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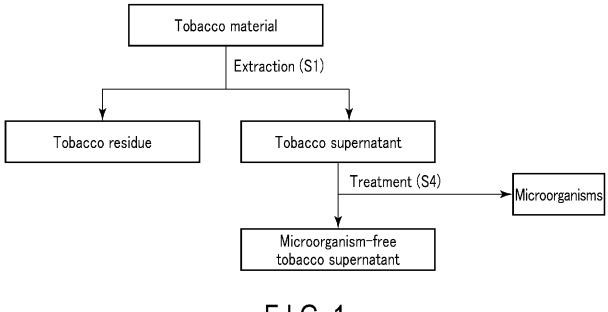
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- (54) TOBACCO-FLAVORED LIQUID MANUFACTURING METHOD, TOBACCO-FLAVORED LIQUID, AND FLAVOR INHALER
- (57) Provided is a tobacco-flavored liquid manufacturing method that includes processing a tobacco liquid extract using a material containing an inorganic porous body, and removing microorganisms from the tobacco liquid extract.



F I G. 1

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#### Description

**FIELD** 

[0001] The present invention relates to a method for producing a tobacco flavor liquid, a tobacco flavor liquid, and a flavor inhaler.

#### **BACKGROUND**

10 [0002] Dried leaves of a tobacco plant are used as a flavor source of a flavor inhaler such as a cigarette or a heating-type tobacco product, and are called leaf tobacco. Leaf tobacco contains various flavor components. The leaf tobacco itself may be used directly as a flavor source of a flavor inhaler; alternatively, a tobacco extract liquid obtained by extracting a tobacco flavor component from the leaf tobacco may be used as a flavor source of a flavor inhaler. Since the tobacco extract liquid contains microorganisms such as yeast, it is desirable to remove the microorganisms if the tobacco extract liquid is used as a flavor source of a flavor inhaler.

**[0003]** Also, it is known that a tobacco extract liquid is reacted with microorganisms such as yeast to increase the amounts of flavor components contained in the tobacco extract liquid (for example, Patent Literature 1). In this case as well, it is desirable to remove the microorganisms if the tobacco extract liquid after the reaction is used as a flavor source of a flavor inhaler.

CITATION LIST

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PATENT LITERATURE

[0004] Patent Literature 1: U.S. Patent No. 4895175

**SUMMARY** 

#### **TECHNICAL PROBLEM**

**[0005]** An object is to provide a technique of removing microorganisms such as yeast from a tobacco extract liquid by a simple method without impairing a tobacco flavor.

#### SOLUTION TO PROBLEM

**[0006]** According to one aspect, there is provided a method for producing a tobacco flavor liquid, the method comprising treating a tobacco extract liquid with a material including an inorganic porous body, thereby removing microorganisms from the tobacco extract liquid.

**[0007]** According to another aspect, there is provided a tobacco flavor liquid obtainable by the above-mentioned method.

**[0008]** According to further another aspect, there is provided a flavor inhaler comprising the above-mentioned tobacco flavor liquid.

## ADVANTAGEOUS EFFECTS OF INVENTION

**[0009]** According to the present invention, there is provided a technique of removing microorganisms such as yeast from a tobacco extract liquid by a simple method without impairing a tobacco flavor.

# BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

- FIG. 1 is a flowchart showing an example of a method for producing a tobacco flavor liquid.
- FIG. 2 is a flowchart showing another example of a method for producing a tobacco flavor liquid.
- FIG. 3 is a cross-sectional view showing an example of a combustion-type flavor inhaler.
  - FIG. 4 is a perspective view showing an example of a heating-type flavor inhaler.
  - FIG. 5 is a diagram showing an internal structure of a tobacco stick.
  - FIG. 6 is a diagram showing an internal structure of an aerosol-generation device.

#### DETAILED DESCRIPTION

[0011] Hereinafter, the present invention will be described in detail; however, the description below is intended to provide a description of the present invention, and not intended to limit the present invention.

<1. Method for Producing Tobacco Flavor Liquid>

[0012] A method for producing a tobacco flavor liquid includes treating a tobacco extract liquid with a material including an inorganic porous body, thereby removing microorganisms from the tobacco extract liquid.

[0013] Since the microorganisms contained in the tobacco extract liquid are removed by this method, the resulting tobacco flavor liquid has reduced amounts of microorganisms as compared with the tobacco extract liquid. As used herein, the term "microorganism" refers to any microorganism contained in a tobacco extract liquid immediately before the tobacco extract liquid is treated with a material including an inorganic porous body, and includes bacteria and fungi.

[0014] In a first embodiment, a tobacco extract liquid is a tobacco supernatant obtained by extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent. The method according to the first embodiment will be detailed in <1-1. First Embodiment> below.

[0015] In a second embodiment, a tobacco extract liquid is a useful-component eluate obtained by a method including the following steps (a) to (c):

- (a) extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent, thereby obtaining a tobacco supernatant;
- (b) culturing yeast in the tobacco supernatant, thereby obtaining a yeast-containing culture liquid; and
- (c) mixing the yeast-containing culture liquid with an elution solvent including an organic solvent, and eluting, from the yeast contained in the resultant mixture and into a liquid portion of the mixture, a useful component included in fungal cells of the yeast, thereby obtaining a useful-component eluate.
- [0016] The method according to the second embodiment will be detailed in <1-2. Second Embodiment> below.

#### <1-1. First Embodiment>

[0017] According to the first embodiment, a method for producing a tobacco flavor liquid includes:

- (S1) extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent, thereby obtaining a tobacco supernatant; and
- (S4) treating the tobacco supernatant with a material including an inorganic porous body, thereby obtaining a microorganism-free tobacco supernatant.

[0018] The method according to the first embodiment is shown in FIG. 1. The method according to the first embodiment will be described in the order of steps (S1) and (S4) with reference to FIG. 1. The "microorganism-free tobacco supernatant" obtained by the method according to the first embodiment can be used as a "tobacco flavor liquid".

[Extraction Step (S1)]

[0019] In the extraction step (S1), a water-soluble component contained in a tobacco material is extracted from the tobacco material by using an aqueous solvent, whereby a tobacco supernatant is obtained. In the extraction step (S1), a tobacco residue is also obtained at the same time as the tobacco supernatant is obtained (see FIG. 1).

[0020] As the tobacco material, cut tobacco which is ready to be incorporated into a tobacco product, such as a combustion-type or heating-type flavor inhaler, can be used. The "cut tobacco which is ready to be incorporated into a tobacco product" refers to cut tobacco which is ready to be incorporated into a tobacco product through various processes including drying in a farm house, subsequently one to several years of long-term aging in a leaf processing facility, and, subsequently to that, blending and cutting in a manufacturing facility.

[0021] The cut tobacco consists of cut pieces of leaf tobacco. The cut tobacco may be any of the following: cut pieces of stemmed leaves, cut pieces of midrib, cut pieces of reconstituted tobacco (i.e., a tobacco material obtained by processing leaf scraps, cut tobacco scraps, midrib scraps, fine powder, etc., generated in the facility processes into a reusable shape), or a mixture thereof. A pulverized product obtained by pulverizing the cut tobacco may be used for the extraction in order to increase extraction efficiency.

[0022] As the cut tobacco, cut tobacco derived from any tobacco variety can be used. For example, cut tobacco derived from flue-cured tobacco, Burley tobacco, Oriental tobacco or the like can be used. As the cut tobacco, cut tobacco

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derived from a single variety, or a mixture of cut tobacco derived from different varieties may be used.

**[0023]** As the aqueous solvent, water or water-containing ethanol can be used. As the water-containing ethanol, a mixture of ethanol and water at a volume ratio of 1:1, for example, can be used. The aqueous solvent is generally water, and preferably water having a room temperature (e.g., approximately 20 °C) to a temperature of 70 °C. The aqueous solvent can be used, for example, in an amount of 500 to 5000% by mass with respect to the tobacco material. **[0024]** The extraction can be performed by, for example, immersing the tobacco material in warm water having a temperature of 40 to 60 °C for 30 to 180 minutes, or shaking the tobacco material (at, e.g., 200 rpm) in warm water having a temperature of 40 to 60 °C for 30 to 180 minutes.

**[0025]** The extraction may also be performed by repeating the extraction multiple times. Specifically, the extraction may be performed by extracting, from the tobacco material, the water-soluble component contained in the tobacco material by using the aqueous solvent, and then placing the resultant tobacco residue in a new aqueous solvent to perform the second extraction, and as necessary, repeating the extraction using a new aqueous solvent.

**[0026]** A mixture of the tobacco residue and the tobacco supernatant is obtained by the extraction. The tobacco supernatant contains a water-soluble component contained in the tobacco material. Examples of the "water-soluble component contained in the tobacco material" include components serving as a nutrient source for microorganisms such as yeast (e.g., saccharides, amino acids, proteins, and nutrient salts) and components that contribute to tobacco flavor (e.g., organic acids, foliar resins, terpenoids, and polyphenols).

[0027] After the extraction, the tobacco residue and the tobacco supernatant are separated, and the tobacco supernatant is used as a raw material for obtaining a tobacco flavor liquid. On the other hand, the tobacco residue can be used for preparing a tobacco filler by mixing the tobacco residue with the finally obtained tobacco flavor liquid (the "microorganism-free tobacco supernatant" in this embodiment) and appropriately processing the resultant mixture. For example, the tobacco residue may be used for preparing a tobacco-molded body such as sheet tobacco from a mixture obtained by mixing the tobacco residue with the finally obtained tobacco flavor liquid. Alternatively, the tobacco residue may be used for preparing a tobacco powder by mixing the tobacco residue with the finally obtained tobacco flavor liquid and drying and pulverizing the resultant mixture.

[Treatment Step (S4)]

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**[0028]** In the treatment step (S4), the tobacco supernatant is treated with a material including an inorganic porous body, thereby removing microorganisms from the tobacco supernatant. Thus, a microorganism-free tobacco supernatant is obtained as a tobacco flavor liquid.

