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(54) Title: DEODORIZATION METHOD

(57) Abstract: Disclosed are a deodorization method comprising applying a deodorizing base, which has been subjected to a contact treatment with an enzyme, to an offensive odor source; a method of eliminating an offensive odor comprising holding an enzyme and a deodorizing base in a single container in a state of isolating the enzyme from the deodorizing base, then bringing the deodorizing base into contact treatment with the enzyme in the container, and applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source; and a deodorant composition containing as the active ingredient a deodorizing base which has been brought into contact with an enzyme.



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DESCRIPTION

DEODORIZATION METHOD

Technical Field

This invention relates to a deodorization method whereby an offensive odor is eliminated or relieved (hereinafter referred to as "deodorized") with the use of a deodorizing base which has been enzymatically treated or the use of the deodorant composition containing such deodorizing base. More specifically, it relates to a deodorization method wherein a phenolic compound, which has been brought into contact with a phenolic compound-oxidizing enzyme, or the composition containing such phenolic compound, is applied to an offensive odor source giving off an offensive odor. Moreover, the invention relates to deodorant compositions which are substantially free from enzyme or contain only small amounts of enzyme and deodorizing devices having the deodorizing base which has been enzymatically treated or deodorant compositions containing such deodorizing base.

Background art

In these days, there might arise various offensive odors in our surrounding. To eliminate or relieve these offensive odors, numerous studies on deodorants have been

reported. For example, it is reported that extracts of plants such as red turnip, cocoa beans, coffee beans and parsley extract (see, for example, Japanese Patent Application Laid-Open No. JP-A-60-207664) are used as deodorants. Although these plant extracts, which contain polyphenol compound mixtures as the main component in many cases, exert deodorizing effect to a certain extent, however sufficient deodorizing effect has not been obtained. It has been required to develop deodorants having an improved deodorizing effect.

On the other hand, there have been reported deodorant compositions containing a plant extract having phenolic compounds as the main component together with phenol oxidase (see, for example, Japanese Patent Application Laid-Open Nos. H09-038183 and H10-212221) as constituents. Although these deodorant compositions have excellent deodorizing effect, it is pointed out that some of the enzymes employed therein might exert some undesirable effects on humans. That is, in case of spraying such a deodorant to eliminate or relieve an offensive odor, namely, there arises a risk that the enzyme contained in the deodorant is scattered into the atmosphere. It is pointed out that repeated inhalation of the scattered enzyme by humans might sometimes induce asthma or fever. Accordingly, enzymes usable in these compositions are restricted. Moreover, there arises a problem that it is

necessary to call people's attention to minimize the inhalation of the enzymes.

Disclosure of the Invention

Under these circumstances, an object of the invention is to provide a deodorization method whereby a deodorizing effect comparable to the deodorizing effect exerted by deodorant compositions containing a plant extract having phenolic compounds as the main component together with phenol oxidase as constituent can be achieved without any fear of undesirable effects on humans in case of applying a deodorant composition to an offensive odor source. Another object of the invention is to provide a deodorant or composition comprising the deodorant which is substantially free from any enzymes and shows a deodorizing effect comparable to deodorant compositions containing an enzyme and a deodorizing base. Furthermore, the invention aims at providing a deodorizing device with the use of the deodorant or the use of the composition comprising the deodorant.

The inventors have conducted intensive studies to solve these problems. As a result, they have found out that, in the known deodorant compositions, a deodorizing effect can be established even in case where a phenolic compound-oxidizing enzyme does not coexist with phenolic compounds. Based on this finding, they have continued studies, thereby

completing the invention.

Namely, the invention includes the following each invention.

(1) A deodorization method comprising applying a deodorizing base, which has been subjected to a contact treatment with an enzyme, to an offensive odor source.

(2) A deodorization method according to the above (1) wherein the deodorizing base is a phenolic compound and the enzyme is a phenolic compound-oxidizing enzyme.

(3) A deodorization method according to the above (1) or (2) wherein a fragrance component and/or a flavor component are added to the deodorizing base.

(4) A deodorization method according to any one of the above (1) to (3) wherein the enzyme is an immobilized phenolic compound-oxidizing enzyme.

(5) A method of eliminating an offensive odor comprising holding an enzyme and a deodorizing base in a single container in a state of isolating the enzyme from the deodorizing base, then bringing the deodorizing base into contact treatment with the enzyme in the container, and applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source.

(6) A method of eliminating an offensive odor comprising holding an enzyme and a deodorizing base in

separate containers, then transferring any one of the enzyme and the deodorizing base into the container having the other therein, bringing the deodorizing base into contact treatment with the enzyme, and applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source.

(7) A deodorant composition containing as the active ingredient a deodorizing base which has been brought into contact with an enzyme.

(8) A deodorizing device comprising a unit of maintaining an enzyme and a deodorizing base in a single container in a state of isolating the enzyme from the deodorizing base, and a unit of, after a contact treatment of the deodorizing base with the enzyme, applying the deodorizing base which has been subjected to the contact treatment with the enzyme to an offensive odor source.

(9) A deodorizing device comprising a unit of holding an enzyme and a deodorizing base in separate containers, a unit of transferring any one of the enzyme and the deodorizing base into the container having the other therein, and a unit of bringing them into contact treatment with each other and then applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source.

Best Mode for Carrying out the Invention

Now, the invention will be described in greater detail.

First, a deodorizing base which is subjected to a contact treatment with an enzyme in the invention will be described.

The deodorizing base to be used in the invention which is subjected to a contact treatment with an enzyme is a fundamental component constituting a deodorant composition and has an important role of establishing a deodorizing effect. Typical examples of the components of the deodorizing base include phenolic compounds, for example phenolic compounds having at least one phenolic hydroxyl group. Among those, polyphenol is the most preferable. The term "phenolic hydroxyl group" as used herein means a hydroxyl group directly attached to an aromatic ring such as a benzene ring. The aromatic ring may be selected from among benzene ring, pyridine ring, thiophene ring, naphthalene ring, biphenyl ring and other aromatic rings. Among all, benzene ring is most desirable.

Appropriate examples of the phenolic compound include diphenols such as catechol, 4-methylcatechol, 5-methylcatechol, resorcinol, 2-methylresorcinol, 5-methylresorcinol and hydroquinone, biphenyloids such as 4,4'-biphenyldiol and 3,4'-diphenyldiol and catechol

derivatives such as dopa, dopamine, chlorogenic acid, caffeinic acid, caffeinic acid esters, paracoumalic acid and tyrosine. In particular, catechol, tyrosine and chlorogenic acid are preferable. In this invention, it is preferable to use a solution of the phenolic compound as a deodorizing base. Among all, an aqueous solution, a water-containing alcohol solution or an alcohol solution of the phenolic compound is preferable.

These phenolic compounds may be either prepared from natural sources or artificially synthesized. The extract can be obtained by a known method. Phenolic compounds from the natural sources can be obtained by known methods, such as a method comprising selecting the plants rich in phenolic compounds, obtaining an extract containing a phenolic compound by ordinary method and using it as the deodorizing base. The extract can be further refined and the refined compound can be used as the deodorizing base. There is no definition of what extent the refining has to be made.

