SPECIFICATION

Title of Invention: Method for evaluating perceived freshness and/or palatability

TECHNICAL FIELD

[0001] The present invention relates to a method for evaluating a fresh feeling (fresh feeling) and/or palatability, and the like.

BACKGROUND ART

[0002] In recent years, palatability involving a fresh sensation has been growing, with an increasing number of products claiming to have a “raw texture” sensation. Its scope of appeal is very broad, and includes not only juices and *Shochu* highballs which claim to have a sensation of freshly squeezed fruit, but also dairy products such as fresh cheese and fresh butter, and even “raw texture” bread and “raw texture” cookies in the category of bread and confectionery that involve a baking process.

[0003] A perceived freshness can also be considered, in a broad sense, as a sensation associated with freshly made or freshly prepared foods. For example, the sensation of toasty, freshly baked bread, the sensation of sweet, freshly cooked rice, and the sensation of rich, freshly brewed coffee can be considered some kind of perceived freshness. In other words, a perceived freshness in terms of foods is not necessarily dependent on the food being unheated. Rather, it can be regarded as a sensation of freshness triggered by freshly made or freshly prepared foods, and plays a very important role in food palatability.

[0004] Substances associated with impartment or enhancement of a perceived freshness include acetaldehyde, butyraldehyde, and isobutyraldehyde. According to the website of the Food Safety Commission of Japan, these aldehydes are also easily generated by fermentation and heating, and are contained in a variety of foods, including fruits, vegetables, legumes, alcoholic beverages, tea leaves, bread, baked confectionery, soft beverages, meats, and dairy products. Acetaldehyde, in particular, is a highly volatile compound that volatilizes along with other aroma components of fruits, allowing a stronger fresh aroma of fruits to be sensed (Patent reference 1), and in fermented foods such as bread, butter, and cheese, it is said to be a component that contributes to a freshly prepared sensation and a fresh fermented taste (Non-patent reference 1, Patent reference 2). In addition to aldehydes, a wide variety of compounds, including ketone compounds such as 2,3-pentanedione and diacetyl, alcohol compounds such as 3,6-nonadienol, and even spice extracts such as fenugreek, are said to be associated with impartment or enhancement

of a perceived freshness and a freshly prepared sensation (Patent references 3 and 4).

[0005] As described above, a number of ingredients associated with impartment or enhancement of a perceived freshness have been reported. However, regarding the case of a perceived freshness of the fruit described in Patent reference 4, the current situation is that "while many ingredients that can reproduce the aroma of the fruit itself have been found, an ingredient that can distinctly convey the perceived freshness of the fruit has not clearly been found." This seems to be mainly due to the lack of a biological indicator for objectively evaluating a perceived freshness, and the fact that a perceived freshness is only evaluated by subjective sensory evaluation, which is ambiguous.

[0006] In recent years, an exhaustive screening of responses of all of the approximately 400 types of human olfactory receptors to a variety of odorants has provided a general understanding of which olfactory receptors respond to which fragrance notes (Non-patent reference 2, Patent reference 5). Specifically, the olfactory receptor OR5AN1 is known to respond very specifically to odorants with a musk aroma, and since OR5AN1 responds more strongly to stronger musk aromas, the OR5AN1 response can be used as an objective indicator of an odor strength of a musk aroma (Non-patent reference 3).

[0007] In addition, responses of some olfactory receptors have been found to cause emotions and behaviors such as like or dislike, or attraction or avoidance (Non-patent reference 4). Since genome-wide association studies (GWAS) of a population of several hundred thousand human subjects, has indicated an association between certain eating habits and food preferences and olfactory receptor genes (Non-patent references 5 and 6), some olfactory receptors seem to be involved in the formation of eating habits or food preferences by triggering specific emotions or sensations.

[0008] To date, no olfactory receptor has been shown to have a clear "causal relationship" with the perception of a perceived freshness. Although Patent reference 5 discloses OR1D2, OR10A2, and OR9A4 as olfactory receptors that are associated with a fresh aroma, the reference only mentions associations based on "correlations" and does not rule out pseudo-correlations or confounding factors. For example, OR1D2, which is associated with a fresh aroma in Patent reference 5, responds to a wide range of compounds, including lactone and citrus compounds contained in fruit (Non-patent reference 6), resulting in the confounding of various fruit-related aroma expressions, such as sweet, fresh, fruity, apricot, pineapple, peach, orange flower, and sulfurous. This makes it unclear whether OR1D2 is truly related to a fresh aroma. Patent reference 5 also states that a bready aroma is associated with OR6B1.

[0009] Patent reference 6 discloses that acetaldehyde, propanal, butanal, and pentanal,

which are considered malodorous substances, induce the response of OR6B1. However, since acetaldehyde odor is not suppressed by OR6B1 response inhibitors (antagonists), it has been pointed out that OR6B1 may not actually be involved in the perception of aldehyde odor (Non-patent reference 7). Thus, the function of OR6B1 remains unclear. In addition, Patent reference 7 discloses that aldehydes such as isobutyraldehyde induce the response of OR6B2. Non-patent reference 5 states that a genomic region containing the OR6B1 gene is strongly correlated with fruit intake. However, it does not mention why this genomic region is associated with the fruit intake at all, and the causal relationship is unclear.

PRIOR ART DOCUMENTS

Patent Documents

[0010] Patent reference 1: Japanese Unexamined Patent Application Publication No. 2015-27311

Patent reference 2: Japanese Unexamined Patent Application Publication No. 2014-8049

Patent reference 3: Japanese Unexamined Patent Application Publication No. 2019-106518

Patent reference 4: Japanese Unexamined Patent Application Publication No. 2016-198025

Patent reference 5: WO2023/013792A1

Patent reference 6: Japanese Unexamined Patent Application Publication No. 2023-117130

Patent reference 7: Japanese Unexamined Patent Application Publication No. 2023-84478

Non-Patent Documents

[0011] Non-patent reference 1: Research Report, Toyo Institute of Food Technology, 30, 79-85 (2014)

Non-patent reference 2: Flavor and Fragrance Journal, 2015, 30, 342-361

Non-patent reference 3: The Journal of Neuroscience, April 20, 2016, 36(16): 4482-44

Non-patent reference 4: Nature Communications 10: 209 (2019)

Non-patent reference 5: Nature Communications 11: 1467 (2020)

Non-patent reference 6: Nature Communications 13: 2743 (2022)

Non-patent reference 7: ECRO XXIV DIJON: “OR1D2 IS A BROADLY

TUNED HUMAN OLFACTORY RECEPTOR". September 19, 2014, Posters

Non-patent reference 8: The 95th Annual Meeting of the Japanese Biochemical Society. [1P-370] Identification of key amino acid residues responsible for differences in functional expression between aldehyde-responsive olfactory receptors

SUMMARY OF THE INVENTION

Problem To Be Solved By The Invention

[0012] Although a perceived freshness (fresh feeling) is a very important sensation in food palatability, it is subjective and ambiguous, and its biological entity is unclear. Although compounds that impart a perceived freshness are known, how the perceived freshness is biologically perceived and how it is related to the formation of eating habits and food preferences have never been fully elucidated.