**[0029]** The inorganic porous body is, for example, diatomaceous earth or zeolite. Diatomaceous earth is a deposit consisting of fossilized shells of diatoms and contains silicon dioxide as a main component. Zeolite is a microporous crystalline hydrous aluminosilicate, and may be natural zeolite or synthetic zeolite.

**[0030]** The microorganisms that can be removed in the treatment step (S4) are microorganisms contained in the tobacco supernatant, and examples thereof include yeast, mold, and microalgae. Since the clarity of the tobacco flavor liquid is demonstrated in Example 1 described later, it is considered that any microorganisms contained in the tobacco supernatant can be removed in the treatment step (S4), and that fine particles other than microorganisms, such as fine particles having a size of 1  $\mu$ m or more, can also be removed in the treatment step (S4).

**[0031]** Since the microorganisms in the tobacco flavor liquid change the composition of the tobacco flavor liquid through metabolism and cause quality deterioration of the tobacco flavor liquid, it is preferable that the tobacco flavor liquid not contain microorganisms. Also, if the tobacco flavor liquid is applied to an electronic cigarette (i.e., a smoking article in which the tobacco flavor liquid is heated by a heater and vaporized to be inhaled by a user), it is preferable that the tobacco flavor liquid not contain microorganisms because the microorganisms in the tobacco flavor liquid cause the heater to burn.

**[0032]** The treatment step (S4) may be performed by passing the tobacco supernatant through a material including an inorganic porous body. Alternatively, the treatment step (S4) may be performed by adding a material including an inorganic porous body to the tobacco supernatant and then removing the material including an inorganic porous body from the tobacco supernatant.

**[0033]** In a preferred embodiment, the treatment step (S4) can be performed by passing the tobacco supernatant through a layer including an inorganic porous body. The layer including an inorganic porous body is, for example, a layer including diatomaceous earth or a layer including zeolite. The "layer including an inorganic porous body" may be a layer composed only of an inorganic porous body, or a layer including an inorganic porous body as a main component and an additive as an additional component. Examples of the additive include a molding aid and a binder.

**[0034]** In an embodiment, the "layer including an inorganic porous body" may be composed of a molded body including an inorganic porous body. That is, the treatment step (S4) may be performed by passing the tobacco supernatant through a molded body including an inorganic porous body. The molded body including an inorganic porous body is, for example, a molded body including diatomaceous earth or a molded body including zeolite. The "molded body including an inorganic

porous body" may be a molded body composed only of an inorganic porous body, or a molded body including an inorganic porous body as a main component and an additive as an additional component. Examples of the additive include a molding aid and a binder. More specifically, the "molded body including an inorganic porous body" may be a calcined molded body including an inorganic porous body, or a compressed molded body of particles including an inorganic porous body.

**[0035]** For example, Celite (registered trade name) (GL Sciences Inc.) or SUPRAdisc (Pall Corporation) can be used as the molded body including diatomaceous earth.

**[0036]** Alternatively, in another embodiment, the "layer including an inorganic porous body" may be composed of an aggregate of particles including an inorganic porous body. That is, the treatment step (S4) may be performed by passing the tobacco supernatant through an aggregate of particles including an inorganic porous body. The particles including an inorganic porous body are, for example, particles including diatomaceous earth or particles including zeolite. The "particles including an inorganic porous body" may be particles composed only of an inorganic porous body, or particles including an inorganic porous body as a main component and an additive as an additional component. Examples of the additive include a molding aid and a binder.

[0037] The "particles including an inorganic porous body" have, for example, a particle size of <400  $\mu$ m, generally a particle size of 10 to 400  $\mu$ m, preferably a particle size of <100  $\mu$ m, more preferably a particle size of 10 to 100  $\mu$ m, still more preferably a particle size of 10 to 80  $\mu$ m, and yet more preferably a particle size of 30 to 75  $\mu$ m. A particle size of <X  $\mu$ m means that the particle diameter is smaller than X  $\mu$ m. That is, a particle size of <X  $\mu$ m refers to the particle size of the particle sthat have passed through a sieve with an opening of X  $\mu$ m. Also, a particle size of Y to Z  $\mu$ m means that the particle diameter is Y  $\mu$ m or more and smaller than Z  $\mu$ m. That is, a particle size of Y to Z  $\mu$ m refers to the particle size of the particles that have passed through a sieve with an opening of Z  $\mu$ m but have not passed through a sieve with an opening of Y  $\mu$ m. In the present disclosure, particle size refers to a value measured by a sieving particle size measurement method (JIS Z 8815: 1994).

[0038] For example, a pulverized product of diatomaceous earth (particle size: <354  $\mu$ m) (GL Sciences Inc.) commercially available under the trade name of K-solute can be used as the particles including diatomaceous earth. For example, synthetic zeolite powder (particle size: <75  $\mu$ m) (FUJIFILM Wako Pure Chemical Corporation) can be used as the particles including zeolite.

**[0039]** In a more preferred embodiment, the treatment step (S4) can be performed by passing the tobacco supernatant through a column filled with an aggregate of particles including an inorganic porous body. The "particles including an inorganic porous body" are as described above, and are, for example, particles including diatomaceous earth or particles including zeolite. For example, a column having an inner diameter of 11 to 100 mm and a length of 100 to 1000 mm can be used as the column. The tobacco supernatant can be passed through the column, for example, by a gravity fall.

#### <1-2. Second Embodiment>

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**[0040]** In the second embodiment, a tobacco supernatant is reacted with yeast to increase the amounts of flavor components contained in the tobacco supernatant, and then microorganisms are removed from the tobacco supernatant having increased amounts of flavor components. Specifically, according to the second embodiment, a method for producing a tobacco flavor liquid includes:

- (S1) extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent, thereby obtaining a tobacco supernatant;
- (S2) culturing yeast in the tobacco supernatant, thereby obtaining a yeast-containing culture liquid;
- (S3) mixing the yeast-containing culture liquid with an elution solvent including an organic solvent, and eluting, from the yeast contained in the resultant mixture and into a liquid portion of the mixture, a useful component included in fungal cells of the yeast, thereby obtaining a useful-component eluate; and
- (S4) treating the useful-component eluate with a material including an inorganic porous body, thereby obtaining a microorganism-free useful-component eluate.

[0041] The method according to the second embodiment is shown in FIG. 2. The method according to the second embodiment will be described in the order of steps (S1), (S2), (S3), and (S4) with reference to FIG. 2. The description below omits description of the same elements as those of the method according to the first embodiment and provides description of only the elements differing from those of the method according to the first embodiment. The "microorganism-free useful-component eluate" obtained by the method according to the second embodiment can be used as a "tobacco flavor liquid".

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[Extraction Step (S1)]

**[0042]** In the extraction step (S1), a water-soluble component contained in a tobacco material is extracted from the tobacco material by using an aqueous solvent, whereby a tobacco supernatant is obtained. In the extraction step (S1), a tobacco residue is also obtained at the same time as the tobacco supernatant is obtained (see FIG. 2). The extraction step (S1) can be performed in the same manner as the extraction step (S1) described in the method of the first embodiment.

[Culture Step (S2)]

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[0043] In the culture step (S2), yeast is cultured in the tobacco supernatant obtained in the extraction step (S1), whereby a yeast-containing culture liquid is obtained (see FIG. 2).

[0044] Any type of yeast can be used as the yeast, provided that it can produce a useful component when cultured in the tobacco supernatant.

**[0045]** The term "useful component" as used herein refers to a component which is useful for the finally obtained tobacco flavor liquid. The useful component may be, for example, a component that contributes to the flavor of the tobacco flavor liquid (hereinafter referred to as "a flavor-contributing component"), a component that colors the tobacco flavor liquid (hereinafter referred to as "a coloring component"), or a component that prevents putrefaction or fermentation of the tobacco flavor liquid (hereinafter referred to as "a preservative component").

**[0046]** The useful component is preferably a flavor-contributing component. The "flavor-contributing component" may be a flavor component that releases a flavor, or a precursor that is converted into a flavor component when heated or burned in a flavor inhaler.

**[0047]** Examples of the flavor-contributing component include carotenoids, fatty acids, neutral fats (i.e., glycerin esters of fatty acids), acetic acid esters, fatty acid esters, organic acids, and higher alcohols (e.g., alcohols having 8 to 22 carbon atoms). Examples of the coloring component include carotenoids. Examples of the preservative component include lactic acid, fatty acid glycosides, and benzoic acid.

**[0048]** Therefore, the yeast known to produce the above-described useful components can be used in the method of the present invention.

**[0049]** Examples of the yeast that produces carotenoids include: yeast of the genus Rhodotorula, such as Rhodotorula alborubescens, Rhodotorula araucariae, Rhodotorula babjevae, Rhodotorula dairenensis, Rhodotorula diobovata, Rhodotorula evergladensis, Rhodotorula glutinis, Rhodotorula graminis, Rhodotorula kratochvilovae, Rhodotorula mucilaginosa, Rhodotorula ngohengohe, Rhodotorula pacifica, Rhodotorula paludigena, Rhodotorula sinensis, Rhodotorula sphaerocarpa, Rhodotorula taiwanensis, and Rhodotorula toruloides; and yeast of the genus Xanthophyllomyces, such as Xanthophyllomyces australis, Xanthophyllomyces rhodozyma, and Xanthophyllomyces tasmanica.

**[0050]** Examples of the yeast that produces fatty acids include: yeast of the genus Yarrowia, such as Yarrowia alimentaria, Yarrowia bubula, Yarrowia deformans, Yarrowia divulgata, Yarrowia galli, Yarrowia hollandica, Yarrowia keelungensis, Yarrowia lipolytica, Yarrowia osloensis, Yarrowia parophoni, Yarrowia phangngaensis, Yarrowia porcina, and Yarrowia yakushimensis; and yeast of the genus Lipomyces, such as Lipomyces anomalus, Lipomyces arxii, Lipomyces chichibuensis, Lipomyces doorenjongii, Lipomyces japonicus, Lipomyces kockii, Lipomyces kononenkoae, Lipomyces lipofer, Lipomyces mesembrius, Lipomyces okinawensis, Lipomyces oligophaga, Lipomyces orientalis, Lipomyces smithiae, Lipomyces spencermartinsiae, and Lipomyces starkeyi.