Examples for the plants rich in phenolic compounds include grape peel, apple, banana, strawberry, apricot, peach, plum, citrus fruit, pineapple, pear, persimmon, cherry, papaya, mango, avocado, melon, loquat, fig tree, kiwi, prune, blueberry, blackberry, raspberry, cranberry, coffee beans, cocoa beans, sunflower seeds, grape seeds, sesame, grapefruit seed, pecan nuts, cashew nuts, chestnut,

coconut, peanuts, walnut, rosemary, lemon balm, sage, thyme, clove, artichoke, German kamille, agrimony, licorice, anise, olive, milfoil, eucalyptus, wormwood, angelica, fenugreek, green pepper, fennel, anise seed, red pepper, cardamom, celery seed, caraway seed, fennel seed, lavender, spearmint, peppermint, blessed thistle, hyssop, sweat basil, marigold, dandelion, carrot leaves, green tea leaves, tea leaves, oolong tea leaves, perilla leaves, tabaco leaves, ginger, wasabi, horseradish, majorana, mint, mustard, palsey, pepper, savory, tarragon, burdock, turmeric, tomato, broad bean, spinach, lotus, egg plant, Chinese radish, turnip, beet, hop, onion, chicory, asparagus, pigmet, barely, wheat, corn, potato, sweet potato, yam, taro, sugar beet, alphaalpha, malt, mugwort, chrysanthemum, etc. Among those, rosemary, sage, thyme, spearmint, peppermint, sunflower seed, coffee beans, fresh coffee beans, grape seed, grape peel, apple, carrot seed, green tea leaves, tea leaves, oolong tea leaves are preferable.

Either one of these phenolic compounds or plant extracts or a combination of two or more of the same may be used.

The extract can be obtained by a known method. More specifically, it can be obtained by mixing a plant source with a solvent followed by, if necessary, elimination of the solvent. As appropriate examples of the solvent, water,

alcohols, organic solvents and mixtures thereof can be cited. Plants rich in phenolic compounds are preferable source. Before the extraction step, it is preferable that the plant source is preliminary treated by, for example, drying and cutting into small pieces. The extraction solvent may be adequately selected depending on the desired purpose of using the deodorant composition. It is preferable to use a solvent which can be safely used in foods, for example, hot water, ethanol-containing water.

The term "deodorizing base" as used herein includes water-soluble lignin, in particular, ligninsulfonic acid obtained by the so-called sulfite delignification method.

Namely, wood chips cut into an appropriate size are immersed in an aqueous solution of sulfurous acid and thermally treated at about 130°C. Thus, lignin in the wood chips are sulfonated and eluted into the liquid reaction mixture. Next, various inorganic matters contained in the liquid reaction mixture are eliminated. Thus, ligninsulfonic acid can be obtained.

In the invention, ligninsulfonic acid may be used together with a ligninsulfonic acid salt or a mixture thereof as a component of the deodorizing base.

Next, the enzyme to be used in the invention will be illustrated. The enzyme to be employed herein may be an arbitrary enzyme, so long as it can be brought into contact

with the deodorizing base and impart a function of eliminating or relieving an offensive odor to the deodorizing base.

One of the representative enzyme class is oxidoreductase. Oxidoreductases are enzymes as classified under the Enzyme Classification number E.C. 1 (Oxidoreductases) in accordance with the Recommendations (1992) of the International Union of Biochemistry and Molecular Biology (IUBMB) which are enzymes catalysing oxidoreductions.

According to the invention, the following three types of oxidoreductases are especially contemplated: Laccases or related enzymes such as tyrosinase cover enzymes which act on molecular oxygen (O_2) and yield water (H_2O) without any need for peroxide (e.g. H_2O_2), Oxidases cover enzymes which act on molecular oxygen (O_2) and yield peroxide (H_2O_2), and Peroxidases cover enzymes which act on peroxide (e.g. H_2O_2) and yield water (H_2O).

Also enzyme systems which comprise a combination of the three types of enzymes are contemplated according to the invention. The enzyme systems may e.g. consist of a laccase or a related enzyme and an oxidase; a laccase or a related enzyme and a peroxidase; a laccase or a related enzyme and an oxidase and a peroxidase; or an oxidase and a peroxidase.

Preferred oxidoreductases are plant- or fruit-derived

enzymes and microbial-derived enzymes. These oxidoreductases are superior in terms of deodorant, however microbial-derived enzymes, especially recombinant and/or substantially purified enzymes without any side activity is the most preferable. Microbial-derived enzymes are superior to plant- or fruit-derived enzymes as they can be produced more easily in large amounts by recombinant DNA techniques known in the art. Microbial-derived enzyme means in the context of the present invention enzymes derived from bacteria, filamentous fungi or yeasts. Furthermore, also genetically modified oxidoreductases designed for the purpose of the invention are contemplated. Said modified oxidoreductases can be selected by screening for oxidoreductases variants having a suitable activity-profile. Said variants may be provided by site directed or cassette or by random mutagenesis by using methods well established in the art. In the case of enzyme acting on oxygen (O₂) as the acceptor, said oxygen may be molecular oxygen supplied by the air.

Following is the description of Laccase and related enzymes.

Examples of specifically contemplated enzymes within the group of laccases and related enzymes which are capable of oxidizing the phenolic compounds used in the invention are mono- and diphenolic oxidases, such as catechol oxidase (E.C. 1.10.3.1), laccase (E.C. 1.10.3.2), tyrosinase (E.C.

1.14.18.1) (E.C. 1.10.3.1), and bilirubin oxidase (E.C. 1.3.3.5).

Laccase oxidizes o-diphenol as well as p-diphenol forming their corresponding quinones. Tyrosinase or catechol oxidase catalyses two different reactions: The hydroxylation of monophenols in o-diphenols and the oxidation of o-diphenols in o-quinones.

Laccases employed may be derived from a strain of *Polyporus* sp., in particular a strain of *Polyporus pinsitus* (also called *Trametes villosa*) or *Polyporus versicolor*, or a strain of *Trametes* sp., a strain of *Myceliophthora* sp., e.g. *M. thermophila* or a strain of *Rhizoctonia* sp., in particular a strain of *Rhizoctonia praticola* or *Rhizoctonia solani*, or a strain of *Scytalidium* sp., in particular *S. thermophilum*, or a strain of *Pyricularia* sp., in particular *Pyricularia oryzae*, or a strain of *Coprinus* sp., such as a *C. cinereus*.

The laccase may also be derived from a fungus such as *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Aspergillus*, *Neurospora*, *Podospora*, *Phlebia*, e.g. *P. radiata* (WO 92/01046), *Coriolus* sp., e.g. *C. hirsitus* (JP 2-238885), and *Botrytis*,

In a preferred embodiment of the invention the laccase is derived from a strain of *Myceliophthora* sp., especially the *Myceliophthora thermophila* laccase described in WO 95/33836 (from Novo Nordisk).

Bilirubin oxidase may be derived from a strain of

Myrothecium sp., such as a strain of *M. verrucaria*.

Following is the description of Peroxidases.

Peroxidases must be used in combination with either H_2O_2 or an oxidase to obtain the desired result, i.e. removal or at least reduction of malodour.

Suitable peroxidases can be found within the group of enzymes acting on peroxide as acceptor, e.g. E.C. 1.11.1, especially peroxidase (E.C. 1.11.1.7).