Under these circumstances, it is desirable to clarify the biological entities of the perceived freshness and to evaluate the perceived freshness qualitatively and/or quantitatively.

Means To Solve The Problem

[0013] The present invention was made in consideration of the above circumstances and provides a method for evaluating a perceived freshness and/or palatability, and the like, as stated below.

[0014]

[1] A method for evaluating a perceived freshness and/or palatability, the method comprising the steps of: adding or exposing a substance to be evaluated to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and evaluating the perceived freshness and/or palatability of the substance to be evaluated based on the measured response.

[0015]

[2] A method for screening for a substance that imparts, enhances, or suppresses a perceived freshness and/or palatability,

wherein the screening of the substance that imparts a perceived freshness and/or palatability comprises: adding or exposing a test substance to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide;

and identifying the test substance as a substance that imparts the perceived freshness and/or palatability based on the measured response,

wherein the screening of the substance that enhances a perceived freshness and/or palatability comprises: adding or exposing a test substance, together with an OR6B1 response-inducing substance, to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and identifying the test substance as a substance that enhances the perceived freshness and/or palatability based on the measured response,

or

wherein the screening of the substance that suppresses a perceived freshness and/or palatability comprises: adding or exposing a test substance, together with an OR6B1 response-inducing substance, to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and identifying the test substance as a substance that suppresses the perceived freshness and/or palatability based on the measured response.

[0016]

[3] The method according to [1] or [2] above, wherein the perceived freshness is a perceived freshness of fruit or a freshly prepared sensation of a processed food.

[4] The method according to [1] or [2] above, wherein the response of the olfactory receptor polypeptide is measured on a cell endogenously expressing the olfactory receptor polypeptide or on a cell forcibly expressing the olfactory receptor polypeptide through genetic engineering.

[5] The method according to [1] or [2] above, wherein the response of the olfactory receptor polypeptide is measured by a reporter assay or calcium imaging.

[6] An agent for imparting or enhancing a perceived freshness and/or palatability, comprising a substance that imparts or enhances the perceived freshness and/or palatability, as identified by the method according to [2] above.

[7] The agent for imparting or enhancing a perceived freshness and/or palatability according to [6] above, wherein the substance that imparts or enhances the perceived freshness and/or palatability is methyl isoeugenol.

EFFECTS OF THE INVENTION

[0017] The present invention can provide a method for qualitatively and quantitatively evaluating a perceived freshness and/or palatability, which has been a subjective and ambiguous sensation, using an OR6B1 response. The present invention can also provide a method for screening for a substance that imparts, enhances, or suppresses a perceived freshness and/or palatability, and the substance identified by this method can be used, for example, to control the perceived freshness, enhance the food palatability, etc.

BRIEF DESCRIPTION OF DRAWINGS

[0018] [Figure 1] a) A chart showing gas-phase responses of the olfactory receptor OR6B1 to various fruits. b) A chart showing the results of gas-phase screening of all 454 kinds of human olfactory receptors against pink grapefruit.

[Figure 2] A chart showing the results of screening for flavor ingredients that induce the response of the olfactory receptor OR6B1.

[Figure 3] A chart showing the gas-phase responses of the olfactory receptor OR6B1 to freshly baked bread, butter, and cream cheese.

[Figure 4] Charts showing the results of liquid-phase screening of all 454 kinds of human olfactory receptors against various perceived freshness-imparting ingredients.

[Figure 5] Graphs showing the results of measurement of the concentration-dependent suppression of olfactory receptor OR6B1 responses for various perceived freshness-imparting ingredients.

[Figure 6] A graph showing the results of measurement of the enhancement of olfactory receptor OR6B1 responses over time using an olfactory receptor OR6B1 response-enhancing agent.

[Figure 7] A graph showing the results of measurement of the suppression of olfactory receptor OR6B1 responses over time using an olfactory receptor OR6B1 response-suppressing agent.

[Figure 8] A graph showing differences in the strength of olfactory receptor OR6B1 response to various orange flavors.

[Figure 9] A chart showing the results of sensory evaluation tests of various orange flavors in terms of a perceived freshness.

DESCRIPTION OF EMBODIMENTS

[0019] Overview of the present invention

Hereinafter, the present invention will be described in detail. The scope of the

present invention is not bound by these descriptions, and the present invention may be implemented by suitably modifying in ways other than those illustrated in the following examples, provided that the purpose of the present invention is not impaired.

Note that this specification incorporates the entirety of the specification of Japanese Patent Application No. 2024-029824 (filed on February 29, 2024 (R6)), on which priority is claimed by this application. In addition, all publications cited herein, such as prior art literature, as well as published patent applications, patent publications, and other patent literature, are incorporated herein by reference.

[0020] The present invention can provide a method for qualitatively and quantitatively evaluating a perceived freshness (fresh feeling) and/or palatability, using the response of the olfactory receptor OR6B1 as an indicator. The present invention can also provide a method for screening for a substance that imparts, enhances, or suppresses a perceived freshness and/or palatability, using the response of the olfactory receptor OR6B1 as an indicator, and the substance identified by this method can be used, for example, to control the perceived freshness, enhance food palatability, etc. (i.e., to regulate the perceived freshness and/or palatability).

[0021] Upon reviewing data from genome-wide association studies (GWAS) targeting a human population of several hundred thousand individuals, the present inventor noticed that a genomic region containing OR6B1 (QTL: Quantitative Trait Locus) was strongly associated with the consumption of fresh fruits, but no association was found between this region and the consumption of dried fruits, and anticipated that OR6B1 was associated with the perception of a fresh element (Table 1). The fact that various fruit aromas induce the response of OR6B1 (Figure 1a) suggests that actual fresh fruits contain an OR6B1 response-inducing component, and further the fact that, among all human olfactory receptors, OR6B1 responded most strongly to a fruit aroma (Figure 1b) suggests that OR6B1 was a receptor that strongly influences human olfaction.