**[0051]** Examples of the yeast that produces acetic acid esters, fatty acid esters, or higher alcohols include: yeast of the genus Saccharomyces, such as Saccharomyces cerevisiae, Saccharomyces paradoxus, Saccharomyces bayanus, Saccharomyces uvarum, and Saccharomyces arboricola; yeast of the genus Cyberlindnera, such as Cyberlindnera jadinii, Cyberlindnera saturnus, Cyberlindnera fabianii, Cyberlindnera suaveolens, Cyberlindnera americana, and Cyberlindnera xylosilytyca; and yeast of the genus Wickerhamomyces, such as Wickerhamomyces anomalus, Wickerhamomyces ciferri, and Wickerhamomyces canadensis.

**[0052]** One type of yeast, or two or more types of yeast, may be cultured in the tobacco supernatant. The yeast may also be recombinant yeast which is genetically modified to increase the production amounts of useful components.

**[0053]** The conditions for culturing the yeast are not particularly limited. Conditions suitable for the growth of the yeast to be used and the production of the useful components can be appropriately selected. Prior to the culturing, the yeast can be added to the tobacco supernatant at a concentration of, for example, 10 to 10<sup>8</sup> cells/mL. The culture can be performed at, for example, 10 to 40 °C for, for example, 5 to 168 hours.

**[0054]** The tobacco supernatant contains a component serving as a nutrient source for the yeast and a component serving as a raw material of the useful components, and can provide an environment suitable for the growth of the yeast and the production of the useful components. Thus, it is unnecessary to add an additional component to the tobacco supernatant. However, the method of the present invention does not exclude the addition of an additional component to the tobacco supernatant.

[0055] In the present disclosure, a mixture of the yeast and the tobacco supernatant obtained after the yeast is cultured

in the tobacco supernatant is referred to as "a yeast-containing culture liquid". In the yeast-containing culture liquid, the amounts of useful components produced by the yeast are increased as compared with the "mixture of the yeast and the tobacco supernatant prior to the culture". Also, in the yeast-containing culture liquid, the amounts of substances consumed by the yeast for its own growth and for the production of the useful components are reduced, as compared with the "mixture of the yeast and the tobacco supernatant prior to the culture".

[Elution Step (S3)]

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**[0056]** In the elution step (S3), the yeast-containing culture liquid obtained in the culture step (S2) is mixed with an elution solvent including an organic solvent, and a useful component included in the fungal cells of the yeast contained in the resultant mixture is eluted from the yeast into the liquid portion of the mixture. Thereby, a useful-component eluate is obtained (see FIG. 2).

**[0057]** Herein, the mixture that is obtained after the elution step is completed and that contains the yeast and the elution solvent is referred to as "a useful-component eluate".

**[0058]** An elution solvent including an organic solvent can be used as the elution solvent. The elution solvent may be an organic solvent itself or a mixture of an organic solvent and water. The elution solvent is preferably an elution solvent including an organic solvent miscible with water, and more preferably an elution solvent including an alcohol miscible with water. That is, a preferred elution solvent is an alcohol miscible with water or its water-containing alcohol (i.e., said alcohol with water contained therein).

**[0059]** The organic solvent included in the elution solvent is preferably an organic solvent having an SP value of 10 to 14.5, and more preferably an alcohol having an SP value of 10 to 14.5. Examples of the organic solvent included in the elution solvent include ethanol, isopropanol, methanol, and butanol. The organic solvent included in the elution solvent is more preferably ethanol or isopropanol, and most preferably ethanol. That is, the most preferred elution solvent is ethanol or water-containing ethanol.

**[0060]** The SP value refers to a value of a Hildebrand solubility parameter. The higher the SP value of the solvent, the higher the miscibility with water. The SP value is known for various solvents. For example, the SP value of ethanol is 12.7, the SP value of isopropanol is 11.5, the SP value of methanol is 14.5, and the SP value of butanol is 11.4.

[0061] One kind of organic solvent, or a mixture of two or more kinds of organic solvents, may be used as the organic solvent included in the elution solvent.

[0062] The elution solvent can be added to the yeast-containing culture liquid in such an amount that the concentration of the organic solvent in the mixed solution of the yeast-containing culture liquid and the elution solvent becomes, for example, 50% by volume or more, preferably 50 to 95% by volume, and more preferably 60 to 95% by volume. The concentration of the organic solvent can be appropriately adjusted in consideration of the efficiency of eluting the useful component. Herein, the "mixed solution of the yeast-containing culture liquid and the elution solvent" refers to a mixed solution of the liquid portion of the yeast-containing culture liquid (i.e., the tobacco supernatant after the culture) and the elution solvent, and it does not contain yeast.

**[0063]** The elution solvent can be added in an amount of, for example, 100 to 900% by volume with respect to the yeast-containing culture liquid. The amount of the elution solvent added can be appropriately adjusted in consideration of the efficiency of eluting the useful component.

**[0064]** For example, if ethanol or water-containing ethanol is used as the elution solvent to elute fatty acids as useful components, the ethanol can be added to the yeast-containing culture liquid in such an amount that the concentration of the ethanol in the mixed solution of the yeast-containing culture liquid and the elution solvent becomes, for example, 50% by volume or more, preferably 50 to 90% by volume, more preferably 60 to 90% by volume, still more preferably 60 to 80% by volume, and most preferably 70% by volume.

[0065] For example, if ethanol or water-containing ethanol is used as the elution solvent to elute carotenoids as useful components, the ethanol can be added to the yeast-containing culture liquid in such an amount that the concentration of the ethanol in the mixed solution of the yeast-containing culture liquid and the elution solvent becomes, for example, 50% by volume or more, preferably 50 to 90% by volume, more preferably 60 to 90% by volume, still more preferably 70 to 90% by volume, yet more preferably 80 to 90% by volume, and most preferably 90% by volume.

**[0066]** In the elution step (S3), after the yeast-containing culture liquid is mixed with the elution solvent, a useful component included in the fungal cells of the yeast contained in the resultant mixture is eluted from the yeast into the liquid portion of the mixture. The elution can be performed by stirring the mixture of the yeast-containing culture liquid and the elution solvent for a predetermined period of time, while heating the mixture if necessary.

**[0067]** The elution is preferably performed while stirring the mixture from the standpoint of the efficiency of eluting the useful components; however, it may be performed while allowing the mixture to stand. In the case of stirring the mixture, the stirring rate can be, for example, 60 to 300 rpm.

**[0068]** If heating promotes the elution of the useful components, the elution may be performed while heating; and if heating does not show the effect of promoting the elution by heating, the elution may be performed at room temperature

(for example, 15 to 25 °C) without heating.

**[0069]** The elution temperature and the elution time can be appropriately adjusted in consideration of the efficiency of eluting the useful component.

**[0070]** For example, if ethanol or water-containing ethanol is used as the elution solvent to elute fatty acids as useful components, the elution can be performed by stirring the mixture at room temperature (for example, 15 to 25 °C) for 5 to 60 minutes.

**[0071]** For example, if ethanol or water-containing ethanol is used as the elution solvent to elute carotenoids as useful components, the elution can be performed by stirring the mixture for 15 to 60 minutes while heating the mixture to 80 to 95 °C.

[Treatment Step (S4)]

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**[0072]** In the treatment step (S4), the useful-component eluate obtained in the elution step (S3) is treated with a material including an inorganic porous body, thereby removing microorganisms from the useful-component eluate. Thus, a microorganism-free useful-component eluate is obtained as a tobacco flavor liquid. The treatment step (S4) can be performed in the same manner as the treatment step (S4) described in the method of the first embodiment.

[0073] The microorganisms that can be removed in the treatment step (S4) are the yeast used in the culture step (S2) and the microorganisms contained in the tobacco supernatant. Examples of the microorganisms contained in the tobacco supernatant include yeast, mold, and microalgae. Since the clarity of the tobacco flavor liquid is demonstrated in Example 3 described later, it is considered that the yeast used in the culture step (S2) and any microorganisms contained in the tobacco supernatant can be removed in the treatment step (S4), and that fine particles other than microorganisms, such as fine particles having a size of 1  $\mu$ m or more, can also be removed in the treatment step (S4).

**[0074]** The method according to the second embodiment may further include removing the organic solvent from the "microorganism-free useful-component eluate" after obtaining the "microorganism-free useful-component eluate". The removal of the organic solvent can be performed by a general method such as concentration under reduced pressure, concentration under normal pressure, or spray drying.

<1-3. Effects>

[0075] According to the method of the present invention, it is possible to remove microorganisms such as yeast from a tobacco extract liquid (a "tobacco supernatant" in the first embodiment and a "useful-component eluate" in the second embodiment) by a simple method without impairing a tobacco flavor. Thus, it is possible to produce a tobacco flavor liquid with reduced amounts of microorganisms (a "microorganism-free tobacco supernatant" in the first embodiment and a "microorganism-free useful-component eluate" in the second embodiment) by a simple method without impairing a tobacco flavor.

**[0076]** The effects of the present invention will be described in detail below.

[0077] The method of the present invention only necessitates treatment with a material including an inorganic porous body in order to remove microorganisms from a tobacco extract liquid, and does not necessitate performance of micro-filtration. Microfiltration requires time for filtration and requires labor for maintenance of an apparatus such as elimination of clogging of a filtration membrane. The method of the present invention, which involves low resistance even when a tobacco extract liquid is passed through a layer including an inorganic porous body, is obviously simpler than treatment with a microfiltration membrane and excels in the production efficiency.

