

METHOD OF PRODUCING FLAVOR BY MIXED FERMENTATION OF HETEROLOGOUS MICROORGANISMS

BACKGROUND

5 1. Technical Field

[0001] The present invention relates to a method of producing a flavor by a co-fermentation process using mixed fermentation of two or more different microorganisms producing different products.

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2. Related Art

[0002] L-glutamic acid is an acidic amino acid that gives an umami (or savory) taste, and is naturally present in vegetable foods such as kelp, soybean paste, and soy sauce,
15 as well as animal foods such as dairy products, meat, and fish. This L-glutamic acid is a food flavor that is most frequently used worldwide, and in particular, monosodium glutamate (MSG) produced by adding a salt during purification of L-glutamic acid has an excellent food
20 flavor enhancing effect, and thus is used as a food additive in many processed foods. Recently, as consumer's awareness of health has increased, the market for natural flavors such as yeast extract and natural material extracts has been rapidly growing, but MSG still has an excellent
25 umami taste compared to natural flavors and has high

competitiveness at a low price. Therefore, much effort is required to develop a substitute for MSG.

[0003] Meanwhile, development of flavors using various amino acids such as L-lysine, L-valine, and L-arginine along with L-glutamic acid has been made for the purpose of enhancing an umami taste. In addition, flavors further comprising nucleic acids such as inosinic acid and guanylic acid, or organic acids such as succinic acid and lactic acid have also been developed. These flavors based on L-glutamic acid are generally produced by mixing fermentation broths produced from microorganisms producing the respective products, or by mixing individually produced amino acid, nucleic acid or organic acid powders. In conventional production methods, it is easy to control the concentration or content of the final product such as L-glutamic acid, but a high production cost is required because the production process must be operated twice before mixing. In addition, large amounts of medium components and fermentation by-products remain in the produced flavor, making it difficult to solve off-flavor and off-odor problems, and there is still a limitation in that it is insufficient to give a rich umami taste. Accordingly, a lot of research and development is needed to develop a flavor with an enhanced umami taste.

[0004] [Prior Art Documents]

[0005] [Patent Documents]

[0006] Korean Patent No. 10-1758332

[0007] Korean Patent No. 10-1328091

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SUMMARY

[0008] An object of the present invention is to provide a method of producing a flavor by mixed fermentation of two or more different microorganisms producing different products.

10 **[0009]** Another object of the present invention is to provide a method of producing a flavor containing L-glutamic acid and L-lysine by mixed fermentation of a glutamic acid-producing microorganism and a lysine-producing microorganism.

15 **[0010]** Still another object of the present invention is to provide a flavor produced by the above method.

[0011] Yet another object of the present invention is to provide a food composition containing the above flavor.

[0012] One aspect of the present invention provides a method
20 for producing a flavor, the method comprising a step of inoculating a fermentation medium with a first microorganism and a second microorganism and then producing a fermentation broth containing amino acid, nucleic acid and/or organic acid by fermentation of the microorganisms,
25 wherein the first microorganism and the second

microorganism produce different products and each produces one selected from the group consisting of amino acid, nucleic acid and organic acid.

[0013] As used herein, the term "flavor" refers to a substance that is added to enhance the flavor of food, and it is used in a broader sense than a general seasoning that is added in a small amount at the end of a general food cooking process. The flavor may be used to enhance the taste in the process of manufacturing processed foods such as ham, sausage and ramen, as well as when cooking at home. The term "flavor" in the present invention refers to a substance having a richer umami taste and excellent sensory properties by containing one or more amino acids, nucleic acids and/or organic acids as taste components.

[0014] According to one embodiment of the present invention, the amino acid may be at least one selected from the group consisting of L-glutamic acid, L-alanine, L-valine, L-leucine, L-isoleucine, L-proline, L-phenylalanine, L-tryptophan, L-methionine, L-glycine, L-serine, L-threonine, L-cysteine, L-asparagine, L-glutamine, L-aspartic acid, L-lysine, L-arginine, and L-histidine.

[0015] According to one embodiment of the present invention, the nucleic acid may be at least one selected from the group consisting of inosinic acid, guanylic acid, xanthylic acid, and salts thereof.

[0016] For example, the nucleic acid may be inosine monophosphate (IMP), guanosine monophosphate (GMP), xanthosine monophosphate (XMP), etc., without being limited thereto.

5 **[0017]** According to one embodiment of the present invention, the organic acid may be at least one selected from the group consisting of succinic acid, malic acid, citric acid, acetic acid, lactic acid, fumaric acid, tartaric acid, ascorbic acid, gluconic acid, and salts thereof.

10 **[0018]** As used herein, the term "fermentation" is a biological phenomenon in which organic matter contained in a medium is decomposed or changed into other substances by microorganisms, and means that the inoculated microorganisms decompose or convert nutrients in the
15 fermentation medium into amino acids, nucleic acids, organic acids, etc.

[0019] As used herein, the term "fermentation broth" refers to a broth containing substances produced from microorganisms through fermentation. This fermentation
20 broth is obtained by mixed fermentation of two or more types of microorganisms producing different products, and contains not only useful substances, such as amino acids, nucleic acids and organic acids, produced from each microorganism, but also by-products produced in the
25 metabolic process and medium components.

[0020] As used herein, the term "mixed fermentation" refers to a process of fermenting different (or heterologous) microorganisms, which produce different products, that is, different kinds of amino acids, nucleic acids, or organic acids, in a microbial fermentation process in a single fermenter under the same conditions.