[0022] Screening for substances that induce the response of OR6B1 showed that OR6B1 responded not only to a group of perceived freshness-imparting substances common in fruits, such as aldehydes, but also to a group of substances that impart a freshly baked sensation of bread and a perceived freshness of butter (Figure 2 and Table 2). When the genome-wide association studies were reviewed again, the top three eating habits predominantly associated with OR6B1 were found to be the consumption of fruit, bread, and butter (Table 3), which were fully consistent with the OR6B1 response screening. This indicates a causal relationship in which “response” of OR6B1 “imparts” a perceived freshness and forms palatability of fruit, bread, and butter.

[0023] Furthermore, since the group of perceived freshness-imparting substances induced the response of only OR6B1 in common, the possibility that a perceived freshness was imparted by the responses of olfactory receptors other than OR6B1 was ruled out (Figure 4). The OR6B1 responses to the group of perceived freshness-imparting substances were concentration-dependent (Figure 5), which appears to support the idea that the "fresh aroma of fruits" perceived by consumers becomes stronger as the content of the perceived freshness-imparting ingredient, such as acetaldehyde, increases (Patent reference 1).

[0024] The present inventor also identified OR6B1 response regulators, as represented by methyl isoeugenol and cinnamic aldehyde, by screening for an OR6B1 response-enhancing agent and an OR6B1 response-suppressing agent (Figures 6 and 7).

[0025] The present inventor further investigated to clarify the relationship between the strength of an OR6B1 response and the perception of a perceived freshness, and demonstrated, through *in vitro* and sensory evaluation tests, that the stronger the OR6B1 response, the stronger the perception of the perceived freshness (Figures 8 and 9: Comparison of flavors 1-3). In addition, a perceived freshness of a flavor that has been added with or exposed to cinnamic aldehyde, i.e., an OR6B1 response-suppressing agent, was clearly impaired (Figures 8 and 9: comparison between flavors 2 and 4). Thus, the effect of the OR6B1 response-suppressing agent on suppressing a perceived freshness was demonstrated, which, for the first time, showed that the perceived freshness was perceived and/or regulated via the OR6B1 response. These results indicate that the perceived freshness is a sensation triggered through the OR6B1 response, and that a freshly made sensation and a freshly prepared sensation of heat-processed foods, such as bread and butter, are biologically equivalent to a perceived freshness of fruits in a broad sense.

The present invention was found based on these novel findings.

[0026] Hereinafter, a method for evaluating a perceived freshness and/or palatability according to the present invention (hereinafter referred to as an "evaluation method of the present invention") will be described specifically.

The evaluation method of the present invention is a method for evaluating a perceived freshness and/or palatability, using the response of at least one olfactory receptor polypeptide among the olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 (hereinafter referred to as the "olfactory receptor OR6B1, etc.") to a substance to be evaluated (examples including, but are not particularly limited to, foods themselves, and various compounds, compositions, and mixtures, such as flavor compositions), as an indicator.

[0027] Specifically, the evaluation method of the present invention is a method for evaluating a perceived freshness and/or palatability, the method comprising: adding or exposing a substance to be evaluated to the olfactory receptor OR6B1, etc. as described above to measure the response of the olfactory receptor polypeptide; and evaluating a perceived freshness and/or palatability of the substance to be evaluated based on the measured response.

[0028] According to the present invention, a perceived freshness is a sensation that can be evaluated by the above method, and more preferably refers to a perceived freshness of fruits and a freshly prepared sensation of processed foods. According to the present invention, the freshly prepared sensation of a processed food refers to a sensation triggered by a component that volatizes from a food within a few minutes after the completion of cooking that involves fermentation or a heating process. For example, it refers to a fresh sensation that is triggered by an aroma of freshly baked bread, an aroma of freshly made, juicy cream cheese, an aroma of freshly brewed coffee, and the like. The evaluation method of the present invention can be conducted, for example, according to the following embodiments.

[0029] Specifically, one embodiment of the present invention is a method for evaluating a perceived freshness and/or palatability by measuring the response of the olfactory receptor OR6B1, etc. to a test sample (food, flavor composition, etc.) as a substance to be evaluated, the method comprising the steps of:

(i) bringing the aroma of the test sample as the substance to be evaluated into contact with the olfactory receptor OR6B1, etc., to measure the basal activity response of the olfactory receptor before contact with the aroma of the test sample (T0) and the response of the olfactory receptor to the aroma of the test sample at a given time point after contact with the aroma of the test sample (Tx); and

(ii) comparing the measurement results in step (i), i.e., the basal activity response of the olfactory receptor before contact with the aroma of the test sample (T0) and the response of the olfactory receptor to the aroma of the test sample at the given time point after contact with the aroma of the test sample (Tx) to calculate and determine the value of change in the response.

[0030] Here, in one embodiment, the value of change in the response may be evaluated using a fold increase value, which is obtained by dividing the response of the olfactory receptor to the aroma of the test sample at the given time point after contact with the aroma of the test sample (Tx) obtained in step (i) by the measured basal activity response of the olfactory receptor before contact with the aroma of the test sample (T0), as an indicator.

For example, when the response of the olfactory receptor is measured by a reporter assay using a luminescent substance such as luciferase, it can be judged that a response is present when the fold increase value is preferably 1 or more, more preferably 1.2 or more, and still more preferably 2 or more. It can be evaluated that the higher the fold increase value, the stronger the perceived freshness and the higher the palatability of the test sample.

[0031] Hereinafter, a method for screening for a substance that imparts a perceived freshness and/or palatability according to the present invention will be described specifically.

Specifically, the screening method is a method comprising: adding or exposing a test substance to the olfactory receptor OR6B1, etc. to measure the response of the olfactory receptor polypeptide as described above; and identifying the test substance as a substance that imparts a perceived freshness and/or palatability, based on the measured response.

[0032] In the screening method, a “test substance” refers to, but is not particularly limited to, a compound, a composition, a mixture, or a test sample (a food itself, a flavor composition, etc.), as a substance potentially having the effect of triggering the response of the olfactory receptor OR6B1, etc.