**[0078]** Since the method of the present invention can remove microorganisms to the same extent as treatment with a microfiltration membrane, the method excels in the efficiency of removing microorganisms (see Examples 1 and 3 described later).

**[0079]** In addition, since the method of the present invention removes microorganisms and fine particles having the same size as the microorganisms but does not remove tobacco flavor components, the tobacco flavor liquid of the present invention excels as a tobacco flavor source of a flavor inhaler (see Example 4 described later).

**[0080]** In particular, the method according to the second embodiment involves culturing yeast in a tobacco supernatant, followed by eluting useful components contained in the fungal cells of the yeast into the tobacco supernatant and removing the yeast from the obtained useful-component eluate. Therefore, the method according to the second embodiment can produce a tobacco flavor liquid containing large amounts of useful components and having reduced amounts of microorganisms.

<2. Tobacco Flavor Liquid>

**[0081]** According to another aspect, there is provided a tobacco flavor liquid obtainable by the "method for producing a tobacco flavor liquid" described above.

[0082] As described above, the tobacco flavor liquid may be any of the following:

- (i) a "microorganism-free tobacco supernatant" obtained by the method of the first embodiment; and
- (ii) a "microorganism-free useful component eluate" obtained by the method of the second embodiment.

**[0083]** Thus, the tobacco flavor liquid produced by the above-described "method for producing a tobacco flavor liquid" includes the above two types of products as specific examples.

**[0084]** As described above, since microorganisms have been removed from the tobacco flavor liquid, the possibility that the composition of the tobacco flavor liquid will change due to the metabolism of microorganisms can be eliminated, and the tobacco flavor liquid excels in quality stability when used as a flavor source of a flavor inhaler.

**[0085]** In particular, since the tobacco flavor liquid obtainable by the method according to the second embodiment is produced by the method according to the second embodiment described above, it can contain large amounts of useful components. Thus, if this tobacco flavor liquid is incorporated into a flavor inhaler, the effects of the useful components can be remarkably exhibited in the flavor inhaler. For example, if the useful components are flavor-contributing components, the tobacco flavor liquid can contain large amounts of flavor-contributing components and thus provide a user with an enhanced flavor when incorporated into a tobacco product such as a flavor inhaler.

**[0086]** The tobacco flavor liquid produced by the above-described "method for producing a tobacco flavor liquid" can be incorporated into a tobacco product such as a flavor inhaler according to a known technique. Examples of the use of the tobacco flavor liquid will be described below.

**[0087]** For example, the tobacco flavor liquid can be used by adding the tobacco flavor liquid to a tobacco material (e.g., stemmed leaves or leaf tobacco) and drying the resultant mixture.

**[0088]** Alternatively, the tobacco flavor liquid can be added to a tobacco residue obtained in the extraction step (S1) described above, and a tobacco-molded body such as a sheet tobacco or tobacco granules can be prepared from the resultant mixture and used as a tobacco flavor source of a tobacco product.

**[0089]** Alternatively, the tobacco flavor liquid can be used by adding the tobacco flavor liquid to a tobacco residue obtained in the extraction step (S1) described above, drying and pulverizing the resultant mixture to prepare a tobacco powder, and adding the tobacco powder to a tobacco material (e.g., stemmed leaves or leaf tobacco).

**[0090]** Alternatively, the tobacco flavor liquid can be used by adding the tobacco flavor liquid to a tobacco residue obtained in the extraction step (S1) described above, drying and pulverizing the resultant mixture to prepare a tobacco powder, suspending the tobacco powder in water to prepare a tobacco slurry, and adding the tobacco slurry to a tobacco material (e.g., stemmed leaves or leaf tobacco).

**[0091]** Alternatively, the tobacco flavor liquid can be encapsulated according to a known technique, and the resultant flavor capsules can be incorporated into a filter portion of a tobacco product.

#### <3. Tobacco Additive>

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**[0092]** As described above, the tobacco flavor liquid may be used in combination with the tobacco residue obtained in the extraction step (S 1) described above. Thus, according to another aspect, there is provided a tobacco additive containing:

the tobacco flavor liquid obtainable by the above-described "method for producing a tobacco flavor liquid"; and a tobacco residue obtainable when the tobacco supernatant is obtained in the above-described "method for producing a tobacco flavor liquid".

45 [0093] Specific examples of the tobacco additive will be described below.

**[0094]** For example, the tobacco additive may be a product obtained by drying a mixture of the tobacco residue obtained in the extraction step (S1) and the tobacco flavor liquid. This product can be used as a tobacco flavor source of a tobacco product.

**[0095]** Alternatively, the tobacco additive may be a tobacco-molded body obtained by molding a mixture of the tobacco residue obtained in the extraction step (S 1) and the tobacco flavor liquid into a specific shape such as a sheet shape or a granular shape. The tobacco-molded body can be used as a tobacco flavor source of a tobacco product.

**[0096]** Alternatively, the tobacco additive may be a tobacco powder obtained by drying a mixture of the tobacco residue obtained in the extraction step (S1) and the tobacco flavor liquid and then pulverizing the dried mixture into powder form. By adding the tobacco powder to a tobacco material (e.g., stemmed leaves or leaf tobacco), the flavor of the tobacco material can be enhanced.

**[0097]** Alternatively, the tobacco additive may be a tobacco slurry obtained by drying a mixture of the tobacco residue obtained in the extraction step (S1) and the tobacco flavor liquid, pulverizing the dried mixture into powder form, and suspending the resultant powder in water. By adding the tobacco slurry to a tobacco material (e.g., stemmed leaves or

leaf tobacco), the flavor of the tobacco material can be enhanced.

**[0098]** The tobacco additive may contain additives such as a binder, a pH adjuster, a preservative, and an antioxidant, as necessary.

<4. Flavor Inhaler>

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**[0099]** The above-described "tobacco flavor liquid" or the above-described "tobacco additive" can be incorporated into any tobacco product. As a typical example, the above-described "tobacco flavor liquid" or the above-described "tobacco additive" can be incorporated into a flavor inhaler such as a combustion-type flavor inhaler or a heating-type flavor inhaler. Specifically, according to another aspect, there is provided a flavor inhaler which includes the above-described "tobacco flavor liquid" or a flavor inhaler which includes the above-described "tobacco additive".

**[0100]** The above-described "tobacco flavor liquid" or the above-described "tobacco additive" can be incorporated into any position of the tobacco product, provided that a user can enjoy an enhanced flavor when using the tobacco product. **[0101]** Examples of the combustion-type flavor inhaler include a cigarette, a pipe, a kiseru (i.e., a traditional Japanese pipe for fine cut tobacco), a cigar, and a cigarillo. An example of the combustion-type flavor inhaler, that is, a cigarette

having a typical structure, is shown in FIG. 3.

**[0102]** A combustion-type flavor inhaler 1 shown in FIG. 3 includes:

a tobacco rod 2 that includes a tobacco filler 2a and cigarette paper 2b wrapped around the tobacco filler 2a; a filter 3 that includes a filter material 3a and a plug wrapper 3b wrapped around the filter material 3a; and tipping paper 4 wrapped over the tobacco rod 2 and the filter 3 so as to connect the tobacco rod 2 and the filter 3.

**[0103]** The tobacco rod 2 includes a tobacco filler 2a such as cut tobacco or a tobacco-molded body. The tobacco rod can have, for example, a diameter of 5 to 10 mm and a length of 40 to 80 mm, as in the case of an ordinary cigarette.

**[0104]** The filter 3 is a filter made of a single filter material 3a, that is, a so-called plain filter. The filter material 3a can be made of a filter material such as acetate tow, as in the case of an ordinary cigarette. The filter 3 has almost the same diameter as that of the tobacco rod 2, and may have a length of, for example, 15 to 40 mm, as in the case of an ordinary cigarette. The plug wrapper 3b may have a thickness of 10 to 100  $\mu$ m. The plug wrapper 3b may or may not be airpermeable; however, it is common to use air-permeable paper.

**[0105]** The tipping paper 4 is adhered with an adhesive so as to cover the entire plug wrapper 3b and a part of the cigarette paper 2b. The tipping paper 4 may have, for example, a length (width) of 20 to 50 mm in the axial direction of the tobacco rod and a thickness of 10 to 100  $\mu$ m. As in the case of an ordinary cigarette, the tipping paper 4 may have a number of small openings for ventilation (ventilation holes) provided in a single line, in multiple lines, or irregularly along the circumferential direction of the cigarette.

**[0106]** In the case of the combustion-type flavor inhaler 1 shown in FIG. 3, the above-described "tobacco flavor liquid" and the above-described "tobacco additive" can be incorporated into, for example, the tobacco filler 2a or the filter material 3a.

[0107] Next, an example of the heating-type flavor inhaler will be described. Examples of the heating-type flavor inhaler include:

a carbon heat source-type inhaler that heats a tobacco filler with the combustion heat of a carbon heat source (see, for example, WO 2006/073065);

an electric heating-type inhaler having a tobacco stick containing a tobacco filler and a heating device for electrically heating the tobacco stick (see, for example, WO 2010/110226); and

a liquid atomizing-type inhaler in which a liquid aerosol source is heated by a heater to generate aerosol and a flavor derived from a tobacco filler is inhaled together with the aerosol (see, for example, WO 2015/046385).

**[0108]** Hereinafter, an example of the heating-type flavor inhaler will be described with reference to FIGS. 4 to 6. FIG. 4 is a perspective view showing an example of a heating-type flavor inhaler. FIG. 5 is a diagram showing an internal structure of a tobacco stick. FIG. 6 is a diagram showing an internal structure of an aerosol-generation device.

**[0109]** As shown in FIG. 4, a heating-type flavor inhaler 100 includes:

a tobacco stick 110 including a tobacco filler and an aerosol source; and an aerosol-generation device 120 to which the tobacco stick 110 is detachably attached, and which heats the tobacco stick 110 to generate aerosol from the aerosol source and release a flavor component from the tobacco filler by an

stick 110 to generate aerosol from the aerosol source and release a flavor component from the tobacco filler by an action of the aerosol.

