the contents of which are included by reference.

Other Flavouring Ingredients

35 The flavouring ingredient or composition used in the present invention as antifungal agent optionally comprises at least one flavouring ingredient of current use, typically incorporated into the food product or beverage.

The term “flavouring ingredient of current use” is defined here as encompassing flavouring ingredients or compositions of both natural and synthetic origin used in the flavours industry. It includes single compounds and mixtures. Specific examples of such flavour ingredients may be found in the current literature, e.g. in Fenaroli’s Handbook of flavour ingredients, 1975, CRC Press; Synthetic Food adjuncts, 1947 by M.B. Jacobs, edited by Van Nostrand; or Perfume and Flavor Chemicals by S. Arctander, 1969, Montclair, New Jersey (USA). Many other examples of current flavouring ingredients may be found in the patent and general literature available. The flavouring ingredients may be present in the form of a mixture with solvents, adjuvants, additives and/or other components, generally those of current use in the flavour industry.

“Flavouring ingredients” are well known to a person skilled in the art of aromatising as being capable of imparting a flavour or taste to a consumer product, or of modifying the taste and/or flavour of said consumer product, or yet its texture or mouthfeel.

End Products

The flavouring ingredients or compositions can be used in any type of food product or beverage to be preserved.

Examples of beverages include carbonated soft drink, functional soft drinks, juices and nectars, hot drinks and alcoholic drinks. The compositions of the invention are particularly useful when such beverages are sweet beverages, preferably comprising any type of sugar or sugar derivatives. Food products in which the compositions of the invention can be advantageously used are sweet as well as savoury products, and in particular all types of food products comprising water, more preferably comprising water and any type of sugar or sugar derivatives. Examples of such food products include for example soups, sauce, dressings, marinades, dairy products such as desserts, ice-cream, spreads and cheese, refrigerated food, acidified food, such as for example pickles, meat products and prepared fruits and vegetables.

Method for preserving a food product or a beverage

The present invention also provides a method for preserving a food product or a beverage comprising adding to such food product or beverage 3,4-dimethylphenol,

2-methylhexanoic acid and/or (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one or a composition comprising at least one of these flavouring ingredients.

- 5 The flavouring composition, the food product and the beverages are as defined above in any general or preferred aspect of the invention. The proportions in which the flavouring ingredient or composition can be added to the food product or the beverage are also as described above.

10 Antifungal agent

The present invention provides an antifungal agent in the form of a composition comprising at least one ingredient selected from 2-methylhexanoic acid, 3,4-dimethylphenol and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one.

15

- Such composition preferably also comprises at least one ingredient capable of inhibiting the growth of at least two yeast and/or mould strains selected from *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida crusei*, *Dekkera bruxellensis*, *Pichia anomala*, *Zygosaccharomyces bailii*, *Dekkera naardensis* *Candida*
20 *albicans*, *Byssoschlamys nivea*, *Byssoschlamys fulva*, *Neosartorya fischeri*, *Talaromyces harzanium*, *Fusarium solani*, *Aspergillus fumigatis*, *Aspergillus niger*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium roqueforti*, *Fusarium oxysporum*, and *Mucor rouxii* in a medium consisting of a 105g/L sucrose solution with an initial inoculum of 10^2 - 10^3 cfu/mL of such yeast or mould strains, so that the average optical
25 density of the growth medium, as measured at 380nm for yeast strains and at 500nm for mould strains, is maintained below 0.2 after 21 days of culture at 21°C or 25°C under aerobic conditions. Preferably, said yeast and/or mould strain is selected from *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida crusei*, *Dekkera bruxellensis*, *Byssoschlamys nivea*, *Byssoschlamys fulva*,
30 *Neosartorya fischeri*, *Talaromyces harzanium*, *Fusarium solani*, *Aspergillus fumigatis*, *Aspergillus niger*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium roqueforti* and *Fusarium oxysporum*.

Most preferably such additional ingredient is a flavouring ingredient or composition having an antifungal effect, as defined in any of the above embodiments.

Examples

- 5 The invention will now be illustrated by way of the following examples. All amounts are percentages by weight unless otherwise stated.

Example 1

Preparation of a composition for use as an antifungal agent according to the invention

10

Composition A was prepared having the following ingredients in the amount indicated.

Table 1: Composition of Composition A

Ingredient	Parts [%]
3,4-Dimethylphenol	8.0
2-methylhexanoic acid	91.0
(+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one (10% solution in propylene glycol)	1
Total	100

15

Example 2

Preparation of a composition for use as an antifungal agent according to the invention

Composition B was prepared having the following ingredients in the amount indicated.

20 **Table 2: Composition of Composition B**

Ingredient	Parts [%]
3,4-Dimethylphenol	8.0
2-methylhexanoic acid	40.0
(+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one (10% solution in propylene glycol)	1.0
Terpinolene	51.0
Total	100

Example 3

Preparation of a composition for use as an antifungal agent according to the invention

- 5 Composition C was prepared having the following ingredients in the amount indicated.