[0033] In addition, one embodiment of the screening method is a method that comprises the steps of:

(i) bringing a test substance into contact with the olfactory receptor OR6B1, etc., to measure the basal activity response of the olfactory receptor before contact with the test substance (T0) and the response of the olfactory receptor to the test substance at a given time point after contact with the test substance (Tx); and

(ii) comparing the measurement results in step (i), i.e., the basal activity response of the olfactory receptor before contact with the test substance (T0) and the response of the olfactory receptor to the aroma of the test sample at the given time point after contact with the test substance (Tx), to calculate and determine the value of change in the response.

[0034] Here, in one embodiment, the value of change in the response may be evaluated using a fold increase value, which is obtained by dividing the response of the olfactory receptor to the aroma of the test sample at the given time point after contact with the aroma of the test sample (Tx) obtained in step (i) by the measured basal activity response of the olfactory receptor before contact with the aroma of the test sample (T0), as an indicator. For example, when the response of the olfactory receptor is measured by a reporter assay using a luminescent substance such as luciferase, the test sample can be judged to be an OR6B1 response-inducing substance when the fold increase value is preferably 1 or more,

more preferably 1.2 or more, and still more preferably 2 or more. An OR6B1 response-inducing substance with a higher fold increase value can be selected as a more excellent perceived freshness- and/or palatability-imparting substance.

[0035] Hereinafter, a method for screening for a substance that enhances or suppresses a perceived freshness and/or palatability according to the present invention will be described specifically.

Specifically, the screening method is a method comprising: adding or exposing a test substance, together with an OR6B1 response-inducing substance, to the olfactory receptor OR6B1, etc. as described above to measure the response of the olfactory receptor polypeptide; and identifying the test substance as a substance that enhances or suppresses a perceived freshness and/or palatability based on the measured response.

[0036] In the screening method, a “test substance” refers to, but is not particularly limited to, a compound, a composition, a mixture, or a test sample (a food itself, a flavor composition, etc.) as a substance potentially having the effect of enhancing or suppressing the response of the olfactory receptor OR6B1, etc.

[0037] Furthermore, one embodiment of the screening method is a method that comprises the steps of:

(i) measuring the basal activity response of the olfactory receptor OR6B1, etc. before bringing an OR6B1 response-inducing substance into contact with the olfactory receptor (T0) and the response of the olfactory receptor to the test substance at a given time point after contact with the OR6B1 response-inducing substance (Tx);

(ii) comparing the measurement results in step (i), i.e., the basal activity response of the olfactory receptor before contact with the OR6B1 response-inducing substance (T0) and the response of the olfactory receptor to the OR6B1 response-inducing substance at the given time point after contact with the OR6B1 response-inducing substance (Tx), to calculate and determine the value of change in the response;

(iii) mixing a test substance with the OR6B1 response-inducing substance in step (i) and calculating and determining the value of the change in the response in the same manner as in step (ii); and

(iv) selecting the test substance whose value in step (iii) increased or decreased compared to that in step (ii) as a substance that enhances or suppresses a perceived freshness and/or palatability.

[0038] Here, in one embodiment, the value of change in the response may be evaluated using a fold increase value, which is obtained by dividing the measured result for Tx by the measured result for T0 obtained in steps (i) and (iii), as an indicator. For example, when

the response of the olfactory receptor is measured by a reporter assay using a luminescent substance such as luciferase, the test substance can be judged to be an OR6B1 response-enhancing substance when, after normalizing the maximum fold increase value in step (i) to 1, the relative value of the maximum fold increase value in step (iii) is 1 or more, more preferably 1.2 or more, and still more preferably 2 or more, whereas the test substance can be judged to be an OR6B1 response-suppressing substance when the relative value is 1 or less, more preferably 0.8 or less, and still more preferably 0.5 or less. Thus, they can be suitably selected as a substance that enhances or suppresses a perceived freshness and or palatability.

[0039] Hereinafter, each of the steps (steps (i)-(iv)) in each of the above screening methods will be described more specifically.

[0040] <Step (i)>

In step (i), a test substance is brought into contact with the olfactory receptor OR6B1, etc. to measure the basal activity response of the olfactory receptor before contact with the test substance (T0) and the response of the olfactory receptor to the aroma of the test sample at a given time point after contact with the aroma of the test sample (Tx).

[0041] The nucleotide and amino acid sequences of the olfactory receptor OR6B1 are registered in the NCBI (GenBank) website (http://www.ncbi.nlm.nih.gov/) under "Accession number: NM_001005281.1" and "Accession number: NP_001005281.1", respectively, and the protein (polypeptide) consisting of the amino acid sequence (SEQ ID NO: 2) encoded by the 1st to 936th DNAs of the nucleotide sequence represented by SEQ ID NO: 1 is the olfactory receptor OR6B1.

[0042] Since the olfactory receptor OR6B1 increases the intake of foods that have a perceived freshness through the perception of the perceived freshness, measurement using the OR6B1 response as an indicator and screening for a substance that triggers, enhances, or suppresses the OR6B1 response can contribute to the evaluation and regulation of the perceived freshness and palatability.

[0043] As an olfactory receptor polypeptide, not only the olfactory receptor OR6B1 but also a protein (polypeptide) containing an amino acid sequence that shares 90% or more, preferably 95% or more, more preferably 98% or more, and still more preferably 99% or more identity with the amino acid sequence of the olfactory receptor OR6B1, may be used instead of or together with the olfactory receptor OR6B1. A protein (polypeptide) containing an amino acid sequence that shares 80% or more, preferably 85% or more, of such identity may also be used. Herein, the sequence identity of the amino acid sequence is calculated by the BLAST search algorithm (publicly available from NCBI).

[0044] According to the present invention, the method of bringing the olfactory receptor OR6B1, etc. into contact with a test substance to measure the response of the olfactory receptor is not particularly limited. For example, the test substance can be brought into contact on a living body-derived cell expressing the olfactory receptor to measure the response of the olfactory receptor, or the test substance can be brought into contact on a cell forcibly expressing the olfactory receptor through genetic engineering to measure the response of the olfactory receptor. The time for the olfactory receptor to be in contact with the test substance depends on the concentration of the test substance and the method of measurement, and thus it is difficult to say, but it is 0-60 minutes, preferably 0-30 minutes in the luciferase assay according to the present invention, and it is from a few seconds to a few minutes in the calcium imaging method.

[0045] The cell forcibly expressing the olfactory receptor OR6B1, etc. through genetic engineering can be produced by transfecting a cell with a vector incorporating a gene encoding the olfactory receptor.

[0046] In a preferred aspect of the present invention, the N-terminal 20 amino acid residues of bovine rhodopsin may be incorporated along with olfactory receptor OR6B1, etc. Incorporation of the N-terminal 20 amino acid residues of bovine rhodopsin can promote expression of the olfactory receptor in the cell membrane.