[0110] The tobacco stick 110 is a replaceable cartridge and has a columnar shape extending along the longitudinal

direction. The tobacco stick 110 is configured to generate aerosol and a flavor component by being heated while being inserted into the aerosol-generation device 120.

**[0111]** As shown in FIG. 5, the tobacco stick 110 includes a base portion 11A including a filler 111 and first cigarette paper 112 wrapped around the filler 111, and a mouthpiece portion 11B forming an end opposite to the base portion 11A. The base portion 11A and the mouthpiece portion 11B are connected by second cigarette paper 113.

[0112] The mouthpiece portion 11B includes a paper tube portion 114, a filter portion 115, and a hollow segment portion 116 disposed between the paper tube portion 114 and the filter portion 115. The paper tube portion 114 is a paper tube formed by winding paper in a cylindrical shape, and has a hollow inside. The filter portion 115 includes a filter material such as acetate tow. The hollow segment portion 116 includes a filling layer having one or more hollow channels. The filter material of the filter portion 115 and the filling layer of the hollow segment portion 116 are connected by being covered with a plug wrapper 117. The filling layer is formed of fibers and has a high filling density of fibers; therefore, during inhalation, air or aerosol flows only through the hollow channel and hardly flows through the filling layer. In the tobacco stick 110, when the decrease in aerosol components through filtration in the filter portion 115 is desired to be diminished, it is effective to shorten the length of the filter portion 115 and replace it with the hollow segment portion 116 in order to increase a delivery amount of aerosol.

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**[0113]** Although the mouthpiece portion 11B is composed of three segments, the mouthpiece portion 11B may be composed of one or two segments, or may be composed of four or more segments. For example, the hollow segment portion 116 may be omitted, and the paper tube portion 114 and the filter portion 115 may be disposed adjacent to each other to form the mouthpiece portion 11B.

**[0114]** The longitudinal length of the tobacco stick 110 is preferably 40 to 90 mm, more preferably 50 to 75 mm, and still more preferably 50 to 60 mm. The circumference of the tobacco stick 110 is preferably 15 to 25 mm, more preferably 17 to 24 mm, and still more preferably 20 to 23 mm. In addition, in the longitudinal direction of the tobacco stick 110, the base portion 11A may have a length of 20 mm, the paper tube portion 114 may have a length of 20 mm, the hollow segment portion 116 may have a length of 8 mm, and the filter portion 115 may have a length of 7 mm, and the lengths of these individual segments can be changed as appropriate according to production suitability, required quality, and the like.

**[0115]** The filler 111 includes a tobacco filler and an aerosol source. The aerosol source is heated at a predetermined temperature to generate aerosol. The aerosol source may be, for example, glycerin, propylene glycol, triacetin, 1,3-butanediol, and a mixture thereof. The content of the aerosol source in the filler 111 is not particularly limited, and from the viewpoint of generating a sufficient amount of aerosol and providing a good smoking flavor, the content is usually 5% by mass or more, preferably 10% by mass or more, and usually 50% by mass or less, preferably 20% by mass or less. **[0116]** The tobacco filler is, for example, in the form of cut tobacco or in the form of a tobacco-molded body, as described above. If the tobacco filler is in the form of cut tobacco, it may be in the form of cut tobacco obtained by cutting leaf tobacco (i.e., aged tobacco leaves) into widths of, for example, 0.8 to 1.2 mm. Alternatively, if the tobacco into widths of, for example, 0.8 to 1.2 mm, or may be in the form of corrugated sheet tobacco obtained by gathering sheet tobacco without cutting it.

**[0117]** If the base portion 11A has a circumference of 22 mm and a length of 20 mm, the content of the filler 111 in the tobacco stick 110 is, for example, 200 to 400 mg, and preferably 250 to 320 mg. The moisture content of the filler 111 is, for example, 8 to 18% by mass, and preferably 10 to 16% by mass. Such a moisture content suppresses generation of a stain on the wrapping paper and improves roll-up machinability during manufacture of the base portion 11A.

**[0118]** For the first cigarette paper 112, the second cigarette paper 113, and the plug wrapper 117, the same cigarette paper, tipping paper, and plug wrapper as those used in a cigarette can be used.

[0119] In the case of the tobacco stick 110 shown in FIG. 5, the above-described "tobacco flavor liquid" and the above-described "tobacco additive" can be incorporated into, for example, the filler 111 or the filter material of the filter portion 115. [0120] As shown in FIG. 6, the aerosol-generation device 120 includes an insertion hole 130 into which the tobacco stick 110 can be inserted. That is, the aerosol-generation device 120 includes an inner tubular member 132 constituting the insertion hole 130. The inner tubular member 132 may be formed of a heat conductive member such as aluminum or stainless steel (SUS).

**[0121]** Further, the aerosol-generation device 120 may include a lid portion 140 that closes the insertion hole 130. The lid portion 140 is configured to be slidable between a state where the insertion hole 130 is closed and a state where the insertion hole 130 is exposed (see FIG. 4).

**[0122]** The aerosol-generation device 120 may include an air flow path 160 communicating with the insertion hole 130. One end of the air flow path 160 is connected to the insertion hole 130, while the other end of the air flow path 160 communicates with the outside (outside air) of the aerosol-generation device 120 at a portion different from the insertion hole 130.

**[0123]** The aerosol-generation device 120 may include a lid portion 170 that covers an end portion of the air flow path 160 on the side communicating with the outside air. The lid portion 170 can cover the end portion of the air flow path

160 on the side communicating with the outside air, or can expose the air flow path 160.

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**[0124]** The lid portion 170 does not air-tightly close the air flow path 160 even in a state of covering the air flow path 160. That is, even in a state where the lid portion 170 covers the air flow path 160, the outside air can flow into the air flow path 160 via the vicinity of the lid portion 170.

**[0125]** In a state where the tobacco stick 110 is inserted into the aerosol-generation device 120, the user holds, in the mouth, one end portion of the tobacco stick 110, specifically, the mouthpiece portion 11B shown in FIG. 5, and performs an inhalation action. The outside air flows into the air flow path 160 through the user's inhalation action. The air flowing into the air flow path 160 passes through the tobacco stick 110 in the insertion hole 130 and is guided into an oral cavity of the user.

**[0126]** The aerosol-generation device 120 may include a temperature sensor in the air flow path 160 or on an outer surface of a wall portion constituting the air flow path 160. The temperature sensor may be, for example, a thermistor, a thermocouple, or the like. When the user sucks the mouthpiece portion 11B of the tobacco stick 110, the internal temperature of the air flow path 160 or the temperature of the wall portion constituting the air flow path 160 decreases because of the influence of the air flowing through the air flow path 160 from the lid portion 170 side toward a heater 30 side. The temperature sensor can detect the user's inhalation action by measuring this temperature decrease.

**[0127]** The aerosol-generation device 120 includes a battery 10, a control unit 20, and a heater 30. The battery 10 stores electric power for use in the aerosol-generation device 120. The battery 10 may be a chargeable and dischargeable secondary battery. The battery 10 may be, for example, a lithium ion battery.

**[0128]** The heater 30 may be provided around the inner tubular member 132. The space accommodating the heater 30 and the space accommodating the battery 10 may be separated from each other by a partition wall 180. This can prevent the air heated by the heater 30 from flowing into the space accommodating the battery 10. Therefore, an increase in the temperature of the battery 10 can be suppressed.

**[0129]** The heater 30 preferably has a tubular shape capable of heating the outer periphery of the columnar tobacco stick 110. The heater 30 may be, for example, a film heater. The film heater may include a pair of film-like substrates and a resistance heating element sandwiched between the pair of substrates. The film-like substrate is preferably made of a material excellent in heat resistance and electrical insulating properties, and is typically made of polyimide. The resistance heating element is preferably made of one or two or more metal materials such as copper, nickel alloy, chromium alloy, stainless steel, and platinum rhodium, and may be formed of, for example, a base material made of stainless steel. Further, in order to connect the resistance heating element to a power source by a flexible printed circuit (FPC), copper plating may be applied to a connection portion and a lead portion thereof.

**[0130]** Preferably, a heat-shrinkable tube may be provided outside the heater 30. The heat-shrinkable tube is a tube that shrinks in a radial direction through heat, and is made of, for example, a thermoplastic elastomer. The heater 30 is pressed against the inner tubular member 132 by the contraction action of the heat-shrinkable tube. This increases the adhesion between the heater 30 and the inner tubular member 132, thereby increasing conduction of the heat from the heater 30 to the tobacco stick 110 via the inner tubular member 132.

[0131] The aerosol-generation device 120 may include a tubular thermal insulator on the outer side of the heater 30 in the radial direction, preferably on the outer side of the heat-shrinkable tube. The thermal insulator may serve to prevent the outer surface of the housing of the aerosol-generation device 120 from reaching an excessively high temperature by blocking the heat of the heater 30. The thermal insulator may be made of an aerogel such as a silica aerogel, a carbon aerogel, or an alumina aerogel. The aerogel as a thermal insulator may typically be a silica aerogel having high thermal insulation performance and relatively low manufacturing costs. However, the thermal insulator may be a fiber-based thermal insulator such as glass wool or rock wool, or a foam-based thermal insulator such as urethane foam or phenolic foam. Alternatively, the thermal insulator may be a vacuum thermal insulator.

**[0132]** The thermal insulator may be provided between the inner tubular member 132 facing the tobacco stick 110 and an outer tubular member 134 outside the thermal insulator. The outer tubular member 134 may be formed of a heat conductive member such as aluminum or stainless steel (SUS). It is preferable that the thermal insulator be provided in the sealed space.