Specific examples of suitable enzymes acting on peroxide as acceptor include peroxidases derived from a strain of the fungus species *Coprinus*, in particular a strain of *Coprinus cinereus* or *Coprinus macrorhizus*, or derived from a strain of the bacteria genus *Bacillus*, in particular a strain of *Bacillus pumilus*.

Haloperoxidases are also suitable according to the invention. Haloperoxidases form a class of enzymes which are able to oxidise halides (Cl^- , Br^- , I^-) in the presence of hydrogen peroxide to the corresponding hypohalous acids. A suitable haloperoxidase is derivable from *Curvularia* sp., in particular *C. verruculosa*.

Following is the description of Oxidases

Oxidases yielding peroxide (H_2O_2) must be used in combination with a peroxidase to be able to remove or at least reduce malodour.

Suitable oxidases include glucose oxidase (E.C.

1.1.3.4), hexose oxidase (E.C. 1.1.3.5), L-amino-acid oxidase (E.C. 1.4.3.2), xylitol oxidase, galactose oxidase (E.C. 1.1.3.9), pyranose oxidase (E.C. 1.1.3.10), alcohol oxidase (E.C. 1.1.3.13).

Hexose oxidases from the red sea-weed *Chondrus crispus* (commonly known as Irish moss) (Sullivan and Ikawa, (1973), Biochim. Biophys. Acts, 309, p. 11-22; Ikawa, (1982), Meth. in Enzymol. 89, carbohydrate metabolism part D, 145-149) oxidises a broad spectrum of carbohydrates, such as D-glucose, D-galactose, maltose, cellobiose, lactose, D-glucose 6-phosphate, D-mannose, 2-deoxy-D-glucose, 2-deoxy-D-galactose, D-fucose, D-glucuronic acid, and D-xylose.

Also the red sea-weed *Iridophycus flaccidum* produces easily extractable hexose oxidases, which oxidise several different mono- and disaccharides (Bean and Hassid, (1956), J. Biol. Chem, 218, p. 425; Rand et al. (1972, J. of Food Science 37, p. 698-710).

Another suitable group of enzyme is xylitol oxidase (see e.g. JP 80892242) which oxidises xylitol, D-sorbitol, D-galactitol, D-mannitol and D-arabinitol in the presence of oxygen. A xylitol oxidase can be obtained from strains of *Streptomyces* sp. (e.g. *Streptomyces* IKD472, FERM P-14339). Said enzyme has a pH optimum at 7.5 and is stable at pH 5.5 to 10.5 and at temperatures up to 65°C.

If a L-amino acid oxidase is used it may be derived

from a *Trichoderma* sp. such as *Trichoderma harzianum*, such as the L-amino acid oxidase described in WO 94/25574 (from Novo Nordisk A/S), or *Trichoderma viride*.

A suitable glucose oxidase may originate from *Aspergillus* sp., such as a strain of *Aspergillus niger*, or from a strain of *Cladosporium* sp. in particular *Cladosporium oxysporum*.

Further, the plant- or fruit-derived enzymes can also be used in the present invention. Preferred examples of plants or fruits include fungi (*Agaricus*, *Boletus*), apple, banana, pear, strawberry, persimmon, pineapple, grape, apricot, peach, plum, papaya, quince, avocado, mango, cherry, melon, loquat, fig, prune, kiwi (Chinese gooseberry), blueberry, blackberry, raspberry, cranberry, edible burdock, egg plant, tomato, tansy, lotus root, lettuce, cabbage, hop, parsnip, spinach, radish, turnip, cauliflower, chicory, onion, celery, carrot, asparagus, horseradish, ginger, aloe, sweet pepper, barley, wheat, sweet corn, alfalfa, malt, broad bean, soybean, azuki bean, garden bean, snap bean, mung bean, potato, sweet potato, sugarcane, elephant ear, tea, tobacco, olive, mum, etc.

In this invention, either one of these enzymes or a combination of two or more thereof may be used.

So long as the desired object can be achieved, use may be made of an enzyme or an enzyme-containing composition

obtained by a method of extracting a plant.

It is preferable in the invention to use a so-called immobilized enzyme. More specifically, enzyme such as Oxidoreductases may be immobilized, e.g., by adsorption on particulate silica, by covalent linkage with glutaraldehyde to particulate silica, by adsorption on a particulate macroporous weakly basic anion exchange resin, by adsorption on polypropylene or by cross-linking, particularly with glutaraldehyde, e.g. with addition of MgSO_4 .

The immobilization may be carried out as described in EP 140452, WO 8902916, WO 9005778, WO 9015868, EP 232933 or US 4665028. Several oxidoreductases may be mixed before immobilization, or they may be immobilized separately. In the latter case, the immobilized oxidoreductases may be mixed, or they may be used separately in consecutive steps.

For example, an immobilized laccase preparation can be prepared as follows.

Laccase concentrate from *Myceliophthora thermophila* was adjusted its pH to alkaline and then sprayed onto cassava starch granules (0,5-2 mm size) using a mixer for distributing the liquid. Following the wet granules were dried.

Laccase loaded granules obtained above were sprayed with 3-6% glutaraldehyde solution to form a cross link. The wet granules were following dried to give a cross linked

immobilized laccase preparation.

A large characteristic of the invention resides in that first the deodorizing base is brought into contact with the enzyme and then obtained deodorizing base, which has been subjected to the contact treatment with the enzyme, is applied to an offensive odor source. It means that the deodorizing base applied to the offensive odor source is substantially free from the enzyme or contains only small amounts of the enzyme. Since the application of the deodorizing base to the offensive odor source brings about no fear of scattering the enzyme, undesirable effects of the enzyme on humans can be thus avoided. Further, in the case of applying the deodorizing base which has been enzymatically treated to the offensive odor source, the allowable maximum amount of the enzyme to be scattered into the air from the deodorizing base cannot be defined since it depends on the kinds of enzyme and the conditions to be applied. It is preferable that the amount of the enzyme to be scattered into the air is set as small as possible. As the standards on the amount of the enzyme to be scattered into the air (i.e., maximum amount), there is known as TLV (Threshold Limit Value). It is described in "American Conference of Governmental Industrial Hygienists, Documentation of the Threshold Limit Values", 5th edition, pp. 540-541 (1986) in more detail. The deodorizing base applied

to the offensive odor source is substantially free from enzyme or contains only small amounts of enzyme. Nevertheless, an excellent effect of eliminating or relieving an offensive odor can be thus established.

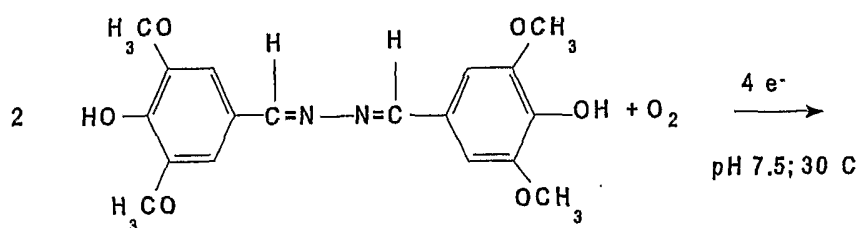
The deodorizing base can be brought into contact with the enzyme under arbitrary conditions without restriction, so long as the effect of eliminating or relieving an offensive odor can be imparted to the deodorizing base thereby.