[0047] The nucleotide and amino acid sequences of bovine rhodopsin are registered in the NCBI (GenBank) website under “Accession number: NM_001014890.2” and “Accession number: NP_001014890.1”, respectively. Bovine rhodopsin is the protein (polypeptide) consisting of the amino acid sequence (SEQ ID NO: 4) encoded by the 1st to 1047th DNAs of the nucleotide sequence represented by SEQ ID NO: 3.

[0048] Instead of or together with bovine rhodopsin, a protein (polypeptide) that contains an amino acid sequence that shares 80% or more, preferably 85% or more, more preferably 90% or more, still more preferably 95% or more, and particularly preferably 98% or more identity with the amino acid sequence represented by SEQ ID NO: 4 and that can promote expression of the olfactory receptor in the cell membrane may be used.

[0049] Note that amino acid residues of a protein (polypeptide) other than bovine rhodopsin may also be used as long as it can promote expression of the olfactory receptor OR6B1, etc. in the cell membrane.

[0050] The method for measuring the response of the olfactory receptor OR6B1, etc. is not particularly limited and any method used in this field can be used. For example, it is known that when an aroma compound binds to an olfactory receptor, it activates the intracellular G protein, which in turn activates adenylate cyclase to convert ATP to cyclic

AMP (cAMP), thereby increasing the intracellular cAMP level. Therefore, the response of the olfactory receptor can be measured by determining the cAMP level. ELISA, a reporter assay, etc. is used as the method of measuring the cAMP level. Among them, it is preferable to measure the response of the olfactory receptor by a reporter assay using a luminescent substance such as luciferase. Any other substances involved in olfactory receptor signaling could also be the subject of measurement, as their levels increase or decrease depending on the response.

[0051] <Step (ii)>

In step (ii), the measurement results in step (i), i.e., the basal activity response of the olfactory receptor OR6B1, etc. before contact with the test substance (T0) and the response of the olfactory receptor to the test substance at the given time point after contact with the test substance (Tx), are compared, to calculate and determine the value of change in the response.

[0052] In one embodiment of the present invention, the change in the response may be evaluated using the fold increase value, which is obtained by dividing the response of the olfactory receptor to the test substance at the given time point after contact with the test substance (Tx) obtained in step (i) by the measured basal activity response of the olfactory receptor before contact with the test substance (T0), as an indicator. For example, when the response of the olfactory receptor is measured by a reporter assay using a luminescent substance such as luciferase, it can be judged that an OR6B1 response is present and that the test substance can be evaluated to be an OR6B1 response-inducing substance, and ultimately a perceived freshness-imparting substance or a palatability-imparting substance, when the fold increase value is preferably 1 or more, more preferably 1.2 or more, and still more preferably 2 or more.

[0053] <Step (iii)>

In step (iii), provided that the test substance in steps (i) and (ii) is a specific OR6B1 response-inducing substance, the specific OR6B1 response-inducing substance is mixed with a test substance to calculate and determine the value of the change in the response in the same manner as in step (ii).

[0054] <Step (iv)>

In step (iv), provided that the test substance in steps (i) and (ii) is a specific OR6B1 response-inducing substance, a test substance, which increases or decreases the value of change when the test substance is mixed with the specific OR6B1 response-inducing substance obtained in step (iii), compared to the value of change using the specific OR6B1 response-inducing substance alone obtained in step (ii), is selected as a

substance that enhances or suppresses the perceived freshness and/or palatability.

[0055] In one embodiment of the present invention, the change in the response may be evaluated using the fold increase value, which is obtained by dividing the response of the olfactory receptor to the test substance at the given time point after contact with the test substance (Tx) obtained in steps (i) and (iii) by the measured basal activity response of the olfactory receptor before contact with the test substance (T0), as an indicator. For example, when the response of the olfactory receptor is measured by a reporter assay using a luminescent substance such as luciferase, the test substance can be judged to be an OR6B1 response-enhancing substance when, after normalizing the maximum fold increase value in step (i) to 1, the relative value of the maximum fold increase value in step (iii) is 1 or more, more preferably 1.2 or more, and still more preferably 2 or more, and the test substance can be judged to be an OR6B1 response-suppressing substance, when the relative value is 1 or less, more preferably 0.8 or less, and still more preferably 0.5 or less. Each of these test substances are suitably selected as a substance that enhances or suppresses a perceived freshness and or palatability.

[0056] The specific descriptions of steps (i) and (ii) above may also be employed for an evaluation method of the present invention as appropriate.

[0057] According to the present invention, the strength of an OR6B1 response can be evaluated as an indicator of the strength of a perceived freshness or the level of palatability by calculating the value of change in the response against various test substances as the measurement result in step (ii). If an increase in the value of change in the response is observed, the test substance can be evaluated to be an OR6B1 response-inducing substance, and ultimately a perceived freshness-imparting substance or a palatability-imparting substance. Moreover, according to the present invention, provided that the test substance in steps (i) and (ii) is a specific OR6B1 response-inducing substance, the test substance used in step (iii) can be evaluated as a substance that enhances or suppresses a perceived freshness and palatability when an increase or decrease in the value of change in the response is observed upon comparing the measurement results in steps (ii) and (iii).

[0058] A perceived freshness and palatability of a test substance can be qualitatively and quantitatively evaluated in terms of an OR6B1 response in the above manner. A substance that imparts, enhances, or suppresses an OR6B1 response can be selected from test substances, and the selected substance can be used as a substance that imparts, enhances, or suppresses a perceived freshness and/or palatability, and can be added to a food to regulate the perceived freshness and/or palatability thereof. Based on the selected substance, modification or the like of the OR6B1 response can be made as necessary to

develop a new compound that has an optimal odor. Furthermore, it is also possible to blend the selected substance with other flavor ingredient to develop a flavor ingredient that offers an optical odor and that can claim the function of enhancing a perceived freshness and/or palatability. The evaluation method or each screening method of the present invention can be used to develop or evaluate a new flavor ingredient with an enhanced perceived freshness and/or palatability.

[0059] The present invention can also provide an agent for imparting, enhancing, or suppressing a perceived freshness and/or palatability, which contains the substance that imparts, enhances, or suppresses the perceived freshness and/or palatability described above, which is obtained by each screening method of the present invention. Here, examples of such an imparting or enhancing agent include, but are not limited to, methyl isoeugenol, while examples of such a suppressing agent include, but are not limited to, cinnamic aldehyde.