**[0133]** The control unit 20 may include a control board, a CPU, a memory, and the like. The aerosol-generation device 120 may include a notification unit for notifying the user of various kinds of information under the control of the control unit 20. The notification unit may be, for example, a light emitting element such as an LED, a vibration element, or a combination thereof

**[0134]** Upon detecting an activation request from the user, the control unit 20 starts supplying power from the battery 10 to the heater 30. The activation request from the user is made by, for example, an operation of a push button or a slide switch by the user, or an inhalation action of the user. The activation request from the user may be made by pressing a push button 150. More specifically, the activation request from the user may be made by pressing the push button 150 in a state where the lid portion 140 is opened. Alternatively, the activation request from the user may be made by detection of an inhalation action of the user. The user's inhalation action can be detected by, for example, such a temperature sensor as described above.

#### <5. Preferred Embodiments>

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[0135] Hereinafter, preferred embodiments will be described.

- [A1] A method for producing a tobacco flavor liquid, the method including treating a tobacco extract liquid with a material including an inorganic porous body, thereby removing microorganisms from the tobacco extract liquid.

  [A2] A method for producing a tobacco flavor liquid, the method including:
  - extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent, thereby obtaining a tobacco supernatant; and
  - treating the tobacco supernatant with a material including an inorganic porous body, thereby obtaining a microorganism-free tobacco supernatant.
  - [A3] A method for producing a tobacco flavor liquid, the method including:

extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent, thereby obtaining a tobacco supernatant;

culturing yeast in the tobacco supernatant, thereby obtaining a yeast-containing culture liquid;

mixing the yeast-containing culture liquid with an elution solvent including an organic solvent, and eluting, from the yeast contained in the resultant mixture and into a liquid portion of the mixture, a useful component included in fungal cells of the yeast, thereby obtaining a useful-component eluate; and

- treating the useful-component eluate with a material including an inorganic porous body, thereby obtaining a microorganism-free useful-component eluate.
- <sup>25</sup> [A4] The method according to [A1], wherein the treatment is performed by passing the tobacco extract liquid through a layer including an inorganic porous body.
  - [A5] The method according to [A2], wherein the treatment is performed by passing the tobacco supernatant through a layer including an inorganic porous body.
  - [A6] The method according to [A3], wherein the treatment is performed by passing the useful-component eluate through a layer including an inorganic porous body.
  - [A7] The method according to [A1], wherein the treatment is performed by passing the tobacco extract liquid through a molded body including an inorganic porous body.
  - [A8] The method according to [A2], wherein the treatment is performed by passing the tobacco supernatant through a molded body including an inorganic porous body.
  - [A9] The method according to [A3], wherein the treatment is performed by passing the useful-component eluate through a molded body including an inorganic porous body.
  - [A10] The method according to [A1], wherein the treatment is performed by passing the tobacco extract liquid through an aggregate of particles including an inorganic porous body.
  - [A11] The method according to [A2], wherein the treatment is performed by passing the tobacco supernatant through an aggregate of particles including an inorganic porous body.
  - [A12] The method according to [A3], wherein the treatment is performed by passing the useful-component eluate through an aggregate of particles including an inorganic porous body.
  - [A13] The method according to [A1], wherein the treatment is performed by passing the tobacco extract liquid through a column filled with particles including an inorganic porous body.
- [A14] The method according to [A2], wherein the treatment is performed by passing the tobacco supernatant through a column filled with particles including an inorganic porous body.
  - [A15] The method according to [A3], wherein the treatment is performed by passing the useful-component eluate through a column filled with particles including an inorganic porous body.
  - [A16] The method according to any one of [A10] to [A15], wherein the particles have a particle size of <400  $\mu$ m, generally a particle size of 10 to 400  $\mu$ m, preferably a particle size of <100  $\mu$ m, more preferably a particle size of 10 to 80  $\mu$ m, and yet more preferably a particle size of 30 to 75  $\mu$ m. [A17] The method according to any one of [A1] to [A16], wherein the inorganic porous body is diatomaceous earth
  - [A18] The method according to any one of [A10] to [A17], wherein the particles including an inorganic porous body are particles composed only of diatomaceous earth or particles composed only of zeolite.
  - [A19] The method according to any one of [A2] to [A18], wherein the tobacco material is cut tobacco.
  - [A20] The method according to any one of [A2] to [A19], wherein the aqueous solvent is water or water-containing ethanol, preferably water, more preferably water having a temperature of 20 to 70 °C.

- [A21] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A20], wherein the yeast is at least one kind selected from the group consisting of yeast of the genus Rhodotorula, yeast of the genus Xanthophyllomyces, yeast of the genus Yarrowia, yeast of the genus Lipomyces, yeast of the genus Saccharomyces, yeast of the genus Cyberlindnera, and yeast of the genus Wickerhamomyces.
- <sup>5</sup> [A22] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A21], wherein the elution solvent is an organic solvent or a mixture of an organic solvent and water.
  - [A23] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A22], wherein the organic solvent is an organic solvent miscible with water, preferably an alcohol miscible with water.
  - [A24] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A23], wherein the elution solvent is an alcohol miscible with water or its water-containing alcohol (i.e., said alcohol with water contained therein).
  - [A25] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A24], wherein the organic solvent is an organic solvent having an SP value of 10 to 14.5, preferably an alcohol having an SP value of 10 to 14.5, more preferably ethanol, isopropanol, methanol, or butanol, still more preferably ethanol or isopropanol, and most preferably ethanol.
- [A26] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A25], wherein the elution solvent is ethanol or water-containing ethanol.
  - [A27] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A26], wherein the elution solvent is added to the yeast-containing culture liquid in such an amount that the concentration of the organic solvent in a mixed solution of the yeast-containing culture liquid and the elution solvent becomes 50% by volume or more, preferably 50 to 95% by volume, and more preferably 60 to 95% by volume.
  - [A28] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A27], wherein the elution is performed by stirring a mixture of the yeast-containing culture liquid and the elution solvent.
  - [A29] The method according to any one of [A3], [A6], [A9], [A12], [A15] to [A28], wherein the elution is performed by heating a mixture of the yeast-containing culture liquid and the elution solvent.
- <sup>25</sup> [A30] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A29], further including removing the organic solvent from the microorganism-free useful-component eluate.
  - [A31] The method according to any one of [A3], [A6], [A9], [A12], and [A15] to [A30], wherein the useful component is a flavor-contributing component.
  - [A32] The method according to [A31], wherein the flavor-contributing component is at least one kind selected from the group consisting of carotenoids, fatty acids, neutral fats (i.e., glycerin esters of fatty acids), acetic acid esters, fatty acid esters, organic acids, and higher alcohols (e.g., alcohols having 8 to 22 carbon atoms).
  - [B1] A tobacco flavor liquid obtainable by the method according to any one of [A1] to [A32].
  - [B2] The tobacco flavor liquid according to [B1], wherein the tobacco flavor liquid is the microorganism-free tobacco supernatant according to [A2].
  - [B3] The tobacco flavor liquid according to [B1], wherein the tobacco flavor liquid is the microorganism-free useful-component eluate according to [A3].
  - [C1] A tobacco additive including:
    - the tobacco flavor liquid obtainable by the method according to any one of [A2] to [A32]; and a tobacco residue obtainable when the tobacco supernatant is obtained in the method according to any one of [A2] to [A32].
  - [C2] The tobacco additive according to [C1], wherein the tobacco flavor liquid is the microorganism-free tobacco supernatant according to [A2].
  - [C3] The tobacco additive according to [C1], wherein the tobacco flavor liquid is the microorganism-free usefulcomponent eluate according to [A3].
    - [D1] A flavor inhaler including the tobacco flavor liquid according to any one of [B1] to [B3].
    - [D2] A flavor inhaler including the tobacco additive according to any one of [C1] to [C3].
    - [D3] The flavor inhaler according to [D1] or [D2], wherein the flavor inhaler is a combustion-type flavor inhaler.
  - [D4] The flavor inhaler according to [D1] or [D2], wherein the flavor inhaler is a heating-type flavor inhaler.

[Examples]

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[Example 1]

**[0136]** In Example 1, the clarity of a tobacco flavor liquid produced according to the method of the present invention was evaluated.

#### 1-1. Method

[0137] Flue-cured leaf tobacco was pulverized and used as a "tobacco material". The cut pieces of the flue-cured leaf tobacco (100 g) were pulverized to a size of 100  $\mu$ m or less by a pulverizer, 600 mL of water having a temperature of 60 °C was added thereto, and a shaking process (200 rpm, 2 hours) was performed. In this manner, a water-soluble component contained in the leaf tobacco was extracted. Subsequently, a tobacco supernatant and a tobacco residue were separated by suction filtration. Advantec No. 60 was used as filter paper. The filtrate obtained is called a "tobacco supernatant".

[0138] The tobacco supernatant was subjected to the following treatment to prepare samples.

Sample 1:

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**[0139]** A 5 mL tobacco supernatant was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size: <354  $\mu$ m) (GL Sciences Inc.) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 1.

Comparative Sample 1:

[0140] A tobacco supernatant was used as a comparative sample 1.

Reference Sample 1:

**[0141]** A 5 mL tobacco supernatant was microfiltered through a filter having a pore size of 0.2  $\mu$ m. The obtained filtrate was used as a reference sample 1.

Sample 2:

[0142] A 100 mL tobacco supernatant was sterilized in an autoclave, and yeast of the genus Saccharomyces (Saccharomyces cerevisiae) was added to the sterilized tobacco supernatant so that the tobacco supernatant had a concentration of  $10^5$  cells/mL, followed by an overnight shake culture (28 °C, 240 rpm). A 5 mL yeast-containing culture liquid obtained after the culture was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size: <354  $\mu$ m) (GL Sciences Inc.) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 2.

35 Comparative Sample 2:

**[0143]** A 100 mL tobacco supernatant was sterilized in an autoclave, and yeast of the genus Saccharomyces (Saccharomyces cerevisiae) was added to the sterilized tobacco supernatant so that the tobacco supernatant had a concentration of 10<sup>5</sup> cells/mL, followed by an overnight shake culture (28 °C, 240 rpm). A yeast-containing culture liquid obtained after the culture was used as a comparative sample 2.