[0021] The mixed fermentation may be performed using microorganisms producing different kinds of amino acids, nucleic acids or organic acids, or using a combination of an amino acid-producing microorganism and a nucleic acid-producing microorganism, a combination of an amino acid-producing microorganism and an organic acid-producing microorganism, or a combination of an organic acid-producing microorganism and a nucleic acid-producing microorganism, but the present invention is not limited thereto.

[0022] The fermentation broth produced by this mixed fermentation may contain two kinds of amino acids, nucleic acids or organic acids, or contain one kind of amino acid and one kind of nucleic acid, one kind of amino acid and one kind of organic acid, or one kind of organic acid and one type of nucleic acid, but the present invention is not limited thereto.

[0023] In one example, when the first microorganism is a glutamic acid-producing microorganism, the second

microorganism may be a lysine-producing microorganism, an arginine-producing microorganism, a histidine-producing microorganism, a tryptophan-producing microorganism, a glycine-producing microorganism, an alanine-producing
5 microorganism, a succinic acid-producing microorganism , a lactic acid-producing microorganism, a guanylic acid-producing microorganism, or an inosinic acid-producing microorganism.

[0024] In addition, when the first microorganism is an
10 inosinic acid-producing microorganism, the second microorganism may be a lysine-producing microorganism, an arginine-producing microorganism, a histidine-producing microorganism, a tryptophan-producing microorganism, a glycine-producing microorganism, an alanine-producing
15 microorganism, a succinic acid-producing microorganism, a lactic acid-producing microorganism, or a guanylic acid-producing microorganism.

[0025] According to one embodiment of the present invention, the step may comprise further inoculating the fermentation
20 medium with a third microorganism, which produces a product different from products produced from the first microorganism and the second microorganism and produces one selected from the group consisting of amino acids, nucleic acids and organic acids.

25 **[0026]** More specifically, the fermentation broth produced

from the first microorganism, the second microorganism and the third microorganism contains three kinds of amino acids, nucleic acids or organic acids, or may contain one kind of amino acid and two kinds of nucleic acids, two kinds of amino acids and one kind of nucleic acid, one kind of amino acid and two kinds of organic acids, two kinds of amino acids and one kind of organic acid, one kind of organic acid and two kinds of nucleic acids, two kinds of organic acids and one kind of nucleic acid, or one kind of amino acid, one kind of nucleic acid and one kind of organic acid, but the present invention is not limited thereto.

[0027] For example, when the first microorganism is a glutamic acid-producing microorganism and the second microorganism is a guanylic acid-producing microorganism, the third microorganism may be an inosinic acid-producing microorganism.

[0028] These microorganisms that are used for the production of a flavor may be microorganisms producing amino acids, nucleic acids and/or organic acids, and may be naturally occurring wild-type microorganisms, or mutant strains modified to improve the production ability of the wild-type microorganisms. As the microorganisms producing amino acids, nucleic acids or organic acids, microorganisms known in the art may be used without limitation, and may be, for example, microorganisms of the genus *Corynebacterium*, the genus

Brevibacterium, the genus *Lactobacillus*, the genus of *Bifidobacterium*, the genus *Bacillus* spp., etc. These microorganisms may be of the same genus or species, or may be of different genera or species, and may be selected by a
5 user.

[0029] According to one embodiment of the present invention, the first microorganism, the second microorganism and the third microorganism may be microorganisms of the genus *Corynebacterium*.

10 **[0030]** More specifically, the microorganisms of the genus *Corynebacterium* may be *Corynebacterium glutamicum*, *Corynebacterium crudilactis*, *Corynebacterium deserti*, *Corynebacterium callunae*, *Corynebacterium suranareeae*, *Corynebacterium lubricantis*, *Corynebacterium doosanense*,
15 *Corynebacterium efficiens*, *Corynebacterium uterequi*, *Corynebacterium stationis*, *Corynebacterium pacaense*, *Corynebacterium singulare*, *Corynebacterium humireducens*, *Corynebacterium marinum*, *Corynebacterium halotolerans*, *Corynebacterium spheniscorum*, *Corynebacterium freiburgense*,
20 *Corynebacterium striatum*, *Corynebacterium canis*, *Corynebacterium ammoniagenes*, *Corynebacterium renale*, *Corynebacterium pollutisoli*, *Corynebacterium imitans*, *Corynebacterium caspium*, *Corynebacterium testudinoris*, *Corynebacterium pseudopelargi* or *Corynebacterium*
25 *flavescens*, without being limited thereto.

[0031] In order to perform mixed fermentation of these two or more microorganisms, isolated and selected microorganisms may be used individually, or a microbial mixture obtained by mixing these microorganisms may be used, 5 and in some cases, an appropriate mixture of the isolated microorganisms and the microbial mixture may be used. Each of the microorganisms may be used for fermentation in a state in which it has been grown and the ability thereof to produce amino acids, nucleic acids and/or organic acids has 10 been activated. In addition, each of the microorganisms is preferably subjected to seed culture for microbial activation.

[0032] According to one embodiment of the present invention, the first microorganism, the second microorganism and the 15 third microorganism may be in a seed culture broth state obtained by individual culture or co-culture.

[0033] As used herein, the term "seed culture" means culturing a microorganism(s) in a small volume of medium before mass-culturing the microorganism, and the term "seed 20 culture broth" refers to a culture containing medium components, a microorganism proliferated through seed culture, and its metabolites.