By utilizing such an imparting, enhancing, or suppressing agent, it is possible to regulate a perceived freshness and/or palatability (impart, enhance, or suppress a perceived freshness and/or palatability) of, for example, a desired food, flavor composition, etc.

EXAMPLES

[0060] Hereinafter, the present invention will be described in more detail by means of examples, although the present invention should not be limited in any way to these examples.

[0061] [Example 1] Extraction and prediction of genes involved in perceived freshness (fresh feeling)

(1-1) Table 1 shows the lead SNP in the genomic region (QTL) most predominantly associated with the “population that consumes fresh fruit daily”, the p-value thereof, and the name of the nearest olfactory receptor gene, which were obtained by extraction from the back data analyzed in Non-patent reference 5 mentioned above. For comparison, the table also includes information on the “population that consumes dried fruit daily” for the same lead SNP. In addition, the table shows the lead SNPs in the genomic regions associated with the consumption of various kinds of fruits, the p-values thereof, and the names of the nearest olfactory receptor genes, which were obtained by extraction from the back data analyzed in Non-patent reference 6 mentioned above.

[0062] [Table 1]

Trait	Lead SNP	P-value	Nearest Gene	Reference
Pieces of fresh fruit per day	rs10249294	5.70E-65	OR6B1	Non-patent reference 5
Pieces of dried fruit per day	rs10249294	No significance (<E-5)	OR6B1	
Cherries	rs7789218	1.67E-31	OR6B1	Non-patent reference 6
Plums	rs10249294	2.37E-29	OR6B1	
Fruit	rs7787699	3.85-24	OR6B1	
Banana	rs7787699	4.78E-18	OR6B1	
Kiwi	rs6965087	1.32E-15	OR6B1	
Strawberries	rs768798559	2.06E-11	OR6B1	

[0063] [Example 2] Proving that "response" of OR6B1 "imparts" perceived freshness

(2-1) Olfactory receptor gene cloning

Each of the human olfactory receptor genes was cloned by PCR from Human Genomic DNA: Female (Promega) based on the nucleotide sequence information registered in the NCBI (GenBank) website mentioned above. As the OR6B1 gene, the sequence represented by SEQ ID NO: 1 (NM_001005281.1) was used. DNA encoding the N-terminal 20 amino acid residues of bovine rhodopsin (DNA consisting of the 1st to 60th nucleotides of the nucleotide sequence represented by SEQ ID NO: 3) was incorporated into pME18S vector and the obtained human olfactory receptor gene was further incorporated downstream of it, thereby obtaining a human olfactory receptor gene expression vector.

[0064] (2-2) Luciferase assay

0.05 μg of the human olfactory receptor gene expression vector, 0.01 μg of RTP1S vector, and 0.01 μg of firefly luciferase vector pGloSensor™-22F cAMP Plasmid (Promega) containing CMV promoter were dissolved in 10 μL of Opti-MEM I (Gibco) to prepare a gene solution (per well). HEK293T cells were seeded at 100 μL per 96-well plate (Biocoat, Corning) at a number of cells that reached subconfluence after 24 hours, and the gene solution was added or exposed to each well by lipofection according to the usage of Lipofectamine 3000 so that the cells were transfected with the gene and cultured at 37°C in a 5% carbon dioxide atmosphere for 24 hours. After removal of the culture solution, the cells were washed once with HEPES buffer. 30 μL (for gas-phase measurement) or 100 μL (for liquid-phase measurement) of GloSensor™ cAMP Reagent (Promega), which was adjusted to a predetermined concentration with HEPES buffer supplemented with FBS, was added or exposed to each well. The top surface of the plate was immediately sealed and left to sit at room temperature for 2 hours. Then, the response value (T0) of each well

immediately before odor exposure was measured using a plate reader, and the luminescence value was measured by measuring the response value (Tx) every minute starting immediately after odor stimulation.

[0065] (2-3) Measurement of gas-phase response of olfactory receptor to fresh fruit

In the luciferase assay, a subject to be measured was placed on the back of a plate lid before the lid was placed to cover the top of the plate, sealing each well and saturating it with the aroma of the subject to be measured. Ten minutes after the sealed exposure (T10), the response value of each well was measured again using the plate reader. The strength of the response of each olfactory receptor was evaluated as a fold increase, which was expressed as T10/T0 from the screening of all 454 kinds of human olfactory receptors against pink grapefruit (Figure 1a), and the responses of the human olfactory receptor OR6B1 to various fruits were evaluated as ΔF/F0 values, which were expressed as (T10-T0)/T0 (Figure 1b).

[0066] (2-4) Screening for flavor ingredients that induce response of OR6B1

A luciferase assay was performed using a human olfactory receptor OR6B1 gene expression vector. The response value of the human olfactory receptor OR6B1 to each flavor ingredient from a library of flavor ingredients was acquired by adding or exposing 10 μL of each flavor ingredient solution in a HEPES buffer at 10 × 30 ppm to each well, and measuring the change in the response value of the luciferase activity over time, i.e., before the addition (T0) and every minute starting immediately after the addition, such as T1, T2, ..., T20. The strength of the response of the olfactory receptor was evaluated by first calculating the FIsample (MAX) value, which was determined by dividing the maximum response value of the luciferase activity that occurred after stimulation with each flavor ingredient in each well by the luciferase activity before the stimulation with each flavor ingredient, and then dividing this value by the average FIcontrol (MAX) of a control obtained by stimulation with only a solvent provided in each plate to give a normalized response value. The results of responses to all 746 kinds of flavor ingredients that were screened are shown in Figure 2, and the top ingredients that gave responses above a certain level were extracted and are shown in Table 2, along with information on their fragrance notes.