The treatment condition cannot be determined in general, since it varies depending on the deodorizing base and the enzyme employed. For example, the enzyme can be applied to deodorizing base in such an amount as giving 10 units (LAMU) or more of the enzyme activity per 0.1 g of the deodorizing base and brought into contact at room temperature. Reaction time is irrespective, however a method which gives a better deodorant effect can be used. For example, when enzyme is used in a tube as fixed bed condition, a small diameter tube gives better deodorant effect since it gives a longer reaction time than tube with bigger diameter.

This level of the enzyme is considerably lower than the required level of enzymes in publicly known enzyme-containing deodorant compositions.

The term "one unit of enzymatic activity (LAMU/g)" as

used herein means an amount of an enzyme which can oxidize 1 μmol of syringaldazine, as represented by the following formula, which is selected as a substrate when it is allowed to react for one minute under conditions of a pH value of 7.5 and a temperature of 30°C:

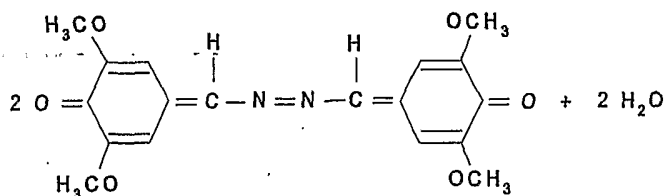


syringaldazine

yellow

reaction temperature: 30°C

reaction time: 120 seconds



tetramethoxy-azo bis methylene quinone

purple

Detailed conditions will be described below.

Enzymatic activity measuring method (LAMU method)

(Procedure of enzymatic activity measurement)

240 μl of a substrate solution as described below is added to an absorbance measurement cell and kept warm at 30°C. Then, 80 μl of a sample enzyme solution as described

below is added to the cell and, at the same time, 3.20 ml of a buffer having a pH value of 7.50 which has previously been kept warm at 30°C is added thereto and mixed.

The cell is set on an absorptiometer to measure absorbance at 530 nm after 60 seconds and 120 seconds, respectively. A value which is obtained by subtracting a measurement of the absorbance after 60 seconds from that of the absorbance after 120 seconds is defined as an increase of the absorbance.

The unit of the enzymatic activity in the sample is determined based on the following formula:

$$\text{Act} = A/W$$

Wherein Act represents a unit (LAMU/g) of the enzymatic activity in the sample;

A represents the enzymatic activity (LAMU/ml) read on a reference curve; and

W represents an amount (g) of a sample enzyme in 1 ml of a sample solution.

(1) Sample enzyme solution

PEG (50 g/l) is added to a sample enzyme, to thereby be a sample enzyme solution.

(2) Substrate solution

4.40 ml of a 0.56 mM syringaldazine (prepared such that 10.0 mg of syringaldazine is added with a 96% ethanol solution to make an entire volume up to be 50 ml and, then,

stirred for about 3 hours until completely dissolved) is added with demineralized water, Milli-Q to make an entire volume up to be 10 ml.

(3) Buffer; pH 7.50

25.0 ml of a second liquid as described below is added with 700 ml of demineralized water, Milli-Q and, further, added with 10 ml of a first liquid as described below and 5 ml of a third liquid as described below. After a pH value of the resultant mixture is adjusted to be 7.50 by an acid (free of chlorine ion) or alkali (free of chlorine ion), the mixture is added with demineralized water, Milli-Q to be made up to be 1000 ml.

The first liquid: 23.2 g of maleic acid is added with demineralized water, Milli-Q to make an entire volume up to be 200 ml.

The second liquid: 121.1 g of tris[hydroxymethyl]-aminomethane is added with demineralized water, Milli-Q to make an entire volume up to be 1000 ml.

The third liquid: 25.0 g of TRITON X-100 is added with demineralized water, Milli-Q to make an entire volume up to be 250 ml.

(Preparation of reference curve of enzymatic activity)

0.9 g of LAMU reference enzyme (61-1100; available from Novozymes A/S) is added with PEG (50 g/l) to make an entire volume up to be 50 ml. Take above solutions of 10 μ l,

20 μl , 30 μl , 40 μl , 50 μl , and 60 μl and each solution is added up to be 1 ml by PEG (50 g/l).

240 μl of the above-described substrate solution is added to an absorbance measuring cell and kept warm at 30°C. Then, 80 μl of the above-described LAMU reference enzyme solution is added to the cell and, at the same time, 3.20 ml of a buffer having a pH value of 7.50, as described below, which has previously been kept warm at 30°C is also added thereto and mixed.

The cell is set on an absorptiometer to measure absorbance at 530 nm after 60 seconds and 120 seconds, respectively. A value which is obtained by subtracting a measurement of the absorbance after 60 seconds from that of the absorbance after 120 seconds is defined as an increase of the absorbance. A reference curve of an enzyme activity is, then, constructed with the enzyme activity (LAMU/ml) in abscissa and the increase of the absorbance in ordinate.

Another characteristic of the invention resides in that the enzyme is isolated from the deodorizing base. The term "isolated" as used herein means in a state that the enzyme and the deodorizing base can be brought into contact with each other and, in case of applying the deodorizing base which has been enzymatically treated to the offensive odor source, the deodorizing base alone can be transferred separately from the enzyme, if necessary.

The isolation method is not particularly restricted. For example, the enzyme may be held on a material which is permeable to the deodorizing base but not to the enzyme. Examples of such a material include woven fabrics, nonwoven fabrics and open-cell resins. Namely, use can be made of sheets, laminates, bags and containers made of these materials.

It is also possible to isolate the enzyme from the deodorizing base using a tube opened at both ends. In this case, an enzyme-supporting unit is constructed in the tube with the use of, for example, absorbent cotton. After placing the enzyme on the supporting unit, a unit of covering the enzyme is constructed with the use of absorbent cotton. Thus the isolated state as described above can be established.

In these cases, the enzyme may be immobilized, if needed. It is also possible to use so-called an acetone freeze-dried enzyme powder preparation prepared from plant stems, leaves or roots.

For example, it is possible that the enzyme is preliminarily immobilized and the immobilized enzyme is kept isolated from deodorizing base. Following, the immobilized enzyme is brought into contact with the deodorizing base and the deodorizing base having been subjected to the contact treatment with the enzyme can be separated from the

immobilized enzyme and the deodorizing base having been subjected to the contact treatment with the enzyme alone can be applied onto the offensive odor source.

The offensive odor source according to the invention means, for example, a substance giving off an odor which is offensive for humans, a container holding such a substance, a substance with a risk of giving off an offensive odor and a space involving the same, etc. Moreover, a place which is to be deodorized is involved in the scope of the offensive odor source as used in the invention. The application of the deodorizing base treated with the enzyme means that the deodorizing base having been subjected to the contact treatment with the enzyme may be sparged, sprayed, dropped (or any other optional way can be used) over to the offensive odor source.

Main examples of the components to be deodorized include nitrogen-containing compounds such as ammonia, trimethylamine, and indole, sulfur-containing compounds such as methylmercaptan, hydrogen sulfide, dimethyl sulfide, and diallyl sulfide, and lower fatty acids such as isovaleric acid and isobutyric acid.