[0034] The seed culture may be performed using an appropriate medium and culture conditions known in the art 25 in consideration of the characteristics of each

microorganism, and those skilled in the art can easily adjust the medium and culture conditions for use.

[0035] More specifically, the medium used for seed culture may contain nutrients necessary for microbial growth and proliferation, and may be a liquid medium.

[0036] The culture temperature in the seed culture may be usually 20 to 45°C, for example, 25 to 40°C, or 27 to 37°C, and the culture may be continued until each microorganism actively grows and proliferate. The culture time may be, for example, 10 to 160 hours, 18 to 120 hours, or 20 to 80 hours.

[0037] In addition, during culture, compounds such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid and sulfuric acid may be added to the medium or culture broth in an appropriate manner to adjust the pH of the culture broth. In addition, it is possible to suppress the formation of air bubbles by using an antifoaming agent for food during culture. Additionally, in order to maintain the medium or culture broth in an aerobic state, oxygen or oxygen-containing gas (e.g., air) may be injected into the culture broth.

[0038] This seed culture may be performed depending on a microbial concentration desired by the user, and the optical density (OD) value of the seed culture broth is measured to predict the microbial concentration and

determine whether to continue culture.

[0039] According to one embodiment of the present invention, the seed culture broth may have $OD_{610} = 10$ to 80 .

[0040] In the step of producing a fermentation broth by
5 fermentation of two or more different microorganisms, a fermentation broth containing different products may be produced by mixed fermentation (main fermentation) using different microorganisms or seed culture broths thereof.

[0041] The mixed fermentation is a concept opposite to a
10 conventional individual fermentation process of producing a fermentation broth containing the product of each microorganism by culturing each microorganism individually.

[0042] In the present invention, through mixed fermentation, for example, it is possible to produce L-glutamic acid by a
15 glutamic acid-producing microorganism, L-lysine by a lysine-producing microorganism, L-arginine by an arginine-producing microorganism, and IMP by an inosinic acid-producing microorganism. In addition, it is possible to produce a fermentation broth capable of exhibiting a rich
20 umami taste and excellent sensory properties due to the interaction between these products and due to taste components such as ions, nucleic acids, organic acids, and other peptides, which are additionally produced in the microbial fermentation process.

25 **[0043]** According to one embodiment of the present invention,

in the step, it is preferable to adjust the inoculum of each microorganism in order to control the ratio between the products of the microorganisms, that is, amino acids, nucleic acids and/or organic acids, in the fermentation
5 broth.

[0044] More specifically, when the fermentation medium is inoculated with two different microorganisms, the inoculation ratio between the first microorganism and the second microorganism may be a ratio of 0.05 to 99.95 (first
10 microorganism): 99.95 to 0.05 (second microorganism) relative to the total inoculum. When the fermentation medium is inoculated with three different microorganisms, the inoculation ratio between the first microorganism, the second microorganism and the third microorganism may be
15 0.05 to 99.95 (first microorganism): 99.95 to 0.05 (second microorganism): 99.95 to 0.05 (third microorganism) relative to the total inoculum.

[0045] For example, when the inoculation ratio between the glutamic acid-producing microorganism and the lysine-producing microorganism is 50 to 99.95: 50 to 0.05, L-
20 glutamic acid and L-lysine in the fermentation broth may be produced at a ratio of 0.83 to 99: 1 through mixed fermentation of the two microorganisms so that they can give an appropriate taste as a flavor. When the inoculation
25 ratio between the glutamic acid-producing microorganism and

the arginine-producing microorganism is 30 to 99.95: 70 to 0.05, L-glutamic acid and L-arginine in the fermentation broth may be produced at a ratio of 1.04 to 99: 1 through mixed fermentation of the two microorganisms. When the inoculation ratio between the glutamic acid-producing microorganism and the inosinic acid-producing microorganism is 0.05 to 99.95: 99.95 to 0.05, L-glutamic acid and IMP in the fermentation broth may be produced at a ratio of 0.02 to 99.8: 1 through mixed fermentation of the two microorganisms. When the inoculation ratio between the inosinic acid-producing microorganism and the lysine-producing microorganism is 0.05 to 99.95: 99.95 to 0.05, IMP and L-lysine in the fermentation broth may be produced at a ratio of 0.01 to 92.3: 1 through mixed fermentation of the two microorganisms. When the inoculation ratio between the inosinic acid-producing microorganism and the arginine-producing microorganism is 65 to 99.95: 35 to 0.05, IMP and L-arginine in the fermentation broth may be produced at a ratio of 1.04 to 95.1: 1 through mixed fermentation of the two microorganisms.

[0046] The mixed fermentation may be performed using an appropriate medium and fermentation conditions known in the art in consideration of the characteristics of each microorganism, and those skilled in the art may easily adjust the medium and fermentation conditions for use.

[0047] More specifically, the fermentation medium used for the mixed fermentation may contain nutrients necessary for microbial growth and proliferation, and may be a liquid medium.

5 **[0048]** The fermentation medium is a medium used during the main fermentation for mass production of amino acids, nucleic acids and/or organic acids, and contains nutrients necessary for the growth of each microorganism. In the present invention, since the fermentation broth containing
10 the fermentation medium is used as a flavor without a separate purification process after completion of the fermentation, it is preferable that the fermentation medium is composed of substances usable as food substances and contains the minimum components and amounts required for
15 culturing microorganisms.

[0049] According to one embodiment of the present invention, the fermentation medium may be based on molasses and contain raw sugar and/or glucose.