[0067] [Table 2]

ITEM Name	Odor description	6B1 response
ACETALDEHYDE	**Fresh** aldehydic green pungent. Exist in **fruity** odor foods.	31.7
Fenugreek Extract	**Caramellic**, **Bready**, **Roasted**. Grainy with savory, vegetative and whiskey nuances	18.3
Butyl Aldehyde	**Fresh**, **Fruity**. Pungent cocoa musty green malty, **Caramellic**, **Bready**, grainy fermented.	8.0
2,4,5-Trimethyl-3-oxazoline	**Bready** and **Caramellic**. **Fresh** confectionery, musty, nutty, vegetative, cocoa, brown	6.5
Cyclotene	**Bready** maple sweet burnt caramellic coffee	6.0
Isobutyl Aldehyde	**Fresh** aldehydic herbal green malty. Exist in **fruity** odor foods	4.8
ALDEHYDE C-6	**Fresh** green fatty aldehydic grassy leafy **fruity** sweaty	4.6
3,6-NONADIENOL	**Fresh** green violet cucumber	3.5
3,4-HEXANEDIONE	**Buttery toasted** almond nutty caramellic	3.0
2,3-PENTANDIONE	**Buttery** nutty **toasted** caramellic	2.7
DIACETYL	Sweet **Buttery** creamy milky	2.7

[0068] (2-5) Extraction of eating habits that are predicted to be affected by genomic region containing OR6B1

Table 3 shows the eating habits, lead SNPs, and p-values that were predominantly associated with the genomic region containing the OR6B1 gene, obtained by extraction from the back data analyzed in Non-patent references 5 and 6 mentioned above.

[0069] [Table 3]

Trait	Lead SNP	P-value	Nearest Gene	Reference
Pieces of fresh fruit per day	rs10249294	5.70E-65	OR6B1	Non-patent reference 5
Slices of bread per week	rs7802468	2.90E-30	OR6B1	
Spread type: butter vs. never	rs6978121	4.10E-09	OR6B1	
Butter on bread	rs10680869	9.52E-10	OR6B1	Non-patent reference 6

[0070] (2-6) Measurement of OR6B1 gas-phase response to freshly baked bread, butter, and cheese

In a luciferase assay, a subject to be measured was placed on the back of a plate lid before the lid was placed to cover the top of the plate, sealing each well and saturating

it with the aroma of the subject to be measured. Ten minutes after the sealed exposure (T10), the response value of each well was measured again using the plate reader. The response of the human olfactory receptor OR6B1 to each fruit was evaluated as ΔF/F0 value, which was expressed as (T10-T0)/T0. The results are shown in Figure 3.

[0071] [Example 3] Proving that olfactory receptor OR6B1 is the only perceived freshness-imparting receptor

(3-1) Screening for olfactory receptor against perceived freshness-imparting ingredients

Each of expression vectors of a total of 454 kinds of human olfactory receptor genes was cloned to perform a luciferase assay using HEK293T cells expressing each olfactory receptor gene. Representative samples of perceived freshness-imparting ingredients, which were OR6B1 response-inducing compounds shown in Figure 2: i.e., a) acetaldehyde, b) butyraldehyde, c) 2,4,5-trimethyl-3-oxazoline, d) isobutyraldehyde, and e) diacetyl, were prepared in a HEPES buffer supplemented with 300 μM $CuCl_2$ and FBS, at a concentration of 10 × 1 mM or 10 × 3 mM. 10 μL of each sample was added or exposed to each well, and changes in the luciferase activity over time were measured before the addition and every minute starting immediately after the addition to acquire the response of each of the human olfactory receptors over time. The strength of the response of each olfactory receptor was evaluated using the maximum fold increase value, which was determined by dividing the luciferase activity that occurred upon stimulation with the aroma compound by the luciferase activity before the stimulation with the aroma compound. The results are shown in Figure 4.

[0072] (3-2) Measurement of concentration-dependent OR6B1 responses to perceived freshness-imparting ingredients

An expression vector of the human olfactory receptor OR6B1 gene was cloned to perform a luciferase assay using HEK293T cells expressing the olfactory receptor OR6B1 gene. As representative samples of perceived freshness-imparting ingredients, which were OR6B1 response-inducing compounds shown in Figure 2, a) acetaldehyde, b) fenugreek extract, c) butyraldehyde, d) isobutyraldehyde, e) 3,6-nonadienol, and f) diacetyl were added or expose at a final concentration of 1 mM, 700 μM, 300 μM, 100 μM, or 700 μM, 300 μM, 70 μM, 30 μM, 0 μM, or 100 ppm, 70 ppm, 30 ppm, 10 ppm, 0 ppm, and changes in the luciferase activity over time were measured every minute starting immediately after the addition to acquire the response of the olfactory receptor OR6B1 over time. The strength of the response of the olfactory receptor OR6B1 was evaluated using the Δfold increase value, which was determined by calculating a fold increase value by dividing the

luciferase activity that occurred upon stimulation with the aroma compound at a given time point by the luciferase activity before the stimulation with the aroma compound, and then subtracting the fold increase value in a 0 μM added well at each given time point. The results are shown in Figure 5.

[0073] (3-3) Screening for flavor ingredients that enhance OR6B1 response

HEK293T cells expressing the olfactory receptor OR6B1 gene were used in a luciferase assay to screen for flavor ingredients that enhance the OR6B1 response in the presence of acetaldehyde. 827 kinds of test substances were added or exposed to 275 μM acetaldehyde in each well to a final concentration of 30 ppm, and changes in the luciferase activity over time were measured every minute starting immediately after the addition to acquire the responses of each human olfactory receptor over time. The fold increase enhancement value (FIE value) was determined as the relative value of the fold increase value obtained in the test that involved mixing each test substance, provided that the maximum fold increase value obtained in the control test, in which only 275 μM acetaldehyde was added or exposed without mixing each test substance, was 1. Flavor ingredients identified in (2-4) of Example 2, which do not trigger the OR6B1 response by themselves, but have a FIE value of 1.5 or higher, were determined as OR6B1 response-enhancing agents. As an example, Figure 6 shows the results of the response enhancement with methyl isoeugenol over time.

[0074] (3-4) Screening for flavor ingredients that suppress OR6B1 response

HEK293T cells expressing the olfactory receptor OR6B1 gene were used in a luciferase assay to measure the effects of test substances on suppressing the OR6B1 response. 827 kinds of test substances were added or exposed to 600 μM acetaldehyde in each well to a final concentration of 30 ppm, and changes in the luciferase activity over time were measured every minute starting immediately after the addition to acquire the responses of each human olfactory receptor over time. The relative suppression value (RS value) was determined as the relative value of the fold increase value obtained in the test that involved mixing each test substance, provided that the maximum fold increase value obtained in the control test, in which only 600 μM acetaldehyde was added or exposed without mixing each test substance, was 1. Flavor ingredients with a RS value of 0.5 or less were determined as OR6B1 response-suppressing agents. As an example, Figure 7 shows the results of the response suppression with cinnamic aldehyde over time.