In prior to the contact treatment of deodorizing base with the enzyme described above, deodorizing base may be formulated with other components. Also the deodorizing base having been subjected to the contact treatment with the

enzyme may be formulated with other compounds and the thus obtained deodorizing base may be applied to the offensive odor source. The example of compounds to be formulated with are various stabilizers, anti-oxidants such as BHT (butylated hydroxy toluene), BHA (butylated hydroxy anisole), Vitamin E, and Vitamin C, fragrance or flavors described later, silicic acid anhydride, sulfate anhydride, various inorganic chlorides, various fillers such as saccharide and polysaccharide, antimicrobial agents such as benzoic acid and sodium benzoate, coloring matter and known water-soluble deodorizing agents.

The method of applying the deodorizing base having been subjected to contact treatment with the enzyme described in above process or the deodorant composition (hereby referred as deodorant) comprising such deodorizing base to the offensive odor source is not particularly restricted. Namely, the deodorant composition may be sparged or sprayed over the offensive odor source. Alternatively, the deodorant composition may be dropped or coated. Although it is preferable that the deodorant composition is in the form of a liquid, it may be any other forms so long as the desired object can be achieved thereby.

As a preferred embodiment of the application of the deodorant composition according to the invention, spray type may be cited. The spray type involves aerosol type and non-

aerosol type. Both of these types are usable in the invention. The aerosol type is characterized by using a propellant and a pressure container, while the non-aerosol type is divided into a trigger system, a pump system and a squeeze system.

First, the aerosol type will be illustrated.

A typical aerosol container consists of a pressure container, a mounting unit composed of a spray unit provided in the upper part of the pressure container and a pump unit, a dip tube going downward from the bottom of the mounting unit, and a cap.

A deodorizing base-containing liquid and a propellant are pressed into the pressure container. In the dip tube, an enzyme is maintained in an immobile state using a porous material.

When the spray unit of the spray container is pressed downward, a mixture of the deodorizing base-containing liquid and the propellant, which has been contained therein under elevated pressure, is pushed out and passes through the dip tube. After the deodorizing base-containing liquid is brought into contact with the enzyme, the mixture is released at once from holes provided in the spray unit. The method of holding enzyme is not restricted to the case as described above. Namely, it is also possible that the enzyme, in particular in the immobilized state, is wrapped

in a sheet having small pores through which the enzyme cannot permeate and thus held in the pressure container. Alternatively, the enzyme may be held in another place in an immobile state.

Next, the trigger type will be illustrated.

A typical spray container of the trigger type consists of a container holding a deodorizing base therein, a cap attached to the opening of the container, a valve case provided with a primary valve, a tube inserted into the valve case and fixed therein, a housing extrapolated to the valve case and fixed thereto, a nozzle fixed to a support, a plunger formed in the support, a trigger pressing the plunger, a spring elastically holding the support and a secondary valve inserted into a valve case of the support.

In the container, an enzyme is held in an immobile state using a porous material. Also, a deodorizing base is held therein.

When the trigger of the container is pulled with human fingers, the plunger is pressed and the piston compresses the deodorizing base having been subjected to the contact treatment with the enzyme that contained in the housing. The thus compressed deodorizing base passes through the gap between the housing and the valve case and released at once from the nozzle tip via the secondary valve. When the fingers are released from the trigger, it returns

to the original state owing to the elasticity of the spring and thus the deodorizing base having been subjected to the contact treatment with the enzyme in the container is filled into the cylinder.

As an example of a preferable deodorizing device with the application of the deodorant composition according to the invention, citation may be made of a deodorizing device consisting of a container having a deodorizing base therein, an outlet for a liquid which is provided in the upper part of the deodorizing base container and has a layer made of an enzyme, and a cap. The layer made of the enzyme may be in an arbitrary form, so long as the enzyme cannot migrate outward from the layer.

When the deodorizing base container is lifted up and leaned after taking off the cap, the deodorizing base in the container is brought into contact with the enzyme maintained at the outlet and then flows out from the outlet. After a definite amount of the deodorizing base having been subjected to the contact treatment with the enzyme has flown out, the leaned container is put up again and capped.

The deodorizing base having been subjected to the contact treatment with the enzyme flowing out from the outlet is applied to an offensive odor source. Thus, the offensive odor can be eliminated or relieved.

Next, another preferable example of a deodorizing

device with the application of the deodorant composition according to the invention will be illustrated. An enzyme, in particular an immobilized enzyme, is placed on a sheet which is permeable to a deodorizing base but not to the enzyme and the same sheet is further placed thereon. Thus an enzyme-containing sheet can be obtained. This enzyme-containing sheet is placed above or close to an offensive odor source. Next, a deodorizing base is poured into the enzyme-containing sheet. Thus, the deodorizing base having been subjected to the contact treatment with the enzyme flows out from the bottom face of the enzyme-containing sheet and applied to the offensive odor source. Thus, the offensive odor can be eliminated or relieved.

Also, citation may be made of a deodorizing device consisting of a container having a deodorizing base therein and provided with a deodorizing base (having been subjected to the contact treatment with the enzyme) outlet, a container having an enzyme therein and provided with a deodorizing base inlet in the lower part of the container and a deodorizing base outlet in the upper part of the container, and a connecting unit such as a pipe connecting these two containers.

The deodorizing base contained in the deodorizing base container is poured into the enzyme container from the lower part with the use of a transfer unit such as a pump as

needed. Thus, the deodorizing base having been subjected to the contact treatment with the enzyme is brought into contact with the enzyme and then the deodorizing base and the enzyme are discharged from the upper part of the enzyme container. The deodorizing base is then applied to an offensive odor source by an arbitrary method.

It is also possible to use an enzyme-containing sheet as a substitute for the enzyme container.

The deodorizing base obtained by subjecting the deodorizing base to the contact treatment with the enzyme serves as an excellent deodorant per se. Also, compositions with the deodorizing base and an enzyme which can be separated from the deodorizing base such as an immobilized enzyme are usable as deodorants.

The deodorant compositions can eliminate or relieve an odor which is offensive to humans, especially effective on eliminating a sulfur-containing offensive odor (e.g., hydrogen sulfide, dimethyl sulfide, diallyl sulfide, methyl mercaptan) or a nitrogen-containing offensive odor (e.g., ammonia, trimethylamine, indole). Specific examples of such an odor include halitosis, body odor, odor in a refrigerator, characteristic odors of foods such as natto (fermented soybeans), kitchen odor such as from garbage or used cooking utensils, rubbish odor, odor in a plant, odor of waste liquor from a plant and odor of excreta of animals including

humans and odor of permanent wave and hair dye.

In the invention, the deodorizing base, the deodorizing base having been subjected to the contact treatment with the enzyme or the deodorant composition comprising such deodorizing base may be further blended with a flavor component, a fragrance component or both of the same. As a result, a pleasant smell can be imparted to the deodorizing base having been subjected to the contact treatment with the enzyme or deodorant composition comprising such deodorizing base. Some of components constituting the deodorant composition would give off a little offensive odor characteristic to the substrate. In such a case, the flavor and/or the fragrance can mask the offensive odor to thereby exert a sufficient deodorizing effect.

Although the content of the flavor component or the fragrance component is not particularly restricted, favorable results can be obtained by using from 0.01 to 5% by weight thereof based on, for example, the deodorizing base having been subjected to the contact treatment with the enzyme.

Preferable examples of the flavor or the fragrance include the following compounds and essential oils.