[0050] More specifically, the fermentation medium may
20 contain molasses, raw sugar, and glucose as both sugar sources and nutrient sources for microorganisms, and may contain molasses in an amount of 1 to 30 wt% based on the total sugar amount. Molasses used herein may be derived from sugar cane or sugar beets.

25 **[0051]** The fermentation medium may further contain nutrients

for nutrient enhancement of microorganisms, in addition to the sugar sources.

[0052] According to one embodiment of the present invention, the fermentation medium may further contain at least one
5 selected from the group consisting of yeast extract, phosphoric acid, and betaine.

[0053] The fermentation temperature in the mixed fermentation may be usually 20 to 45°C, for example, 25 to 40°C, or 30 to 38°C, and the fermentation may be continued
10 until the microorganisms actively grow and proliferate. The fermentation time may be, for example, 10 to 160 hours, 18 to 120 hours, or 20 to 100 hours.

[0054] In addition, during culture, compounds such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, ammonia,
15 phosphoric acid and sulfuric acid may be added to the medium or culture broth in an appropriate manner to adjust the pH of the culture broth. In addition, it is possible to suppress the formation of air bubbles by adding an antifoaming agent for food during culture. Additionally, in
20 order to maintain the medium or culture broth in an aerobic state, oxygen or oxygen-containing gas (e.g., air) may be injected into the culture broth.

[0055] The fermentation broth produced through this mixed fermentation is used as the raw material of a flavor, is
25 characterized by being used intact without being mixed with

additional components, and contains large amounts of amino acids, nucleic acids and/or organic acids produced from the microorganisms.

[0056] According to one embodiment of the present invention,
5 the fermentation broth may contain total microbial products in an amount of 3 to 90 wt% based on the total solid content.

[0057] More specifically, the fermentation broth may contain amino acids, nucleic acids and/or organic acids in an
10 amount of 3 to 90 wt%, 10 to 90 wt%, 20 to 90 wt%, 30 to 90 wt%, 40 to 90 wt%, 50 to 90 wt%, 60 to 90 wt%, 70 to 90 wt%, or 80 to 90 wt%, based on the total solid content. This fermentation broth may contain 5 to 150 g/L of amino acids, nucleic acids and/or organic acids.

[0058] Another aspect of the present invention provides a
15 method for producing a flavor containing L-glutamic acid and L-lysine, the method comprising a step of inoculating a fermentation medium with a glutamic acid-producing microorganism and a lysine-producing microorganism and then
20 producing a fermentation broth containing L-glutamic acid and L-lysine by fermentation of the microorganisms.

[0059] The glutamic acid-producing microorganism and the lysine-producing microorganism may be naturally occurring wild-type microorganisms or mutant strains modified to
25 improve the amino acid production ability of the wild-type

microorganisms. As this glutamic acid- or lysine-producing microorganism, a microorganism known in the art may be used without limitation. For example, the glutamic acid- or lysine-producing microorganism may be a microorganism of
5 the genus *Corynebacterium*, the genus *Brevibacterium*, the genus *Lactobacillus*, the genus *Bifidobacterium*, the genus *Bacillus*, etc. The microorganisms in the present invention may be of the same genus or species, or different genera or species, and may be selected by the user.

10 **[0060]** According to one embodiment of the present invention, the glutamic acid-producing microorganism and the lysine-producing microorganism may be microorganisms of the genus *Corynebacterium*.

[0061] In an example of the present invention,
15 *Corynebacterium glutamicum* strains were used as the glutamic acid-producing microorganism and the lysine-producing microorganism.

[0062] In order to perform mixed fermentation of the glutamic acid-producing microorganism and the lysine-producing microorganism, isolated and selected
20 microorganisms may be used individually, or a microbial mixture obtained by mixing these microorganisms may be used, and in some cases, an appropriate mixture of the isolated microorganisms and the microbial mixture may be used. This
25 glutamic acid-producing microorganism or lysine-producing

microorganism may be used for fermentation in a state in which it has been grown and the ability thereof to produce amino acids has been activated. In addition, each of the microorganisms is preferably subjected to seed culture for
5 microbial activation.

[0063] According to one embodiment of the present invention, the glutamic acid-producing microorganism and lysine-producing microorganism may be in a seed culture broth state obtained by individual culture or co-culture.

10 **[0064]** The seed culture may be performed using an appropriate medium and culture conditions known in the art in consideration of the characteristics of each microorganism, and any person skilled in the art can easily adjust the medium and culture conditions for use.

15 **[0065]** More specifically, the medium used for the seed culture may contain nutrients necessary for microbial growth and proliferation, and may be a liquid medium.

[0066] According to one embodiment of the present invention, the medium for seed culture of the glutamic acid-producing
20 microorganism may contain, based on the total weight of the medium, 4.5 to 5.5 wt% of molasses, 3 wt% of glucose, 0.85 wt% of yeast extract paste, 100 ppm of methionine, 0.6 wt% of H₃PO₄, 0.1 wt% of sodium succinate, 50 ppm of vitamin C, 12 ppm of thiamine HCl, 20 ppb of vitamin B₁₂, 10 ppm of
25 biotin, 0.4 wt% of MgSO₄, and 0.01 wt% of an antifoaming

agent for food.