[0075] (3-5) Evaluation of OR6B1 response to orange flavor

[0076] [Table 4]

	Amount blended (parts by mass)			
Formulation	FLAVOR-1	FLAVOR-2	FLAVOR-3	FLAVOR-4
Orange peel oil	4	4	4	4
Acetaldehyde	0.4	2	2	2
Methyl isoeugenol	-	-	0.1	-
Cinnamic aldehyde	-	-	-	0.1
Ethanol	71	71	71	71
Water	24.6	23	22.9	22.9
TOTAL	100	100	100	100

[0077] HEK293T cells expressing the olfactory receptor OR6B1 gene were used in a luciferase assay to measure the OR6B1 responses to orange flavors 1-4, which were prepared according to the formulations shown in Table 4 above. Each of the orange flavors 1-4 was added or exposed to each well to a final concentration of 0.1%, and changes in the luciferase activity over time were measured every minute starting immediately after the addition to calculate the response (fold increase value) of each human olfactory receptor over time. The results are shown in Figure 8.

[0078] (3-6) Sensory evaluation tests for orange flavors on perceived freshness

Sensory evaluation was conducted to determine the relationship between the strength of the OR6B1 response and the sensation. Sensory evaluation tests were conducted by a consumer panel of 9 members on the perceived freshness of orange sensed immediately after drinking water containing 0.1% of the above orange flavors 1-4. The evaluation of the strength of the perceived freshness was conducted on a 10-point scale, ranging from 0 (no sensation) to 9 (very strong sensation). The results are shown in Figure 9.

[0079] Results from examples

The genomic region containing OR6B1 was strongly associated with the consumption of fresh fruit, but no association was found between this region and the consumption of dried fruits, suggesting that OR6B1 was strongly associated with the perception of the perceived freshness of fruits (Table 1). The fact that various fruit aromas respond to OR6B1 (Figure 1a) suggests that actual fresh fruits contain OR6B1 response-inducing components. The fact that OR6B1 was the most strongly responsive receptor among all human olfactory receptors (Figure 1b) indicates that OR6B1 is an olfactory receptor that has the strongest influence on the perception of fruit aromas by humans.

[0080] Screening for substances that induce response of OR6B1 showed that OR6B1 responded not only to a group of perceived freshness-imparting substances common in

fruits, such as aldehydes, but also to a group substances that impart a freshly baked sensation of bread and a perceived freshness of butter (Figure 2 and Table 2). This result was fully consistent with the data from the population genomic analysis showing that the top eating habits predominantly associated with OR6B1 were consumption of fruits, bread, and butter (Table 3). This demonstrates the causal relationship where the "response" of OR6B1 leads to the "impartation" of a perceived freshness and the formation of palatability of fruits, bread, and butter.

[0081] Since the group of perceived freshness-imparting substances induced the response of only OR6B1 in common, the possibility that the perceived freshness was imparted to the group in common via a response of an olfactory receptor other than OR6B1 was ruled out (Figure 4). The OR6B1 responses to the group of the perceived freshness-imparting substances were concentration-dependent (Figure 5). OR6B1 response regulators, as represented by methyl isoeugenol and cinnamic aldehyde, were identified by screening for OR6B1 response-enhancing agents and OR6B1 response-suppressing agents (Figures 6 and 7). When they were used, the perceived freshness increased in flavors in which the OR6B1 response was enhanced, while the perceived freshness was suppressed in flavors in which the OR6B1 response was suppressed, in the sensory evaluation tests (Figures 8 and 9). Accordingly, the effect of suppressing a perceived freshness with the OR6B1 response-suppressing agent was demonstrated, which, for the first time, revealed that the perceived freshness is perceived via the OR6B1 response, and that a freshly made sensation and a freshly prepared sensation of heat-processed foods, such as bread and butter, are also biologically equivalent to a perceived freshness of fruits in a broad sense.

INDUSTRIAL APPLICABILITY

[0082] The present invention can provide a method for qualitatively and quantitatively evaluating a perceived freshness and/or palatability, which has been a subjective and ambiguous sensation, using an OR6B1 response. The present invention can also provide a method for screening for a substance that imparts, enhances, or suppresses a perceived freshness and/or palatability, and the substance identified by this method can be used, for example, to control the perceived freshness, enhance the food palatability, etc. Furthermore, the method is expected to contribute to the development of food palatability-enhancing techniques.

CLAIMS

[Claim 1] A method for evaluating a perceived freshness and/or palatability, the method comprising the steps of: adding or exposing a substance to be evaluated to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and evaluating the perceived freshness and/or palatability of the substance to be evaluated based on the measured response.

[Claim 2] A method for screening for a substance that imparts, enhances, or suppresses a perceived freshness and/or palatability,

wherein the screening of the substance that imparts a perceived freshness and/or palatability comprises: adding or exposing a test substance to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and identifying the test substance as a substance that imparts the perceived freshness and/or palatability based on the measured response,

wherein the screening of the substance that enhances a perceived freshness and/or palatability comprises: adding or exposing a test substance, together with an OR6B1 response-inducing substance, to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and identifying the test substance as a substance that enhances the perceived freshness and/or palatability based on the measured response,

or

wherein the screening of the substance that suppresses a perceived freshness and/or palatability comprises: adding or exposing a test substance, together with an OR6B1 response-inducing substance, to at least one olfactory receptor polypeptide among an olfactory receptor OR6B1 and polypeptides having an amino acid sequence that shares at least 90% identity with an amino acid sequence of the olfactory receptor OR6B1 to measure the response of the olfactory receptor polypeptide; and identifying the test substance as a substance that suppresses the perceived freshness and/or palatability based

on the measured response.

[Claim 3] The method according to claim 1 or 2, wherein the perceived freshness is a perceived freshness of fruit or a freshly prepared sensation of a processed food.

[Claim 4] The method according to claim 1 or 2, wherein the response of the olfactory receptor polypeptide is measured on a cell endogenously expressing the olfactory receptor polypeptide or on a cell forcibly expressing the olfactory receptor polypeptide through genetic engineering.

[Claim 5] The method according to claim 1 or 2, wherein the response of the olfactory receptor polypeptide is measured by a reporter assay or calcium imaging.

[Claim 6] An agent for imparting or enhancing a perceived freshness and/or palatability, comprising a substance that imparts or enhances the perceived freshness and/or palatability, as identified by the method according to claim 2.

[Claim 7] The agent for imparting or enhancing a perceived freshness and/or palatability according to claim 6, wherein the substance that imparts or enhances the perceived freshness and/or palatability is methyl isoeugenol.