Amyl salicylate, benzylacetone, benzyl salicylate, 1,1,2,3,3-pentamethyl-2,3,5,6,7-pentahydroinden-4-one,

2,6,6,8-tetramethyltricyclo[5.3.1.0(1,5)]undecan-8-ol,
 citronellol, tricyclo[5.2.1.0(2,6)]-4-decen-8-yl acetate,
 tricyclo[5.2.1.0(2,6)]-4-decen-8-yl propanoate, 2,6-
 dimethyloct-7-en-2-ol, 2,6-dimethylheptan-1-ol,
 phenoxybenzene, 4,6,6,7,8,8-hexamethyl-6,7,8-trihydroxy-
 cyclopenta[1,2-g]isochroman, galbanum oil, geranyl acetate,
 3-ethoxy-1,1,5-trimethylcyclohexane, hexyl cinnamic aldehyde,
 hexyl salicylate, isobornyl acetate, 1-(3,4,10,10-
 tetramethylbicyclo[4.4.0]-5-decen-3-yl)ethan-1-one, 2-
 ((2Z)pent-2-enyl)-3-methylcyclopentane-2-en-1-one, 3-[4-
 (tert-butyl)phenyl]-2-methylpropanal, linalool, methyl 2-
 aminobenzoate, (1E)-1-(6,6-dimethyl-2-methylenecyclohexyl)-
 1-penten-3-one, methyl 2-((1E)-1-aza-8-hydroxy-4,8-dimethyl-
 1-nonenyl)benzoate, 2-noninal-dimethylacetal, 2-phenylethan-
 1-ol, α -terpineol, 1-((6S,1R)-2,2,6-
 trimethylcyclohexyl)hexan-3-ol, 2-(tert-butyl)cyclohexyl
 acetate, 4-(tert-butyl)cyclohexyl acetate, 2-
 methoxynaphthalene, 1-(2,6,6,8-
 tetramethyltricyclo[5.3.1.0(1,5)]-8-undecen-9-yl)ethan-1-one,
 acetylisoegenol, allylamyl glycolate,
 1,6,10,10-tetramethyl-5-oxatricyclo[7.4.0.0(2,6)]tridecane,
 (1S,2R,6R)-1,6,10,10-tetramethyl-5-
 oxatricyclo[7.4.0.0(2,6)]tridecane, amyl- α -cinnamaldehyde,
 anisaldehyde, benzyl acetate, bergamot oil, 7-methyl-2H,4H-
 benz[b]1,4-dioxepin-3-one, cinnamyl alcohol, citronellol, 2-

methyl-3-[4-(methylethyl)phenyl]propanal, 2-oxabicyclo[4.4.0]decan-3-one, (2E)-1-(2,6,6-trimethylcyclohexan-3-enyl)butan-2-en-1-one, 2,6-dimethyloctan-7-en-2-ol, 1,1-dimethyl-2-phenylethyl acetate, 2,6-dimethylheptan-1-ol, eugenol, 2-oxacyclohexadecan-1-one, 3-(4-ethylphenyl)-2,2-dimethylpropanal, geraniol, methyl 2-(3-oxo-2-pentylcyclopentyl)acetate, hexyl acetate, hexyl salicylate, 2H,4H,4aH,9aH-indano[2,1-d]1,3-dioxane, (3E)-4-(2,6,6-trimethylcyclohexan-2-enyl)butan-3-en-2-one, (3E)-4-(6,6-dimethyl-2-methylenecyclohexyl)butan-3-en-2-one, 1-(3,4,10,10-tetramethylbicyclo[4.4.0]-5-decen-3-yl)ethan-1-one, 2-((2Z)-2-pentenyl)-3-methylcyclo-2-penten-1-one, 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene carbaldehyde, 3-[4-(tert-butyl)phenyl]-2-methylpropanal, (5E)-2,6-dimethylheptan-5-enal, methylchavicol, (1E)-1-(6,6-dimethyl-2-methylenecyclohexyl)pentan-1-en-3-one, 2,5-dioxacycloheptadecan-1,6-dione, trans-2-tridecenal, phenylethyl acetate, phenylethyl alcohol, styralyl acetate, dimethylcyclohexanal, 5-heptyl-3,4,5-trihydrofuran-2-one, 2-(tert-butyl)cyclohexyl acetate, α -fenchyl alcohol, 1-decanal, 2,6-dimethyl-7-octen-2-ol, 4,6,6,7,8,8-hexamethyl-6,7,8-trihydrocyclopenta[1,2-g]isochroman, benzyl benzoate, methyl 2-(3-oxo-2-pentylcyclopentyl)acetate, 3,7-dimethyl-2,6-octadien-1-al, linalyl acetate, tetrahydrolinalool, vaniline, caryophyllene, menthol, isopregol, eucalyptol, p-menthane-

3,8-diol, vinyl butyl ether, apple oil, apricot oil, cassia oil, cinnamyl aldehyde, allyl hexanoate, isoamyl acetate, amyl alcohol, anethol, benzaldehyde, benzyl acetate, isobutyl acetate, butyl butyrate, isobutyl butyrate, camphor, carvone, β -caryophyllene, cinnamaldehyde, cinnamyl alcohol, citral, citronellyl acetate, cumminaldehyde, cymene, decalactone, decanal, diacetyl, ethyl acetoacetate, ethyl anthranate, ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl 2-methyl lactate, ethyl salicylate, ethyl vaniline, ethyl menthol, eugenol, isoeugenol, furfural, furfuryl alcohol, hexanal, hexenal, hexynyl acetate, hexyl alcohol, ionone, irone, limonene, linalol, maltol, menthol, menthone, methyl acetate, methyl anthranate, methyl cinnamate, methyl salicylate, nerol, nerolidol, nonalactone, nonanal, octalactone, octanal, octanol, octenol, octyl acetate, phenyl acetate, phenetyl alcohol, pinene, piperanal, propyl acetate, thymol, undecalactone, anise oil, anise star oil, basil oil, laurel leaf West Indian oil, camphor tree oil, buchu leaf oil, cardamon seed oil, cassie bark oil, camomile roman oil, cinnamon bark oil, cinnamon leaf oil, clove bud oil, cognac green oil, coriander oil, cubeb oil, caraway oil, fennel oil, garlic oil, ginger oil, petitgrain oil, lemon oil, lime oil, orange oil, citrus oil, cedar tree oil, camphor tree oil, citronella oil, patchouli oil, eucalyptus oil, bay oil, grapefruit oil, mandarin oil, sandalwood oil,

juniper berry oil, rose oil, ylang-ylang oil, tangerine oil, geranium oil, limonene, Japanese mint oil and peppermint oil, etc. Use can be also made of mixtures of two or more of them.

The deodorant composition according to the invention may further contain other components. More specifically speaking, use may be made of, for example, various stabilizers, silicic acid anhydride, sulfate anhydride, various inorganic chloride, various fillers such as saccharide and polysaccharide, coloring matter, surfactants, anti-oxidant such as BHT, BHA, Vitamin E and Vitamin C, antimicrobial agent such as benzoic acid and sodium benzoate, commercially available deodorant agent such as activated carbon and cyclodextrin. These compounds can be formulated so long as the desired object can be achieved thereby.