[0067] According to one embodiment of the present invention, the medium for seed culture of the lysine-producing microorganism may contain, based on the total weight of the medium, 1.5 to 3 wt% of molasses, 9 to 12 wt% of raw sugar, 1 wt% of yeast extract paste, 1.6 wt% of $(\text{NH}_4)_2\text{SO}_4$, 0.3 wt% of H_3PO_4 , 7.3 ppm of $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$, 14 ppm of nicotinamide, 2.5 ppm of thiamin HCl, 1.5 ppm of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.056 ppm of biotin, 0.045 wt% of betaine, and 0.01 wt% of an antifoaming agent for food.

[0068] The culture temperature in the seed culture may be usually 20 to 45°C, for example, 25 to 40°C, or 27 to 37°C, and the culture may be continued until each microorganism actively grows and proliferates. The culture time may be, for example, 10 to 160 hours, 18 to 120 hours, or 20 to 80 hours.

[0069] In addition, during culture, compounds such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid and sulfuric acid may be added to the medium or culture broth in an appropriate manner to adjust the pH of the culture broth. In addition, it is possible to suppress the formation of air bubbles by using an antifoaming agent for food during culture. Additionally, in order to maintain the medium or culture broth in an aerobic state, oxygen or oxygen-containing gas (e.g., air) may be

injected into the culture broth.

[0070] This seed culture may be performed depending on a microbial concentration desired by the user, and the optical density (OD) value of the seed culture broth is
5 measured to predict the microbial concentration and determine whether to continue culture.

[0071] According to one embodiment of the present invention, the seed culture broth may have $OD_{610} = 10$ to 80 .

[0072] In the step of producing a fermentation broth by
10 fermentation of the glutamic acid-producing microorganism and the lysine-producing microorganism, a fermentation broth containing both L-glutamic acid and L-lysine may be produced by mixed fermentation (main fermentation) using the glutamic acid-producing microorganism or a seed culture
15 broth thereof and the lysine-producing microorganism or a seed culture broth thereof.

[0073] The mixed fermentation is a concept opposite to a conventional individual fermentation process of producing a fermentation broth containing the product of each
20 microorganism by culturing each microorganism individually for amino acid production.

[0074] In the present invention, through mixed fermentation, it is possible to produce L-glutamic acid by the glutamic acid-producing microorganism and L-lysine by the lysine-
25 producing microorganism. In addition, it is possible to

produce a fermentation broth capable of exhibiting a rich umami taste and excellent sensory properties due to the interaction between L-glutamic acid and L-lysine and due to taste components such as ions, nucleic acids, organic acids, and other peptides, which are additionally produced in the microbial fermentation process.

[0075] According to one embodiment of the present invention, in the step, it is preferable to adjust the inoculum of each of the glutamic acid-producing microorganism and the lysine-producing microorganism in order to control the ratio between L-glutamic acid and L-lysine in the fermentation broth.

[0076] More specifically, the inoculation ratio between the glutamic acid-producing microorganism and the lysine-producing microorganism may be a ratio of 0.05 to 99.95 (glutamic acid-producing microorganism): 99.95 to 0.05 (lysine-producing microorganism) relative to the total inoculum. Through mixed fermentation of the two microorganisms inoculated at this ratio, L-glutamic acid and L-lysine in the fermentation broth may be produced at a ratio of 1 to 99: 1 so that they may give an appropriate taste as a flavor.

[0077] For example, when the inoculation ratio between the glutamic acid-producing microorganism and the lysine-producing microorganism is 50 to 99.95: 50 to 0.05, L-

glutamic acid and L-lysine in the fermentation broth may be produced at a ratio of 0.83 to 99: 1, and when the inoculation ratio between the glutamic acid-producing microorganism and the lysine-producing microorganism is
5 adjusted to 65: 35, L-glutamic acid and L-lysine in the fermentation broth may be produced at a ratio of about 1: 1.

[0078] The mixed fermentation may be performed using an appropriate medium and fermentation conditions known in the art in consideration of the characteristics of each
10 microorganism, and any person skilled in the art may easily adjust the medium and fermentation conditions for use.

[0079] More specifically, the fermentation medium used for the mixed fermentation may be a liquid medium.

[0080] The fermentation medium is a medium used during the
15 main fermentation for mass production of L-glutamic acid and L-lysine, and contains nutrients necessary for the growth of the glutamic acid-producing microorganism and the lysine-producing microorganism. In the present invention, since the fermentation broth containing the fermentation
20 medium is used as a flavor without a separate purification process after completion of the fermentation, it is preferable that the fermentation medium is composed of substances usable as food substances and contains the minimum components and amounts required for culturing the
25 microorganisms.

[0081] According to one embodiment of the present invention, the fermentation medium may be based on molasses and contain raw sugar and/or glucose.

[0082] More specifically, the fermentation medium may
5 contain molasses, raw sugar, and glucose as both sugar sources and nutrient sources for the microorganisms, and may contain molasses in an amount of 1 to 30 wt% based on the total sugar amount. Molasses used herein may be derived from sugar cane or sugar beets.

10 **[0083]** The fermentation medium may further contain nutrients for nutrient enhancement of microorganisms, in addition to the sugar sources.

[0084] According to one embodiment of the present invention, the fermentation medium may further contain at least one
15 selected from the group consisting of yeast extract, phosphoric acid, and betaine.

[0085] According to one embodiment of the present invention, the fermentation medium may contain, based on the total weight of the medium, 1.5 to 3 wt% of molasses, 2.5 to 4 wt%
20 of glucose, 0.4 to 1 wt% of yeast extract paste, 0.1 to 0.2 wt% of H_3PO_4 , 0.05 to 0.12 wt% of betaine, and 0.001 to 0.01 wt% of an antifoaming agent for food.