Table 3: Composition of Composition C

Ingredient	Parts [%]
Geranic acid	10.0
3,4-Dimethylphenol	6.0
2-methylhexanoic acid	1.0
(+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one (10% solution in propylene glycol)	0.5
Terpinolene	82.5
Total	100

Example 4

- 10 Preparation of a composition for use as an antifungal agent according to the invention

Composition D was prepared having the following ingredients in the amount indicated.

Table 4: Composition of Composition D

Ingredient	Parts [%]
3,4-Dimethylphenol	6.0
2-methylhexanoic acid	1.0
(+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one (10% solution in propylene glycol)	0.5
Terpinolene	87.5
5-Methyl-2-phenyl-2-hexenal	5.0
Total	100

Example 5Determination of the antifungal activity of several flavouring ingredients and compositions

5 The antifungal activity of various flavouring ingredients was evaluated against the following yeast strains: *Zygosaccharomyces rouxii* (DSM70835), *Pichia membranaefaciens* (DSM 70369), *Dekkera Bruxellensis* (DSM70726), *Saccharomyces cerevisiae* (DSM 70869) and *Candida krusei* (origin: Leatherhead Food Research) and against the following moulds strains: *Byssoschlamys nivea*, *Fusarium solani*, *Aspergillus*
10 *fumigatis*, *Byssoschlamys fulva*, *Aspergillus niger*, *Talaromyces Harzanium*, *Penicillium crustosum*, *Penicillium digitatum*, *Neosartorya fischeri*, *Penicillium roquerforti*, *Fusarium oxysporum*.

A 105 g/L solution of sucrose in water at pH 3 $\pm$ 0.2 was prepared. Another sucrose
15 solution at the same concentration at pH 5 $\pm$ 0.2 was also prepared. The sucrose solutions were autoclaved at 121°C for 15 minutes and the pH was re-checked to ensure it remained within the range.

Test samples were then prepared in microtiter plates. An amount of 100 μ L of sucrose
20 solution prepared above, were mixed with an inoculum of 20 μ L of one fungal strain, so as to achieve a microbial level comprised between 10² and 10³ cfu/mL.

The flavouring ingredient or composition was then added to the microtiter plate. Dilutions
were prepared in the microtiter plate for each mixture of sucrose solution and inoculum to
25 afford final concentrations of 2000ppm, 1000ppm, 500ppm, 250ppm and 125ppm of the ingredient or composition to be tested. One well for each mixture of sucrose solution and inoculum was kept free of flavouring ingredient or composition, to serve as a control.

The microtiter plates were then stored in a standard 25°C incubator under aerobic
30 conditions.

The optical density (OD) of each sample was measured after 21 days of incubation using
a Bio-Tek Synergy™ HT Multi-Mode Microplate Reader (from Labtech International,
UK). The wavelength was set to 380 nm for yeast strains and to 500 nm for mould strains.

All tests were done in triplicate to ensure statistical relevance of the results.

The following tables indicate the OD measurements obtained when each flavouring ingredient or composition was used to inhibit growth of yeast and mould strains involved at concentration ranging between 0 and 2000ppm. Measurements corresponding to efficient growth inhibition are indicated by grey cells in the tables below. Results were considered as evidence of efficient growth inhibition when the OD of the growth medium was below 0.2. OD measurements below 0.2 were not considered as sufficient evidence of the antifungal activity of a given flavouring ingredient or composition at a given concentration if OD measurements obtained with a higher concentration of such flavouring ingredient or composition were above 0.2.

Table 5: OD measurements obtained using Composition A as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.986	0.065	0.085	0.103	-0.004	0.005
Pichia membranaefaciens	1.119	0.101	0.156	0.066	-0.005	0.011
Dekkera bruxellensis	1.403	0.975	0.622	0.163	-0.003	0.010
Saccharomyces cerevisiae	0.141	0.381	0.031	0.024	0.002	0.013
Candida krusei	0.995	0.102	0.014	0.003	-0.009	0.012
Byssoschlamys nivea	0.239	0.145	0.246	0.305	-0.003	0.015
Fusarium solani	0.612	0.001	-0.015	-0.006	-0.009	0.024
Aspergillus fumigatis	0.181	0.274	0.254	0.046	0.006	0.036
Aspergillus niger	0.304	0.168	-0.015	0.008	0.022	0.026
Talaromyces harzanium	0.253	0.151	0.120	-0.016	-0.002	-0.002
Penicillium crustosum	0.255	0.103	0.159	-0.011	-0.009	0.003
Penicillium digitatum	0.283	0.172	0.173	-0.011	-0.007	-0.003
Neosartorya fischeri	0.523	0.004	-0.007	-0.009	-0.005	0.003
Penicillium roqueforti	0.402	0.069	0.084	-0.011	-0.011	0.004
Fusarium oxysporum	0.303	0.013	0.003	-0.001	0.001	0.008