Because of being effective in eliminating or relieving odors over a broad scope, the deodorant compositions according to the invention are applicable to various uses. Since the deodorant described in this invention can capture sulfur- or nitrogen-containing compounds, it is possible to eliminate any kind of offensive odor in which the compounds mentioned above is exists as an odor ingredient and therefore can be applied for extensive ways to use. More specifically speaking, they can be applied to, for example, kitchen deodorizers such as rubbish

odor, chopping board, kitchen knife, pan, table ware, hair deodorizers for permanent waving or hair coloring agent, sanitary deodorizers such as diapers, bed sheets and bed mats for bedridden aged people and sanitary products such as napkins, deodorizers for textile products such as sofa, curtain and cloths, deodorizers for pet or related goods, deodorizers for toilet or bath. Specially, use of spray type deodorizers is an easy way to use deodorizers and besides it is quite effective to achieve complete deodorize of offensive odor.

Examples

Now, the invention will be described in greater detail by reference to the following Reference Examples and Examples. However, it is to be understood that the invention is not construed as being restricted thereto. Further, % indicate w/w% when there is no particular definition.

Reference Example 1: Preparation of deodorizing base

1 kg of raw coffee beans were ground in a mill and passed through a 5 mm-mesh filter. After adding 10 l of water, the ground matter thus obtained was extracted at 85 to 95°C for 2 hours. The extract was filtered and the filtrate was adsorbed by a column XAD2 (manufactured by

ORGANO Corporation). After washing with water, it was eluted with methanol. The eluate was concentrated to dryness to thereby give 182 g of a raw coffee bean extract.

20 mg of this raw coffee bean extract was dissolved in water. The obtained solution was referred to as a liquid raw coffee bean extract and employed in the following test.

Reference Example 2: Preparation of enzyme

100 g of burdock and 400 ml of acetone cooled to -20°C were introduced into a mixer and ground together therein followed by filtration under suction. The residue was sufficiently washed with 500 ml of an 80% aqueous solution of acetone at 5°C. Then the solvent was eliminated with the use of an evaporator to thereby give 20 g of a burdock powder.

Reference Example 3: Preparation of immobilized enzyme

527 g *Myceliophthora thermophila* laccase concentrate (4690 LAMU/g) was adjusted to pH 10 with a 1N NaOH solution and sprayed onto 2000 g cassava starch granules (grain size: about 0.7 mm) using a 5 litre Lödige MG5 mixer for distributing the liquid. The wet granules were following dried in a Glatt fluid bed MP1 at 60°C. 1Kg of the dry laccase loaded granules were transferred to a 5 litre Lödige MG5 mixer and sprayed with 22 g of a 4.5 % glutaraldehyde

solution. The wet granules were following dried in a Glatt fluid bed MP1 at 60°C to give a cross linked immobilized laccase preparation with an activity of 344 LAMU/g.

Example 1: Preparation of deodorizing base having been subjected to the contact treatment with enzyme

20 mg of the burdock powder obtained in Reference Example 2 was packed into a tube which was covered with an absorbent cotton stopper permeable to an aqueous solution at about 3 cm above from bottom end and opened at the top end. Then an absorbent cotton stopper permeable to an aqueous solution was put thereon to make the burdock powder immovably packed. 3 ml of the raw coffee bean extract obtained in Reference Example 1 was poured into the tube and the raw coffee bean extract passing through layer of the acetone extract of burdock was collected.

Test 1: Deodorant performance of deodorizing base having been contacted with enzyme

2 ml of the raw coffee bean extract prepared in Example 1 was introduced into a 50 ml brown vial. After adding 2 μ l of a 15% aqueous solution of sodium methylmercaptan, the vial was covered with a parafilm and stirred at 25 °C. After 10 minutes, 50 ml of the head space gas in the vial was passed through a gas detection tube

(manufactured by GASTECH) and the concentration of sulfur-containing compounds remaining in the gas was measured. 1 ml of raw coffee bean extract of Example 1 was added to brown glass with 1 ml water and following applied the same process described above. The deodorant rate was calculated based on the result.

Then the deodorization rate was calculated in accordance with the following formula.

Table 1 shows the results.

$$\text{Deodorization rate (\%)} = 100 \times \{1 - (A)/(B)\}$$

In this formula, A stands for the concentration of the offensive odor components thus measured, while B stands for the concentration of the offensive odor components measured in a control.

The control was prepared by adding 2 ml of water as a substitute for 2 ml of the raw coffee bean extract of Example 1.

In comparative sample (1), 2 ml of raw coffee bean extract of Reference Example 1 was added as a substitute for 2 ml of the raw coffee bean extract of Example 1.

In comparative sample (2), 2 ml of solution prepared by following method was added as a substitute for the raw coffee bean extract of Example 1.

3 ml of water was passed as a substitute for the raw coffee bean extract of Example 1.

In comparative sample (3), 2 ml of a mixture of 1 ml of the solution obtained in comparative sample (2) with 1 ml of the raw coffee bean extract obtained in Reference Example 1 was added as a substitute for the raw coffee bean extract of Example 1.

This invention is a mixture of 20 mg raw coffee bean extract of Example 1 dissolved in 3 ml water.

Table 1

	Deodorization time (min)	Found (ppm)	Deodorization ratio (%)
Control	10	200	-
Comparative sample (1)	10	200	0
Comparative sample (2)	10	200	0
Comparative sample (3)	10	200	0
Invention product 2 ml	10	0	100
Invention product 1 ml + water 1 ml	10	0	100

Example 2: Preparation of deodorizing base having been contacted with enzyme

Immobilized laccase obtained in Reference Example 3 was used in following amount as a substitute for the burdock powder of Example 1. The same tube as Example 1 was prepared.

Next, 5 ml of deodorizing base (if the types of deodorizing base is not defined) described in below was

passed through the layer of immobilized laccase at speed described below to obtain the deodorizing base having being contacted with enzyme.

Amount of immobilized laccase used in the experiment: 500 mg, 1000 mg

Deodorizing base: raw coffee bean extract solution (0.5%, 2.5%, 5%)

Lignin solution (Nacalai Tesque Inc., 0.5%)

Raw coffee bean extract solution is the solution comprising 0.5 w/w% raw coffee bean extract of Reference example 1. Same is applied for raw coffee bean extract solution (2.5%) and raw coffee bean extract solution (5%). Lignin solution (Nacalai tesque, 0.5%) is the solution comprising 0.5 w/w% Nacalai tesque's lignin.

Test 2: Deodorant performance test of deodorizing base having been subjected to the contact treatment with enzyme

The deodorizing base contact treated with enzyme of Example 2 was tested in the same method as Test 1.

The result was shown in Tables 2 to 5. The calculation was made based on control, a deodorizing base which was not subjected to the contact treatment with the immobilized enzyme.

Table 2

1) Enzyme amount and deodorant rate

Enzyme amount	Deodorant rate (%)
500 mg	57.1
1000 mg	100.0

Deodorizing base: raw coffee bean extract solution 0.5%

Flow rate: 2 ml/min.

Table 3

2) Kinds of deodorizing base and deodorant rate

Enzyme amount	Deodorant rate (%)
Raw coffee bean extract solution	57.1
Lignin solution (0.5%)	100

Amount of immobilized laccase: 500 mg

Flow rate: 2 ml/min.