[0086] The fermentation temperature in the mixed fermentation may be usually 20 to 45°C, for example, 25 to
25 40°C, or 30 to 38°C, and the fermentation may be continued

until the contents or concentrations of L-glutamic acid and L-lysine obtained reach desired levels. The fermentation time may be, for example, 10 to 160 hours, 18 to 120 hours, or 20 to 100 hours.

5 **[0087]** In addition, during fermentation, compounds such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, ammonia, phosphoric acid and sulfuric acid may be added to the medium or fermentation broth in an appropriate manner to adjust the pH of the fermentation broth. In addition, it
10 is possible to suppress the formation of air bubbles by using an antifoaming agent for food during fermentation. Additionally, in order to maintain the medium or fermentation broth in an aerobic state, oxygen or oxygen-containing gas (e.g., air) may be injected into the
15 fermentation broth.

[0088] According to one embodiment of the present invention, the fermentation may be performed by fed-batch culture, which starts at a temperature of 30 to 33°C and is maintained at a temperature of 36 to 39°C, a pH of 6.5 to
20 7.5 and a dissolved oxygen concentration of 20 to 70%, for 28 to 40 hours.

[0089] This fermentation broth containing L-glutamic acid and L-lysine is used as the raw material of a flavor containing L-glutamic acid and L-lysine, is characterized
25 by being used intact without being mixed with additional

components, and contains large amounts of L-glutamic acid and L-lysine.

[0090] According to one embodiment of the present invention, the fermentation broth may contain amino acids, including
5 L-glutamic acid and L-lysine, in an amount of 3 to 90 wt% based on the total solid content.

[0091] More specifically, the fermentation broth may contain amino acids, including L-glutamic acid and L-lysine, in an amount of 3 to 90 wt%, 10 to 90 wt%, 20 to 90 wt%, 30 to 90
10 wt%, 40 to 90 wt%, 50 to 90 wt%, 60 to 90 wt%, 70 to 90 wt%, or 80 to 90 wt%, based on the total solid content. This fermentation broth may contain 5 to 150 g/L of L-glutamic acid and L-lysine.

[0092] Meanwhile, the method for producing a flavor
15 according to the present invention may further comprise an additional process for using the fermentation broth as a flavor or a flavor containing L-glutamic acid and L-lysine.

[0093] More specifically, the method may further comprise a step of separating cells from the fermentation broth and
20 decolorizing the fermentation broth.

[0094] The cell separation may be performed without limitation by applying a cell separation method and separation conditions known in the art. An example of the cell separation method may be membrane separation,
25 ultrafiltration, centrifugal filtration, or the like, but

is not limited thereto.

[0095] The decolorization may be performed without limitation by applying a decolorization method and decolorization conditions known in the art. For example,
5 the decolorization may be performed using activated carbon or the like, without being limited thereto.

[0096] In addition, the method may further comprise a step of filtering the decolorized fermentation broth.

[0097] The filtration may be performed without limitation by
10 applying a filtration method and filtration conditions known in the art. For example, the filtration may be performed using filter paper, filter net, membrane filtration, ultrafiltration, etc., without being limited thereto.

15 **[0098]** In addition, the method may further comprise a step of concentrating the filtered fermentation broth.

[0099] The concentration may be performed without limitation by applying a concentration method and concentration conditions known in the art. Examples of the concentration
20 method include, but are not limited to, heat concentration, vacuum concentration, freeze concentration, evaporation concentration, vacuum low-temperature concentration, and the like.

[00100] In addition, the method may further comprise a step
25 of drying and powdering the concentrated fermentation broth.

[00101] The drying may be performed without limitation by applying a drying method and drying conditions known in the art. Examples of the drying method include, but are not limited to, freeze drying, vacuum drying, air drying, blown
5 air drying, hot air drying, fluidized bed drying, spray drying, infrared drying, high frequency drying, and the like.

[00102] Through this method, the fermentation broth may be finally obtained in a powder state, and may be used as a
10 natural food flavor without undergoing an additional chemical purification process.

[00103] Still another aspect of the present invention provides a flavor produced by the above-described method for producing a flavor using two or more different
15 microorganisms.

[00104] According to one embodiment of the present invention, the flavor may be a natural flavor containing two or three taste components selected from among amino acid, nucleic acid and organic acid.

[00105] The amino acid may be at least one selected from the group consisting of L-glutamic acid, L-alanine, L-valine, L-leucine, L-isoleucine, L-proline, L-phenylalanine, L-tryptophan, L-methionine, L-glycine, L-serine, L-threonine, L-cysteine, L-asparagine, L-glutamine, L-aspartic acid, L-
20 lysine, L-arginine, and L-histidine.
25

[00106] The nucleic acid may be at least one selected from the group consisting of inosinic acid, guanylic acid, xanthyl acid, and salts thereof.

[00107] For example, the nucleic acid may be inosine
5 monophosphate (IMP), guanosine monophosphate (GMP), xanthosine monophosphate (XMP), etc., but is not limited thereto.

[00108] The organic acid may be at least one selected from the group consisting of succinic acid, malic acid, citric
10 acid, acetic acid, lactic acid, fumaric acid, tartaric acid, ascorbic acid, gluconic acid, and salts thereof.