These results show that the use of Composition A in a sucrose solution at pH 3 has a strong antifungal effect against 15 of the main fungi responsible for beverage and food

spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 6: OD measurements obtained using Composition A as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.631	0.192	0.227	0.043	-0.002	0.016
Pichia membranaefaciens	0.913	0.246	0.153	0.114	-0.011	0.005
Dekkera bruxellensis	1.401	1.098	1.025	0.642	-0.008	-0.002
Saccharomyces cerevisiae	1.215	0.077	0.030	0.002	-0.009	-0.002
Candida krusei	0.875	0.039	0.030	-0.018	-0.015	-0.003
Byssochlamys nivea	0.439	0.187	0.176	-0.003	-0.006	-0.011
Fusarium solani	0.533	-0.010	-0.007	-0.001	-0.001	-0.005
Aspergillus fumigatis	0.211	0.107	0.037	-0.008	-0.005	-0.012
Byssochlamys fulva	0.517	0.071	0.007	0.002	0.002	-0.004
Aspergillus niger	0.412	0.129	-0.007	-0.003	-0.003	-0.001
Talaromyces harzanium	0.247	0.073	-0.009	-0.009	-0.010	-0.018
Pennicillium crustosum	0.456	0.089	-0.004	-0.003	-0.003	-0.015
Penicillium digitatum	0.643	0.106	0.055	-0.011	-0.004	-0.013
Neosartorya fischeri	0.736	-0.002	-0.002	-0.003	-0.001	-0.008
Penicillium roqueforti	0.578	0.035	-0.006	-0.007	-0.006	-0.009
Fusarium oxysporum	0.333	-0.001	-0.001	0.003	0.001	-0.002

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These results show that the use of Composition A in a sucrose solution at pH 5 has a strong antifungal effect against 16 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

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Table 7: OD measurements obtained using Composition B as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.968	0.072	0.107	0.092	0.084	0.051

Candida krusei	0.857	0.167	0.220	0.123	0.104	-0.010
Fusarium solani	0.386	0.080	0.030	0.003	0.001	0.020
Byssoschlamys fulva	0.313	0.177	0.220	0.174	0.011	0.017
Aspergillus niger	0.375	0.126	0.135	0.000	0.005	0.011
Pennicillium crustosum	0.288	0.068	0.268	0.136	-0.012	-0.015
Penicillium digitatum	0.345	0.051	0.177	0.154	-0.009	-0.014
Neosartorya fischeri	0.726	-0.005	-0.003	-0.006	-0.010	-0.009
Penicillium roqueforti	0.475	0.110	0.294	0.107	-0.006	0.000
Fusarium oxysporum	0.255	0.152	-0.002	0.001	-0.003	-0.004

These results show that the use of Composition B in a sucrose solution at pH 3 has a strong antifungal effect against 10 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 8: OD measurements obtained using Composition B as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.872	0.392	0.653	0.177	0.111	0.081
Pichia membranaefaciens	0.992	0.472	0.242	0.197	0.154	0.128
Candida krusei	0.542	0.037	0.044	0.035	0.048	0.021
Fusarium solani	0.451	0.276	0.279	0.253	-0.011	0.007
Aspergillus fumigatis	0.231	0.092	0.109	0.085	0.161	0.006
Aspergillus niger	0.386	0.238	0.088	0.207	-0.004	0.014
Talaromyces harzanium	0.315	0.759	0.510	0.269	0.095	-0.030
Pennicillium crustosum	0.732	0.315	0.176	0.150	0.199	-0.330
Penicillium digitatum	0.676	0.210	0.158	0.426	0.116	-0.028
Neosartorya fischeri	0.524	0.347	0.328	0.368	0.084	-0.039
Penicillium roqueforti	0.569	0.158	0.226	0.133	0.011	-0.038
Fusarium oxysporum	0.653	0.178	0.144	0.354	-0.002	-0.018

These results show that the use of Composition B in a sucrose solution at pH 5 has a strong antifungal effect against 12 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

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Table 9: OD measurements obtained using salicylic acid as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.903	0.005	0.003	0.001	0.013	-0.006
Pichia membranaefaciens	0.909	0.008	0.003	0.013	0.016	-0.035
Dekkera bruxellensis	1.459	0.688	0.013	0.043	0.007	-0.012
Saccharomyces cerevisiae	1.097	0.017	0.006	0.010	0.017	-0.075
Candida krusei	0.970	0.009	-0.002	0.004	0.015	0.010
Byssoschlamys nivea	0.251	0.313	0.308	0.315	0.141	0.065
Talaromyces harzanium	0.111	-0.010	-0.001	0.000	-0.008	-0.003
Penicillium crustosum	0.331	-0.011	-0.003	-0.002	-0.009	-0.004
Penicillium digitatum	0.428	-0.008	-0.001	-0.009	-0.005	0.000
Neosartorya fischeri	0.351	-0.007	0.001	-0.001	-0.005	0.007
Penicillium roqueforti	0.253	-0.009	-0.005	-0.005	-0.009	-0.003
Fusarium oxysporum	0.412	-0.004	0.001	0.004	0.001	0.014

These results show that the use of salicylic acid in a sucrose solution at pH 3 has a strong antifungal effect against 12 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

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Table 10: OD measurements obtained using salicylic acid as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.568	0.017	0.019	0.026	0.055	0.192
Pichia membranaefaciens	1.315	0.020	0.002	0.028	0.064	0.074
Dekkera bruxellensis	1.410	0.516	0.001	0.021	0.025	0.098