Table 4

3) Dosage of deodorizing base and deodorant rate

Enzyme amount	Deodorant rate%	
	Deodorizing base 0.5%	Deodorizing base 2.5%
500 mg	57.1	85.0
1000 mg	100.0	100.0

Deodorizing base: raw coffee bean extract solution

Flow rate: 2 ml/min.

Example 3 : Preparation of deodorizing base having been subjected to the contact treatment with enzyme

5g of Immobilized laccase obtained in Example 3 (344LAMU/g) is immovably packed into a tube which was covered with an absorbent cotton stopper permeable to an aqueous solution and then tube was set into the spray bottle.

The 2.5% of raw coffee bean extract solution obtained in Example 1 was filled into the spray bottle.

Press the knob and guide the raw coffee bean extract solution obtained in Example 1 into the tube, let it pass through the tube contact with immobilized laccase, to obtain a spray type deodorizing base.

Test3 : Deodorant performance test of deodorizing base having been contacted with enzyme

5g of Immobilized laccase obtained in Example 3 (344LAMU/g) and 2 ml of deodorizing base having been contact treated with enzyme was collected and the deodorizing base having been subjected to the contact treatment with enzyme was subjected for the deodorant test as same method described in Test 1.

The calculation was made based on control, a deodorizing base which was not contact treated with

immobilized enzyme. The result of 71.4% deodorant rate was obtained.

Industrial Applicability

The invention provides enzymes, deodorizing base having been subjected to contact treatment with enzyme, deodorant compositions comprising said deodorizing base and a deodorization method showing an excellent deodorizing effect on various offensive odor components. Some of known deodorants contain enzymes. The substantially enzyme-free deodorant compositions according to the invention exert an excellent deodorizing effect and are highly useful in practice.

In case of spraying an enzyme-containing deodorant composition to eliminate or relieve an offensive odor, there arises a risk that the enzyme contained in the deodorant composition is scattered into the atmosphere, and repeated inhalation of the scattered enzyme by humans might sometimes induce asthma or fever.

Thus, it is intended to provide a deodorization method whereby a deodorizing effect comparable to the deodorizing effect exerted by enzyme-containing deodorant compositions can be achieved without any fear of undesirable effects on humans in case of spraying a deodorant composition to eliminate or relieve an offensive odor.

Namely, an offensive odor is eliminated or relieved by subjecting a deodorizing base to a contact treatment with an enzyme and then applying the deodorizing base, which has been subjected to the contact treatment, to an offensive odor source. It is preferable to use a phenolic compound as the deodorizing base and a phenolic compound-oxidizing enzyme as the enzyme. It is still preferable that the deodorizing base is further added and blended with a fragrance component and/or a flavor component.

While the invention has been described in detail with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

CLAIMS

1. A deodorization method comprising applying a deodorizing base, which has been subjected to a contact treatment with an enzyme, to an offensive odor source.
2. A deodorization method according to claim 1 wherein the deodorizing base is a phenolic compound and the enzyme is a phenolic compound-oxidizing enzyme.
3. A deodorization method according to claim 1 or 2 wherein a fragrance component and/or a flavor component are added to the deodorizing base.
4. A deodorization method according to any one of claims 1 to 3 wherein the enzyme is an immobilized phenolic compound-oxidizing enzyme.
5. A method of eliminating an offensive odor comprising holding an enzyme and a deodorizing base in a single container in a state of isolating the enzyme from the deodorizing base, then bringing the deodorizing base into contact treatment with the enzyme in the container, and applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source.

6. A method of eliminating an offensive odor comprising holding an enzyme and a deodorizing base in separate containers, then transferring any one of the enzyme and the deodorizing base into the container having the other therein, bringing the deodorizing base into contact treatment with the enzyme, and applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source.

7. A deodorant composition containing as the active ingredient a deodorizing base which has been brought into contact with an enzyme.

8. A deodorizing device comprising a unit of maintaining an enzyme and a deodorizing base in a single container in a state of isolating the enzyme from the deodorizing base, and a unit of, after a contact treatment of the deodorizing base with the enzyme, applying the deodorizing base which has been subjected to the contact treatment with the enzyme to an offensive odor source.

9. A deodorizing device comprising a unit of holding an enzyme and a deodorizing base in separate containers, a unit of transferring any one of the enzyme and the deodorizing base into the container having the other therein, and a unit

of bringing them into contact treatment with each other and then applying the deodorizing base, which has been subjected to the contact treatment with the enzyme, to an offensive odor source.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/JP2004/007153

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K7/32 A61L9/01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EP0-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 064 956 A (TAKASAGO PERFUMERY CO LTD) 3 January 2001 (2001-01-03) paragraphs '0010!', '0011!', '0027! -----	1-3,7
X	US 5 804 170 A (NEGISHI OSAMU ET AL) 8 September 1998 (1998-09-08) the whole document -----	1-3,7
A	FR 2 798 137 A (CALONE BONNEAU MARGUERITE GABR) 9 March 2001 (2001-03-09) the whole document -----	1-9
A	US 5 342 768 A (PEDERSEN SVEN ET AL) 30 August 1994 (1994-08-30) the whole document ----- -/--	1-9



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Date of the actual completion of the international search

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 141 223 A (HOECHST AG) 15 May 1985 (1985-05-15) the whole document	1-9
A	US 4 665 028 A (AMOTZ SHMUEL) 12 May 1987 (1987-05-12) the whole document	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/JP2004/007153

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 1064956	A	03-01-2001	JP 2001000527 A EP 1064956 A2	09-01-2001 03-01-2001
US 5804170	A	08-09-1998	JP 9038183 A	10-02-1997
FR 2798137	A	09-03-2001	FR 2798137 A1 AU 7297800 A WO 0118188 A2	09-03-2001 10-04-2001 15-03-2001
US 5342768	A	30-08-1994	AT 91503 T CA 2003163 A1 DE 68907611 D1 DE 68907611 T2 DK 85791 A WO 9005778 A1 EP 0444092 A1 JP 2873251 B2 JP 4501664 T	15-07-1993 16-05-1990 19-08-1993 21-10-1993 08-05-1991 31-05-1990 04-09-1991 24-03-1999 26-03-1992
EP 0141223	A	15-05-1985	DE 3334846 A1 AT 30335 T AU 565703 B2 AU 3355784 A CA 1277268 C DE 3466893 D1 DK 460984 A EP 0141223 A1 IL 73059 A JP 60094086 A ZA 8407562 A	04-04-1985 15-11-1987 24-09-1987 04-04-1985 04-12-1990 26-11-1987 28-03-1985 15-05-1985 30-11-1988 27-05-1985 29-05-1985
US 4665028	A	12-05-1987	BE 897926 A1 CA 1205402 A1 DE 3336257 A1 DK 442883 A ,B, ES 8504247 A1 FI 833615 A ,B, FR 2534272 A1 GB 2129809 A ,B GB 2128620 A ,B IE 56079 B1 IT 1163947 B JP 1723280 C JP 4007192 B JP 59130184 A KR 9007631 B1 NL 8303415 A ,B,	05-04-1984 03-06-1986 12-04-1984 07-04-1984 01-07-1985 07-04-1984 13-04-1984 23-05-1984 02-05-1984 10-04-1991 08-04-1987 24-12-1992 10-02-1992 26-07-1984 17-10-1990 01-05-1984