[00109] For example, when the flavor contains two taste components, it may contain glutamic acid and lysine, arginine, histidine, tryptophan, glycine, alanine, succinic
15 acid, lactic acid, guanylic acid, or inosinic acid. In addition, the flavor may contain inosinic acid and lysine, arginine, histidine, tryptophan, glycine, alanine, succinic acid, lactic acid, or guanylic acid.

[00110] In another example, when the flavor contains three
20 taste components, it may contain glutamic acid, guanylic acid, and inosinic acid.

[00111] According to one embodiment of the present invention, the flavor may contain taste components, including amino acids, nucleic acids and/or organic acids, in an amount of
25 3 to 90 wt% based on the total solid content.

[00112] More specifically, the flavor may contain taste components, including amino acids, nucleic acids and/or organic acids, in an amount of 3 to 90 wt%, 10 to 90 wt%, 20 to 90 wt%, 30 to 90 wt%, 40 to 90 wt%, 50 to 90 wt%, 60 to 90 wt%, 70 to 90 wt%, or 80 to 90 wt%, based on the total solid content.

[00113] Yet another aspect of the present invention provides a flavor containing L-glutamic acid and L-lysine, produced by the above-described method for producing a natural flavor containing L-glutamic acid and L-lysine using a glutamic acid-producing microorganism and a lysine-producing microorganism.

[00114] According to one embodiment of the present invention, the flavor containing L-glutamic acid and L-lysine may be a natural flavor.

[00115] According to one embodiment of the present invention, the flavor may have an L-glutamic acid and L-lysine content of 3 to 90 wt% based on the solid content, and contain L-glutamic acid and L-lysine at a ratio of 0.83 to 99: 1.

[00116] Since this flavor contains L-lysine together with L-glutamic acid that gives an umami taste, it may increase the solubility of L-glutamic acid and solve the ammonia odor problem by reducing the production of ammonium glutamate during the fermentation process. In addition, since the flavor also contains metabolites such as organic

acids, inorganic ion components, proteins, peptides, and vitamins produced in the fermentation process, it has a rich umami taste and a strong body feeling and exhibits excellent sensory properties. Thus, the flavor may be added
5 to various foods to maximize the taste of the foods.

[00117] Still yet another aspect of the present invention provides a food composition containing the above-described flavor or the flavor containing L-glutamic acid and L-lysine.

10 **[00118]** As used herein, the term "food composition" refers to a natural product or processed product containing one or more nutrients, and preferably refers to a product that has undergone certain processing and is ready to be eaten directly. In a common sense, the term "food composition"
15 includes all health functional foods, functional foods, beverages, food additives, and beverage additives.

[00119] According to one embodiment of the present invention, the food composition contains a flavor produced without a chemical purification process or a flavor containing
20 natural L-glutamic acid and L-lysine, that is a natural flavor, and it may contain the flavor in an amount of 0.001 to 90 wt%, more specifically 0.01 to 50 wt%, based on the total weight of the composition.

[00120] The food composition in the present invention may be
25 provided in any formulation suitable for food, and may be,

for example, in the form of a solution, emulsion, viscous mixture, powder, granule, tablet, capsule, or the like. In this case, the food composition may contain various bases and/or additives necessary and appropriate for formulation
5 within the range that does not impair the main effect of the present invention. In addition, the food composition may further contain additives such as fragrance, colorants, disinfectants, antioxidants, preservatives, humectants, thickeners, inorganic salts, and emulsifiers, within the
10 range that does not impair the effect thereof. The content of these additives in the food composition may be selected depending on the formulation or intended use of the composition within the range that does not impair the purpose and effect of the present invention. For example,
15 the content of the additives may be 0.01 to 70 wt%, more specifically 0.1 to 50 wt%, based on the total weight of the food composition.

[00121] This food composition may be used as an additive for various foods. As foods to which this food composition may
20 be added, any foods known in the art may be used without limitation. Examples of the foods include, but are not limited to, meat, sausage, bread, chocolate, candy, snacks, confectionery, pizza, ramen, other noodles, gums, dairy products including ice cream, various soups, beverages, tea,
25 drinks, alcoholic beverages, and vitamin complexes.

[00122] The method for producing a flavor according to the present invention may produce a natural flavor capable of improving the taste and aroma of food and the overall sensory properties of food through a fermentation broth
5 containing amino acids, nucleic acids and/or organic acids, which is produced by mixed fermentation of different microorganisms producing different products, that is, different kinds of amino acids, nucleic acids and/or organic acids. This flavor may be used in various food
10 fields.

BRIEF DESCRIPTION OF THE DRAWINGS

[00123] FIG. 1 is a flow chart showing a fermentation process performed using a glutamic acid-producing microorganism and
15 a lysine-producing microorganism in Preparation Examples 1 to 3 according to one embodiment of the present invention.

[00124] FIG. 2 is a flow chart showing a fermentation process performed using a glutamic acid-producing microorganism and an arginine-producing microorganism in Preparation Examples
20 4 to 6 according to one embodiment of the present invention.

[00125] FIG. 3 is a flow chart showing a fermentation process performed using a glutamic acid-producing microorganism and an inosinic acid-producing microorganism in Preparation Examples 7 to 9 according to one embodiment of the present
25 invention.

[00126] FIG. 4 is a flow chart showing a fermentation process performed using an inosinic acid-producing microorganism and a lysine-producing microorganism in Preparation Examples 10 to 12 according to one embodiment of the present invention.

[00127] FIG. 5 is a flow chart showing a fermentation process performed using an inosinic acid-producing microorganism and an arginine-producing microorganism in Preparation Examples 13 to 15 according to one embodiment of the present invention.