Saccharomyces cerevisiae	1.196	0.024	0.019	0.024	0.064	0.072
Candida krusei	0.914	0.079	-0.004	0.012	0.042	0.086
Byssochlamys nivea	0.664	-0.011	-0.013	-0.009	-0.002	0.049
Fusarium solani	0.311	-0.006	-0.009	-0.012	-0.012	0.056
Aspergillus fumigatis	0.348	-0.010	-0.012	-0.006	-0.009	0.061
Byssochlamys fulva	0.951	0.007	-0.006	-0.002	-0.003	0.057
Penicillium crustosum	0.894	-0.010	0.011	0.000	-0.010	-0.004
Penicillium digitatum	0.878	-0.013	0.001	-0.003	0.002	0.003
Neosartorya fischeri	0.394	0.005	0.003	-0.001	-0.014	0.004
Penicillium roqueforti	0.219	0.008	0.003	-0.002	-0.07	-0.009
Fusarium oxysporum	0.089	-0.001	0.009	0.006	-0.003	0.000

These results show that the use of salicylic acid in a sucrose solution at pH 5 has a strong antifungal effect against 14 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 11: OD measurements obtained using carvacrol as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.963	0.096	0.129	0.101	-0.004	-0.001
Pichia membranaefaciens	0.906	0.738	1.235	1.043	-0.004	-0.009
Dekkera bruxellensis	1.444	1.425	1.418	1.113	0.004	0.006
Saccharomyces cerevisiae	1.399	1.155	0.054	-0.006	-0.007	-0.011
Candida krusei	0.821	0.109	0.125	0.042	-0.012	-0.011
Byssochlamys nivea	0.476	0.127	0.404	-0.015	-0.005	0.005
Fusarium solani	0.555	0.007	-0.009	-0.010	-0.009	0.007
Aspergillus fumigatis	0.274	0.418	0.215	-0.014	-0.014	0.007
Byssochlamys fulva	0.371	0.005	0.090	-0.011	-0.007	0.009
Aspergillus niger	0.378	0.084	0.278	-0.013	-0.015	0.010
Penicillium crustosum	0.276	0.494	0.242	-0.016	-0.013	0.001
Penicillium digitatum	0.378	0.002	0.039	-0.016	-0.012	-0.008

Neosartorya fischeri	0.482	0.207	-0.012	-0.016	-0.009	-0.004
Penicillium roqueforti	0.462	0.459	0.045	-0.019	-0.011	-0.003
Fusarium oxysporum	0.389	0.544	0.039	-0.011	0.000	-0.001

These results show that the use of carvacrol in a sucrose solution at pH 3 has a strong antifungal effect against 15 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 12: OD measurements obtained using carvacrol as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.130	0.530	1.217	-0.014	-0.003	-0.007
Pichia membranaefaciens	0.943	1.070	0.962	0.913	0.001	0.009
Saccharomyces cerevisiae	1.310	1.264	0.982	0.054	-0.005	0.006
Candida krusei	1.280	0.298	0.087	0.072	0.030	0.004
Byssoschlamys nivea	0.413	0.267	0.322	-0.014	0.010	-0.002
Fusarium solani	0.562	0.324	0.119	-0.011	-0.006	0.002
Byssoschlamys fulva	0.829	1.023	0.022	-0.013	0.001	0.012
Aspergillus niger	0.479	0.223	0.172	-0.045	-0.016	-0.007
Talaromyces harzanium	0.561	0.252	0.208	0.068	0.001	-0.021
Penicillium crustosum	0.603	0.122	0.173	0.300	-0.005	-0.019
Penicillium digitatum	0.769	0.452	0.392	0.077	0.001	-0.019
Neosartorya fischeri	0.674	0.139	0.144	0.003	-0.002	-0.013
Penicillium roqueforti	0.534	0.238	0.036	0.006	-0.013	-0.023
Fusarium oxysporum	1.034	0.155	0.542	0.010	-0.005	-0.004

These results show that the use of carvacrol in a sucrose solution at pH 5 has a strong antifungal effect against 14 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 13: OD measurements obtained using dihydro eugenol as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.990	0.053	0.064	0.080	0.015	0.032
Pichia membranaefaciens	1.057	0.450	0.704	0.557	0.008	0.047
Dekkera bruxellensis	1.395	1.155	0.929	0.353	0.009	0.032
Saccharomyces cerevisiae	0.772	0.963	0.729	1.135	0.003	0.036
Candida krusei	0.965	0.168	0.100	0.071	0.020	0.041
Byssoschlamys nivea	0.350	0.339	0.506	0.595	-0.002	-0.016
Fusarium solani	0.435	0.391	0.274	0.001	0.009	-0.022
Aspergillus fumigatis	0.370	0.611	0.423	0.000	-0.006	0.000
Byssoschlamys fulva	0.357	-0.002	0.418	0.074	-0.002	-0.011
Aspergillus niger	0.342	0.217	0.187	0.371	-0.010	-0.011
Penicillium crustosum	0.256	-0.005	0.341	0.192	-0.004	0.006
Penicillium digitatum	0.437	-0.007	0.312	0.066	-0.003	0.002
Neosartorya fischeri	0.619	0.456	-0.004	-0.008	-0.001	0.021
Penicillium roqueforti	0.498	0.077	0.081	0.092	-0.009	0.009
Fusarium oxysporum	0.337	0.279	0.409	0.293	0.002	0.012