Reference Sample 2:

[0144] A 100 mL tobacco supernatant was sterilized in an autoclave, and yeast of the genus Saccharomyces (Saccharomyces cerevisiae) was added to the sterilized tobacco supernatant so that the tobacco supernatant had a concentration of 10<sup>5</sup> cells/mL, followed by an overnight shake culture (28 °C, 240 rpm). A 5 mL yeast-containing culture liquid obtained after the culture was microfiltered through a filter having a pore size of 0.2 μm. The obtained filtrate was used as a reference sample 2.

**[0145]** The clarity of each sample was evaluated by measuring the absorbance at 600 nm using an absorptiometer. The reference sample 1 was used as a reference of the clarity of the sample 1 and the comparative sample 1. The reference sample 2 was used as a reference of the clarity of the sample 2 and the comparative sample 2.

1-2. Results

55 [0146] The results of the measurement of the absorbance are shown in the table below.

#### [Table 1]

	Absorbance (OD600)	Reference	
Sample 1	0	Reference Sample 1	
Comparative Sample 1	0.782	Reference Sample 1	
Sample 2	0.002	Reference Sample 2	
Comparative Sample 2	0.553	Reference Sample 2	

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**[0147]** The absorbance shown in Table 1 represents differences in absorbance from the reference sample. Thus, in Table 1, the absorbance values of the samples being closer to 0 indicates that the samples have a clarity comparable to that of the reference sample.

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**[0148]** The clarity of the sample 1 was significantly higher than that of the comparative sample 1. This result indicates that passing the tobacco supernatant through the column filled with diatomaceous earth removed the suspended solids having a large molecular weight that lowered the clarity (including the microorganisms contained in the tobacco supernatant).

**[0149]** The clarity of the sample 2 was significantly higher than that of the comparative sample 2. This result shows that passing the tobacco supernatant through the column filled with diatomaceous earth removed the suspended solids having a large molecular weight that lowered the clarity (including the microorganisms contained in the tobacco supernatant and the added yeast).

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**[0150]** Since the above results demonstrate the clarity of the tobacco flavor liquid produced by the method of the present invention, it is considered that the method of the present invention can remove not only the added yeast but also any microorganisms contained in the tobacco supernatant, such as molds and bacteria.

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[Example 2]

**[0151]** In Example 2, the concentration of the yeast in a tobacco flavor liquid produced according to the method of the present invention was evaluated.

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2-1. Method

**[0152]** A tobacco supernatant was obtained according to the method described in Example 1. The tobacco supernatant was subjected to the following treatment to prepare samples.

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Sample 3A:

obtained

**[0153]** A 100 mL tobacco supernatant was sterilized in an autoclave, and yeast of the genus Saccharomyces (Saccharomyces cerevisiae) was added to the sterilized tobacco supernatant so that the tobacco supernatant had a concentration of 10<sup>5</sup> cells/mL, followed by an overnight shake culture (28 °C, 240 rpm). A yeast-containing culture liquid obtained after the culture was used as a sample 3A.

Sample 3B:

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**[0154]** A 5 mL yeast-containing culture liquid (sample 3A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with glass powder (particle size: 63 to 106  $\mu$ m) (AS ONE CORPORATION) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 3B.

Sample 3C:

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**[0155]** A 5 mL yeast-containing culture liquid (sample 3A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size: <105  $\mu$ m) (IMERYS) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 3C.

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Sample 3D:

[0156] A 5 mL yeast-containing culture liquid (sample 3A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size:  $<50 \mu m$ ) (GL Sciences Inc.) by a

gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 3D.

Sample 3E:

[0157] A 5 mL yeast-containing culture liquid (sample 3A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size: <354 μm) (GL Sciences Inc.) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 3E.

Sample 3F:

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**[0158]** A 5 mL yeast-containing culture liquid (sample 3A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with synthetic zeolite powder (particle size:  $<75\,\mu\text{m}$ ) (FUITFILM Wako Pure Chemical Corporation) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 3F.

15 Sample 4A:

**[0159]** On the other hand, the yeast-containing culture liquid (sample 3A) was centrifuged to obtain a centrifuged supernatant. The centrifuged supernatant was used as a sample 4A.

20 Sample 4B:

**[0160]** A 5 mL centrifuged supernatant (sample 4A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with glass powder (particle size: 63 to 106  $\mu$ m) (AS ONE CORPORATION) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 4B.

Sample 4C:

**[0161]** A 5 mL centrifuged supernatant (sample 4A) was passed through a column (having an inner diameter of 11 mm and a length mm) filled with diatomaceous earth powder (particle size: <105  $\mu$ m) (IMERYS) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 4C.

Sample 4D:

**[0162]** A 5 mL centrifuged supernatant (sample 4A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size:  $<50 \mu m$ ) (IMERYS) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 4D.

Sample 4E:

40 [0163] A 5 mL centrifuged supernatant (sample 4A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size: <354 μm) (GL Sciences Inc.) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 4E.</p>

Sample 4F:

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[0164] A 5 mL centrifuged supernatant (sample 4A) was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with synthetic zeolite powder (particle size: <75  $\mu$ m) (FUJIFILM Wako Pure Chemical Corporation) by a gravity fall, whereby a column eluate was obtained. The column eluate was used as a sample 4F.

**[0165]** The number of fungal cells of the yeast in the samples 3A to 3F and 4A to 4F were measured by a colony counting method.

2-2. Results

[0166] The results of the measurement of the number of fungal cells of the yeast are shown in the table below.

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#### [Table 2]

	CFU[cells/mL]
Sample 3A	2.5×10 <sup>8</sup>
Sample 3B (Glass Powder)	6.8×10 <sup>6</sup>
Sample 3C (Diatomaceous Earth Powder)	N.D.
Sample 3D (Diatomaceous Earth Powder)	N.D.
Sample 3E (Diatomaceous Earth Powder)	N.D.
Sample 3F (Zeolite Powder)	N.D.
Sample 4A	1.6×10 <sup>4</sup>

[0167] It was confirmed that the yeast was indeed removed when the tobacco supernatant was passed through the column filled with diatomaceous earth. The yeast was likewise removed even if the particle size of the diatomaceous earth was varied. It was also confirmed that the yeast was indeed removed when the tobacco supernatant was passed through the column filled with zeolite. On the other hand, the yeast could not be removed when the tobacco supernatant was passed through the column filled with a glass powder.
[20] This result shows that the added yeart say he removed to an undetectable level by treating the tobacco.

**[0168]** This result shows that the added yeast can be removed to an undetectable level by treating the tobacco supernatant with an inorganic porous body according to the method of the present invention. From this result and the result of Example 1, it is considered that the method of the present invention can remove not only the added yeast but also any microorganisms contained in the tobacco supernatant, such as molds and bacteria.

Example 3]

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[0169] In Example 3, the method according to the second embodiment was performed.

3-1. Method

Sample 5:

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**[0170]** According to the method of the second embodiment, the extraction step (S1), the culture step (S2), and the elution step (S3) were performed, and 5 mL of a useful-component eluate thus obtained was passed through a column (having an inner diameter of 11 mm and a length of 25 mm) filled with diatomaceous earth powder (particle size: <354  $\mu$ m) (GL Sciences Inc.) by a gravity fall. The column eluate was used as a sample 5.

Comparative Sample 5:

[0171] According to the method of the second embodiment, the extraction step (S1), the culture step (S2), and the elution step (S3) were performed, and a useful-component eluate thus obtained was used as a comparative sample 5.

Reference Sample 5:

[0172] According to the method of the second embodiment, the extraction step (S1), the culture step (S2), and the elution step (S3) were performed, and 5 mL of a useful-component eluate thus obtained was microfiltered through a filter having a pore size of 0.2 μm. The obtained filtrate was used as a reference sample 5.

[0173] The details of the extraction step (S1), the culture step (S2), and the elution step (S3) are described below.

50 Extraction step (S1)

[0174] Flue-cured leaf tobacco was pulverized and used as a "tobacco material". The cut pieces of the flue-cured leaf tobacco (100 g) were pulverized to a size of 100  $\mu$ m or less by a pulverizer, 600 mL of water having a temperature of 60 °C was added thereto, and a shaking process (200 rpm, 2 hours) was performed. In this manner, a water-soluble component contained in the leaf tobacco was extracted. Subsequently, solid-liquid separation was performed by filtering. Thereby, a tobacco supernatant and a tobacco residue were obtained.

Culture Step (S2)

**[0175]** Yeast of the genus Saccharomyces (Saccharomyces cerevisiae) was added to the obtained 3 mL tobacco supernatant at a concentration of 10<sup>5</sup> cells/mL, and the yeast was cultured in the tobacco supernatant. When culturing the yeast, a shake culture (240 rpm) was performed at 28 °C for 24 hours under an aerobic condition. The "mixture of the yeast and the tobacco supernatant" obtained after the culture was performed is referred to as a "yeast-containing culture liquid".

Elution Step (S3)

**[0176]** After 24 hours from the start of the culture, 7 mL of ethanol was added as an elution solvent to the 3 mL yeast-containing culture liquid. Specifically, ethanol was added in such an amount that the concentration of the ethanol in the mixed solution of the yeast-containing culture liquid and the ethanol would be 70% by volume.

**[0177]** The clarity of each sample was evaluated by measuring the absorbance at 600 nm using an absorptiometer. Reference sample 5 was used as a reference of the clarity of the sample 5 and the comparative sample 5.

#### 3-2. Results

[0178] The results of the measurement of the absorbance are shown in the table below.

[Table 3]

	Absorbance (OD600)	Reference	
Sample 5	0.02	Reference Sample 5	
Comparative Sample 5	2.08	Reference Sample 5	

**[0179]** The absorbance shown in Table 3 represents differences in absorbance from the reference sample 5. Thus, in Table 3, the absorbance values of the samples being closer to 0 indicates that the samples have a clarity comparable to that of the reference sample 5.