These results show that the use of dihydro eugenol in a sucrose solution at pH 3 has a strong antifungal effect against 15 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 14: OD measurements obtained using dihydro eugenol as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.111	0.289	0.380	0.578	-0.007	-0.002
Dekkera bruxellensis	1.361	1.283	1.404	1.296	0.008	0.012
Saccharomyces cerevisiae	0.595	0.684	0.578	0.320	0.007	0.025
Candida krusei	1.112	0.165	0.148	0.029	0.004	0.030
Byssoschlamys nivea	0.859	0.864	0.369	0.265	0.185	0.062

Fusarium solani	0.885	0.620	0.862	0.024	0.027	0.060
Aspergillus fumigatis	0.447	0.240	0.395	0.124	0.207	0.069
Byssosclamyces fulva	0.904	0.457	0.387	0.290	0.133	0.074
Aspergillus niger	0.387	0.389	0.172	0.202	0.021	0.061
Talaromyces harzanium	0.382	0.174	0.435	0.208	-0.004	-0.006
Penicillium crustosum	0.728	0.330	0.334	0.146	0.080	0.009
Penicillium digitatum	0.597	0.324	0.394	0.156	0.063	-0.002
Neosartorya fischeri	0.743	0.705	0.444	-0.012	-0.007	0.006
Penicillium roqueforti	0.616	0.164	0.074	0.008	-0.009	0.006
Fusarium oxysporum	0.717	0.256	0.316	0.309	0.006	0.017

These results show that the use of dihydro eugenol in a sucrose solution at pH 5 has a strong antifungal effect against 15 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 15: OD measurements obtained using farnesol as an antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.947	0.203	0.080	0.144	0.021	0.036
Candida krusei	0.950	0.207	0.159	0.220	0.163	-0.003

These results show that the use of farnesol in a sucrose solution at pH 3 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 16: OD measurements obtained using a mixture of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.740	0.161	0.115	0.021	0.053	0.066

Aspergillus niger	0.275	0.239	0.266	0.184	0.145	0.144
Pennicillium crustosum	0.430	0.158	0.312	0.181	0.121	0.159
Penicillium digitatum	0.331	0.264	0.370	0.218	0.093	0.098
Penicillium roqueforti	0.576	0.264	0.323	0.245	0.157	0.083

These results show that the use of a mixture of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol in a sucrose solution at pH 3 has a strong antifungal effect against 5 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 17: OD measurements obtained using a mixture of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Candida krusei	1.051	0.075	0.188	0.031	0.098	0.076
Penicillium roqueforti	0.581	0.348	0.398	0.293	0.132	0.070

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These results show that the use of a mixture of 2-tert-butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol in a sucrose solution at pH 5 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

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Table 18: OD measurements obtained using thymol as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.996	0.129	0.098	0.123	0.029	-0.002
Candida krusei	0.745	0.154	0.158	0.184	0.134	0.022
Neosartorya fischeri	0.570	0.567	0.619	0.433	0.116	0.003
Penicillium roqueforti	0.492	0.347	0.398	0.351	0.115	0.064

These results show that the use of thymol in a sucrose solution at pH 3 has a strong antifungal effect against 4 of the main fungi responsible for beverage and food spoilage at

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concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 19: OD measurements obtained using 7-methyl-3-octen-2-one as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.789	0.033	0.085	-0.005	0.048	0.000
Pichia membranaefaciens	1.057	0.628	0.325	-0.006	-0.001	-0.003
Dekkera bruxellensis	1.410	0.673	0.493	-0.007	0.001	-0.004
Saccharomyces cerevisiae	0.393	0.204	0.032	0.024	0.000	-0.003
Candida krusei	0.789	0.002	-0.007	-0.011	-0.006	-0.001
Fusarium solani	0.393	0.893	0.447	0.000	0.004	0.011

These results show that the use of 7-methyl-3-octen-2-one in a sucrose solution at pH 3 has a strong antifungal effect against 6 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 20: OD measurements obtained using 7-methyl-3-octen-2-one as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.129	0.548	0.744	0.285	-0.011	0.003
Pichia membranaefaciens	0.788	0.756	0.415	-0.007	-0.010	0.002
Dekkera bruxellensis	1.421	1.363	1.396	0.916	-0.006	0.007
Saccharomyces cerevisiae	0.801	0.602	0.538	-0.001	-0.008	0.008
Candida krusei	0.973	-0.003	-0.010	-0.011	-0.010	0.002
Fusarium solani	0.853	0.666	0.350	0.006	0.018	0.049
Aspergillus fumigatis	0.254	0.576	0.261	0.600	0.196	0.087
Talaromyces harzanium	0.433	0.268	0.486	0.374	0.110	-0.009
Neosartorya fischeri	0.527	0.677	0.456	0.330	-0.010	-0.004

Penicillium roqueforti	0.542	0.272	0.624	0.553	-0.012	-0.004
Fusarium oxysporum	0.706	0.683	0.852	0.002	0.004	0.003

These results show that the use of 7-methyl-3-octen-2-one in a sucrose solution at pH 5 has a strong antifungal effect against 11 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 21: OD measurements obtained using 3,4-dimethylphenol as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.697	0.118	0.137	0.133	0.088	0.056
Candida krusei	0.745	0.156	0.128	0.126	0.103	0.056
Fusarium solani	0.452	0.663	0.375	0.674	0.197	0.020

These results show that the use of 3,4-dimethylphenol in a sucrose solution at pH 3 has a strong antifungal effect against 3 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 22: OD measurements obtained using 2-methylhexanoic acid at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.874	0.064	0.046	0.083	-0.007	-0.011
Pichia membranaefaciens	1.175	0.098	0.192	0.049	-0.002	-0.011
Dekkera bruxellensis	1.418	0.683	0.586	0.250	-0.001	-0.008
Saccharomyces cerevisiae	1.457	0.618	0.452	0.004	0.005	-0.001
Candida krusei	0.854	0.085	0.014	-0.005	-0.002	-0.004
Byssoschlamys nivea	0.229	0.362	-0.002	-0.011	0.002	0.014
Fusarium solani	0.808	0.000	-0.001	-0.008	0.006	0.018
Aspergillus fumigatis	0.210	0.116	0.133	-0.006	0.011	0.020
Byssoschlamys fulva	0.267	0.152	0.138	-0.006	0.007	0.022

Aspergillus niger	0.335	0.001	-0.008	-0.008	-0.001	0.019
Pennicillium crustosum	0.218	0.172	0.045	-0.007	-0.020	-0.011
Penicillium digitatum	0.244	0.148	0.049	-0.007	-0.012	-0.009
Neosartorya fischeri	0.495	0.016	-0.003	0.006	-0.011	-0.012
Penicillium roqueforti	0.481	0.002	-0.06	-0.008	-0.019	-0.011
Fusarium oxysporum	0.291	-0.002	0.003	0.005	-0.010	0.005

These results show that the use of 2-methylhexanoic acid in a sucrose solution at pH 3 has a strong antifungal effect against 15 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 23: OD measurements obtained using 2-methylhexanoic acid as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.068	0.201	0.114	0.077	0.050	0.000
Pichia membranaefaciens	0.823	0.277	0.145	0.189	-0.007	-0.003
Dekkera bruxellensis	1.422	1.06	0.807	0.158	-0.003	-0.015
Saccharomyces cerevisiae	0.823	0.507	0.651	0.229	-0.007	-0.023
Candida krusei	1.067	0.047	0.038	-0.007	-0.013	-0.027
Byssoschlamys nivea	0.587	0.223	0.004	0.155	-0.011	0.027
Fusarium solani	0.586	0.094	0.092	-0.007	-0.004	0.018
Aspergillus fumigatis	0.263	0.130	0.129	-0.013	-0.004	0.009
Byssoschlamys fulva	0.729	0.072	0.130	-0.005	-0.001	0.051
Aspergillus niger	0.402	0.067	0.033	-0.008	-0.021	0.051
Talaromyces harzanium	0.340	0.182	0.105	-0.018	-0.016	-0.009
Pennicillium crustosum	0.483	0.070	0.188	-0.018	-0.009	-0.007
Penicillium digitatum	0.513	0.068	0.140	-0.018	-0.008	-0.005
Neosartorya fischeri	0.789	-0.008	0.001	-0.012	-0.003	0.003
Penicillium roqueforti	0.615	0.016	0.097	-0.014	-0.008	-0.004
Fusarium oxysporum	0.520	0.048	0.006	-0.006	-0.001	0.007

These results show that the use of 2-methylhexanoic acid in a sucrose solution at pH 5 has a strong antifungal effect against 16 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 24: OD measurements obtained using 5-methyl-2-phenyl-2-hexenal as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.256	0.075	0.072	0.074	0.109	0.036
Saccharomyces cerevisiae	1.356	1.052	0.788	0.590	0.111	0.023
Candida krusei	1.423	0.177	0.332	0.127	0.109	0.153
Fusarium solani	0.713	-0.004	0.181	0.000	0.009	0.000
Aspergillus fumigatis	0.386	0.237	0.242	0.196	0.130	0.138
Talaromyces harzanium	0.213	0.402	0.236	0.399	-0.006	-0.001
Pennicillium crustosum	0.400	0.131	0.170	0.293	0.177	-0.009
Neosartorya fischeri	0.425	0.365	0.627	0.506	-0.001	-0.012
Fusarium oxysporum	0.546	0.380	0.377	0.472	0.003	0.010

These results show that the use of 5-methyl-2-phenyl-2-hexenal in a sucrose solution at pH 3 has a strong antifungal effect against 9 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 25: OD measurements obtained using 5-methyl-2-phenyl-2-hexenal as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Aspergillus niger	0.340	0.576	0.281	0.238	0.051	0.040
Talaromyces harzanium	0.354	0.632	0.178	0.301	-0.001	-0.017
Neosartorya fischeri	0.787	0.434	0.455	0.446	-0.011	-0.013

Penicillium roqueforti	0.602	0.215	0.256	0.282	0.176	-0.015
Fusarium oxysporum	0.946	0.216	0.248	0.001	0.000	-0.001

These results show that the use of 5-methyl-2-phenyl-2-hexenal in a sucrose solution at pH 5 has a strong antifungal effect against 5 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 26: OD measurements obtained using orcinyle as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.888	0.223	0.228	0.387	0.094	0.036
Candida krusei	1.023	0.135	0.234	0.036	0.073	0.125