**[0180]** The clarity of the sample 5 was significantly higher than that of the comparative sample 5. This result shows that passing the useful-component eluate obtained by the method according to the second embodiment through the column filled with diatomaceous earth removed the suspended solids having a large molecular weight that lowered the clarity (including the microorganisms contained in the tobacco supernatant and the added yeast).

[Example 4]

[0181] In Example 4, sensory evaluation was performed.

4-1. Method

**[0182]** A sample 1, a comparative sample 1, a reference sample 1, a sample 2, a comparative Sample 2, and a reference sample 2 were prepared as described in Example 1, and used as tobacco flavor liquids. Each sample was mixed in an amount of 1 mL with 1 mL propylene glycol, and the resulting mixture was atomized and inhaled using a commercially available electronic cigarette to evaluate its smoking flavor.

#### 4-2. Results

**[0183]** No difference in smoking flavor was detected between the sample 1 and the comparative sample 1. Also, no difference in smoking flavor was detected between the sample 2 and the comparative sample 2. These results show that passing the tobacco supernatant through the diatomaceous earth column removes suspended solids having a large molecular weight but does not remove low-molecular weight compounds that have an effect on a smoking flavor, and that this treatment step does not affect the smoking flavor of the tobacco supernatant.

**[0184]** No difference in smoking flavor was detected between the sample 1 and the reference sample 1. Also, no difference in smoking flavor was detected between the sample 2 and the reference sample 2. These results show that there is no difference in the smoking flavor of the obtained tobacco flavor liquids between the case where the tobacco supernatant was passed through the diatomaceous earth column and the case where the tobacco supernatant was microfiltered.

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#### Claims

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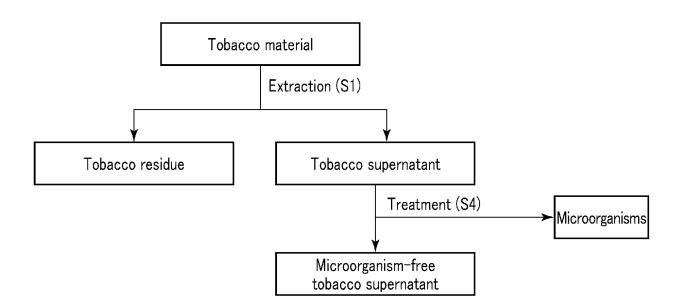
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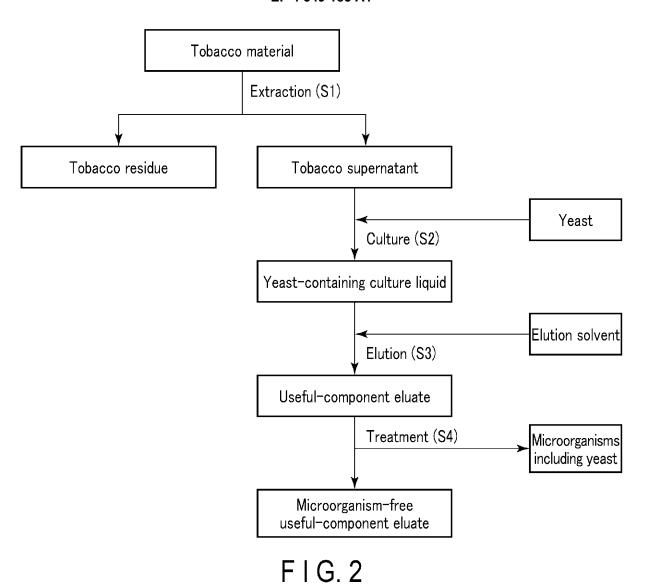
- 1. A method for producing a tobacco flavor liquid, the method comprising treating a tobacco extract liquid with a material including an inorganic porous body, thereby removing microorganisms from the tobacco extract liquid.
- 2. The method according to claim 1, wherein the treatment is performed by passing the tobacco extract liquid through a layer including an inorganic porous body.
- **3.** The method according to claim 1, wherein the treatment is performed by passing the tobacco extract liquid through a molded body including an inorganic porous body.
  - **4.** The method according to claim 1, wherein the treatment is performed by passing the tobacco extract liquid through an aggregate of particles including an inorganic porous body.
- 15. The method according to any one of claims 1 to 4, wherein the inorganic porous body is diatomaceous earth or zeolite.
  - **6.** The method according to any one of claims 1 to 5, wherein the tobacco extract liquid is a tobacco supernatant obtainable by extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent.
  - 7. The method according to any one of claims 1 to 5, wherein the tobacco extract liquid is a useful-component eluate obtainable by a method comprising following steps (a) to (c):
    - (a) extracting, from a tobacco material, a water-soluble component contained in the tobacco material by using an aqueous solvent, thereby obtaining a tobacco supernatant;
    - (b) culturing yeast in the tobacco supernatant, thereby obtaining a yeast-containing culture liquid; and
    - (c) mixing the yeast-containing culture liquid with an elution solvent including an organic solvent, and eluting, from the yeast contained in the resultant mixture and into a liquid portion of the mixture, a useful component included in fungal cells of the yeast, thereby obtaining a useful-component eluate.
  - 8. A tobacco flavor liquid obtainable by the method according to any one of claims 1 to 7.
  - 9. A flavor inhaler comprising the tobacco flavor liquid according to claim 8.
- 35 **10.** A tobacco additive comprising:

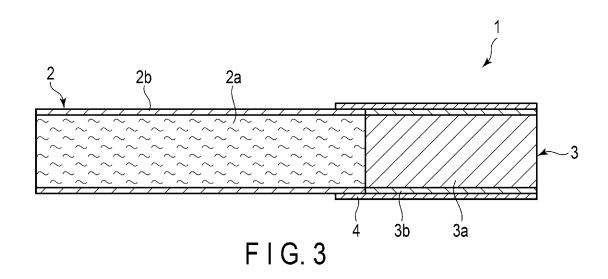
the tobacco flavor liquid obtainable by the method according to claim 6 or 7; and a tobacco residue obtainable when the tobacco supernatant is obtained in the method according to claim 6 or 7.

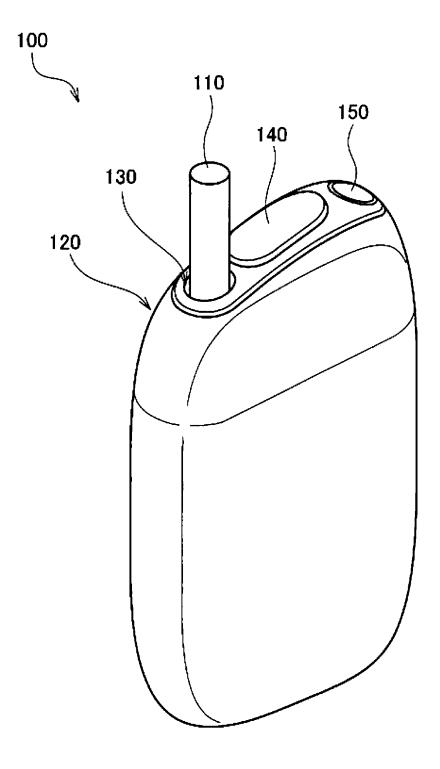
**11.** A flavor inhaler comprising the tobacco additive according to claim 10.



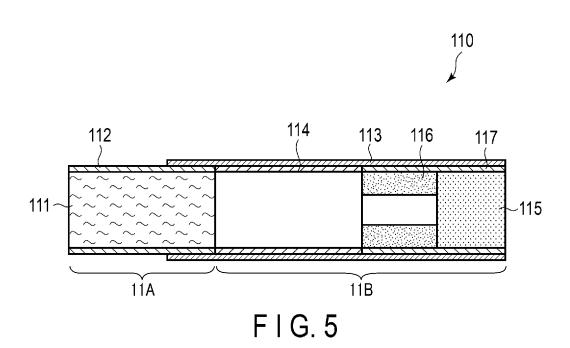
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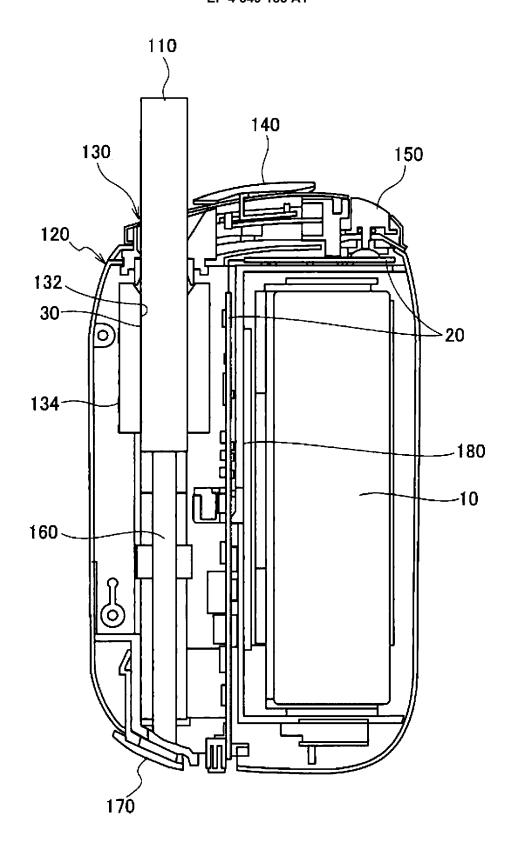






F I G. 4





F I G. 6

		INTERNATIONAL SEARCH REPORT	Inter	national application No.	
5				PCT/JP2021/019623	
	A24B 15/2	CATION OF SUBJECT MATTER 0(2006.01)1; A24B 15/24(2006.0 5/24; A24B15/20	1)i		
10	According to International Patent Classification (IPC) or to both national classification and IPC				
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