These results show that the use of orcinyle in a sucrose solution at pH 3 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 27: OD measurements obtained with (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.263	0.117	0.127	0.147	0.126	0.117
Candida krusei	1.025	0.135	0.232	0.117	0.080	0.012
Talaromyces harzanium	0.230	0.821	0.208	0.240	0.112	0.155

These results show that the use of (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one in a sucrose solution at pH 3 has a strong antifungal effect against 3 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 28: OD measurements obtained using (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Candida krusei	1.153	0.132	0.201	0.094	0.044	0.040
Fusarium oxysporum	0.855	-0.035	-0.040	-0.042	-0.052	-0.020

These results show that the use of (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one in a sucrose solution at pH 5 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 29: OD measurements obtained using maniguette seed extract as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.854	0.061	0.098	0.120	0.121	0.073
Candida krusei	0.796	0.187	0.129	0.092	0.064	-0.054
Fusarium solani	0.447	0.326	0.566	0.556	0.059	0.036

These results show that the use of maniguette seed extract in a sucrose solution at pH 3 has a strong antifungal effect against 3 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 30: OD measurements obtained with maniguette seed extract at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Aspergillus niger	0.606	0.135	0.353	0.260	0.055	0.129
Penicillium roqueforti	0.623	0.132	0.351	0.259	0.111	0.037

These results show that the use of maniguette seed extract in a sucrose solution at pH 5 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 31: OD measurements obtained using orange extract (Tetrarome[®] orange, origin: Firmenich SA, Geneva, Switzerland) as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.796	0.117	0.127	0.147	0.126	0.117
Candida krusei	1.025	0.896	0.232	0.117	0.080	0.047

These results show that the use of Tetrarome[®] orange in a sucrose solution at pH 3 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 32: OD measurements obtained using 2-pentylfuran as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	0.971	0.089	0.064	0.146	0.094	0.036
Saccharomyces cerevisiae	1.443	0.475	0.236	0.721	0.044	0.030
Candida krusei	1.201	0.125	0.171	0.104	0.061	-0.007

These results show that the use of 2-pentylfuran in a sucrose solution at pH 3 has a strong antifungal effect against 3 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 33: OD measurements obtained using 2-pentylfuran as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Candida krusei	1.180	0.248	0.041	0.097	0.150	0.010
Fusarium oxysporum	0.658	0.456	1.236	0.562	-0.054	-0.057

These results show that the use of 2-pentylfuran in a sucrose solution at pH 5 has a strong antifungal effect against 2 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 34: OD measurements obtained using terpinolene as antifungal agent at pH 3

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Zygosaccharomyces rouxii	1.132	0.117	0.120	0.159	0.061	-0.006
Pichia membranaefaciens	1.112	1.074	1.155	1.109	0.198	-0.010
Dekkera bruxellensis	1.460	1.460	1.300	0.023	-0.007	-0.011
Saccharomyces cerevisiae	0.761	0.182	0.065	0.001	-0.003	-0.007
Candida krusei	1.106	0.095	0.045	0.344	-0.012	-0.014
Byssoschlamys nivea	0.952	0.383	-0.003	-0.012	0.008	0.017
Fusarium solani	0.746	0.207	0.590	0.268	-0.002	0.020
Aspergillus fumigatis	0.371	0.327	0.542	-0.010	0.003	0.021
Byssoschlamys fulva	0.275	0.544	0.002	-0.005	0.001	0.033
Aspergillus niger	0.349	0.341	0.282	-0.015	-0.006	0.025
Penicillium crustosum	0.507	-0.007	0.001	-0.011	-0.009	-0.017
Penicillium digitatum	0.382	0.487	-0.003	-0.013	-0.007	-0.018
Neosartorya fischeri	0.688	0.695	0.705	-0.005	-0.009	-0.011
Penicillium roqueforti	0.588	0.364	-0.004	-0.012	-0.009	-0.015
Fusarium oxysporum	0.932	0.774	0.405	-0.001	0.005	-0.005

These results show that the use of terpinolene in a sucrose solution at pH 3 has a strong antifungal effect against 15 of the main fungi responsible for beverage and food spoilage

at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

Table 35: OD measurements obtained using terpinolene as antifungal agent at pH 5

	Flavouring ingredient concentration (ppm)					
	0	125	250	500	1000	2000
Fusarium solani	0.743	0.658	0.854	0.655	-0.004	-0.010
Byssochlamys fulva	0.496	0.733	-0.003	0.000	-0.003	-0.005
Aspergillus niger	0.595	0.658	0.369	-0.004	-0.002	-0.009
Pennicillium crustosum	0.702	0.469	0.739	0.648	-0.005	0.025
Neosartorya fischeri	0.886	0.685	0.004	-0.009	0.006	0.031
Penicillium roqueforti	0.488	0.624	0.397	-0.015	-0.005	0.017
Fusarium oxysporum	0.962	0.741	0.954	-0.062	-0.065	-0.042

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These results show that the use of terpinolene in a sucrose solution at pH 5 has a strong antifungal effect against 7 of the main fungi responsible for beverage and food spoilage at concentrations of 1000 ppm or less. It can therefore be advantageously used as a preservative composition in such food products or beverages.

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Claims

1. Use of at least one flavouring ingredient selected from 2-methylhexanoic acid, 3,4-dimethylphenol, and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one or of a flavouring composition comprising at least one of such flavouring ingredients as an antifungal agent.

2. Use according to claim 1 wherein the flavouring composition further comprises at least one flavouring ingredient capable, at a concentration of 1000 ppm or less, of inhibiting the growth of two or more strains selected from yeast strains *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*, *Candida crusei*, *Dekkera bruxellensis*, *Pichia anomala*, *Zygosaccharomyces bailii*, *Dekkera naardensis* and *Candida albicans* and moulds such as *Byssoschlamys nivea*, *Byssoschlamys fulva*, *Neosartorya fischeri*, *Talaromyces harzanium*, *Fusarium solani*, *Aspergillus fumigatis*, *Aspergillus niger*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium roqueforti*, *Fusarium oxysporum*, and *Mucor rouxii* in a growth medium consisting of a 105g/L sucrose solution with an initial inoculum of 10^2 - 10^3 cfu/mL of such yeast or mould strain, so that the average optical density of the growth medium, as measured at 380nm for yeast strains and at 500nm for mould strains, is maintained below 0.2 after 21 days of culture at 21 °C or 25°C under aerobic conditions.

3. Use according to claim 2, wherein said further flavouring ingredient is selected from salicylic acid, carvacrol, dihydro eugenol, farnesol, a mixture of 2-tert-butyl-4-methoxyphenol and 3-ter-butyl-4-methoxyphenol, thymol, 7-methyl-3-octen-2-one, 5-methyl-2-phenyl-2-hexenal, 3-methoxy-5-methylphenol, maniguette seed extract, orange extract, 2-pentylfuran and terpinolene.

4. Use according to claim 3 wherein said further flavouring ingredient is selected from 5-methyl-2-phenyl-2-hexenal, dihydroeugenol and terpinolene.

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5. Use according to any one of claims 2 to 4, wherein the flavouring composition comprises from 1 to 25% by weight of 3,4-dimethylphenol, 2-methylhexanoic acid and/or (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one.

6. Use according to any one of claims 1 to 5, wherein the flavouring ingredient or composition is used as antifungal agent in a food product or beverage.

5 7. A method for preserving a food product or a beverage comprising adding at least one flavouring ingredient selected from 3,4-dimethylphenol, 2-methylhexanoic acid and (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one or a flavouring composition comprising at least one of such flavouring ingredients to the food product or to the beverage.

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8. A method according to claim 7, wherein the flavouring composition further comprises at least one flavouring ingredient capable, at a concentration of 1000 ppm or less, of inhibiting the growth of two or more strains selected from yeast strains *Zygosaccharomyces rouxii*, *Saccharomyces cerevisiae*, *Pichia membranaefaciens*,
15 *Candida crusei* and *Dekkera bruxellensis* and mould strains *Byssoschlamys nivea*, *Byssoschlamys fulva*, *Neosartorya fischeri*, *Penicillium crusotsum*, *Penicillium digitatum*, *Penicillium roqueforti* and *fusarium oxysporum* in a growth medium consisting of a 105g/L sucrose solution with an initial inoculum of 10^2 - 10^3 cfu/mL of such yeast or mould strain, so that the average optical density of the growth medium, as measured at
20 380nm for yeast strains and at 500nm for mould strains, is maintained below 0.2 after 21 days of culture at 21 °C or 25°C under aerobic conditions.

9. A method according to claim 8, wherein said further flavouring ingredients is selected from salicylic acid, carvacrol, dihydro eugenol, farnesol, a mixture of 2-tert-
25 butyl-4-methoxyphenol and 3-tert-butyl-4-methoxyphenol, thymol, 7-methyl-3-octen-2-one, 5-methyl-2-phenyl-2-hexenal, 3-methoxy-5-methylphenol, maniguette seed extract, orange extract, 2-pentylfuran and terpinolene.

10. A method according to claim 9, wherein said further flavouring ingredient is
30 selected from 5-methyl-2-phenyl-2-hexenal, dihydroeugenol and terpinolene.

11. A method according to any one of claims 7 to 10, wherein the flavouring composition comprises from 1 to 25% by weight of 3,4-dimethylphenol,

2-methylhexanoic acid and/or (+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one.

12. A method according to any one of claims 7 to 11, wherein the beverage is selected
5 from sweet beverages comprising sugar or a sugar derivative.

13. A method according to any one of claims 7 to 12, wherein the beverage is selected
from carbonated soft drinks, functional soft drinks, juices and nectars, hot drinks, and
alcoholic drinks.

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14. A method according to any one of claims 7 to 11, wherein the food product is
selected from soups, sauces, dressings, marinades, dairy products, refrigerated food,
acidified food, meat products and prepared fruits and vegetables.

15 15. An antifungal agent in the form of a composition comprising at least one
ingredient selected from 2-methylhexanoic acid, 3,4-dimethylphenol and
(+)-(3S,3AS,6R,7AR)-perhydro-3,6-dimethyl-benzo[B]furan-2-one.