DETAILED DESCRIPTION

[00128] Hereinafter, the present invention will be described in more detail with reference to the accompanying drawings. However, these descriptions are provided for illustrative purposes only to help the understanding of the present invention, and the scope of the present invention is not limited by these illustrative descriptions.

[00129] Example 1. Mixed fermentation of glutamic acid and lysine

[00130] 1-1. Seed culture

[00131] *Corynebacterium glutamicum* NFG6 (KCCM13164P) that produces L-glutamic acid (GA) was used as a glutamic acid-producing microorganism, and *Corynebacterium glutamicum* NFL21 (KCCM13163P) that produces L-lysine (LYS) was used as

a lysine-producing microorganism.

[00132] For seed culture of the glutamic acid-producing microorganism, the glutamic acid-producing microorganism was inoculated into a 2-L flask containing 0.2 L of a seed culture medium, followed by primary culture for 22 to 24 hours at 30°C and 140 rpm ($OD_{610} = 10$ to 15). Next, 2 to 3% of the primary culture was inoculated into a 5-L jar fermenter, and 2 to 2.5 L of a seed culture medium was added thereto, followed by secondary culture at 32°C, pH 6.9, 600 rpm and an aeration rate of 1.0 vvm for 22 to 24 hours ($OD_{610} = 20$ to 60), thereby preparing a glutamic acid seed culture broth.

[00133] For seed culture of the lysine-producing microorganism, the lysine-producing microorganism was inoculated into a 2-L flask containing 0.2 L of a seed culture medium, followed by primary culture for 16 to 18 hours at 30°C and 140 rpm ($OD_{610} = 11$ to 12). Next, 5% of the primary culture was inoculated into a 5-L jar fermenter, and 2 to 2.5 L of a seed culture medium was added thereto, followed by secondary culture at 32°C, pH 7.0, 650 rpm and an aeration rate of 2.0 vvm for 21 to 24 hours ($OD_{610} = 20$ to 60), thereby preparing a lysine seed culture broth.

[00134] The compositions of the seed culture media used are shown in Table 1 below.

[00135] [Table 1]

	Composition
Seed culture medium for glutamic acid-producing microorganism	4.5 to 5.5% molasses, 3% glucose, 0.85% yeast extract paste, 100 ppm methionine, 0.6% H ₃ PO ₄ , 0.1% sodium succinate, 50 ppm vitamin C, 12 ppm thiamine HCl, 20 ppb vitamin B12, 10 ppm biotin, 0.4% MgSO ₄ , and 0.01% antifoaming agent for food
Seed culture medium for lysine-producing microorganism	1.5 to 3% molasses, 9 to 12% raw sugar, 1% yeast extract paste, 1.6% (NH ₄) ₂ SO ₄ , 0.3% H ₃ PO ₄ , 7.3 ppm MnSO ₄ ·5H ₂ O, 14 ppm nicotinamide, 2.5 ppm thiamine HCl, 1.5 ppm CuSO ₄ ·5H ₂ O, 0.056 ppm biotin, 0.045% betaine, and 0.01% antifoaming agent for food

[00136] 1-2. Main fermentation

[00137] In order to examine the ratio of glutamic acid to lysine in the fermentation broth depending on the inoculums of the glutamic acid seed culture broth and the lysine seed culture broth, the glutamic acid seed culture broth and the lysine seed culture broth were inoculated at various ratios and fermented.

[00138] In main fermentation, 14 to 18 L of a fermentation medium was added to a 50 L fermenter, and inoculated with 1.2 to 1.8 L of the total seed culture broth at an inoculation ratio of 50 to 99.95 (glutamic acid seed culture broth): 50 to 0.05 (lysine seed culture broth),

followed by mixed fermentation by fed-batch culture for 28 to 40 hours. The fermentation medium composition and fermentation conditions used are shown in Table 2 below.

[00139] [Table 2]

Fermentation medium composition	1.5 to 3% molasses, 2.5 to 4% glucose, 0.4 to 1% yeast extract paste, 0.1 to 0.2% H_3PO_4 , 0.05 to 0.12% betaine, and 0.005% antifoaming agent for food
Fermentation conditions	Temperature 32–38°C, pH 6.5 to 7.5, aeration rate 0.8 to 1.2 vvm, internal pressure 0.6 to 1.0 kg/cm ³ , agitation speed 320 to 350 rpm, and dissolved oxygen (DO) concentration 20 to 70%

- 5 **[00140]** This mixed fermentation was performed a total of three times, the average value was calculated, and the results are shown in Table 3 below.

[00141] [Table 3]

Inoculation ratio between seed culture broths (GA: LYS)	Fermentation time	GA: LYS ratio in fermentation broth
99.95 : 0.05	31 hours	99 : 1
75 : 25	29 hours	1.48 : 1
70 : 30	29 hours	1.30 : 1
67 : 33	30 hours	1.10 : 1

65 : 35	30 hours	1.01 : 1
60 : 40	29 hours	0.93 : 1
50 : 50	28 hours	0.83 : 1

[00142] Referring to Table 3 above, it was confirmed that, when the seed culture broths of the amino acid-producing microorganisms were inoculated at a ratio of 50 to 99.95 (glutamic acid-producing microorganism): 50 to 0.05 (lysine-producing microorganism), L-glutamic acid and L-lysine in the fermentation broth were produced at a ratio of 0.83 to 99: 1.

[00143] Example 2. Comparison of glutamic acid-lysine fermented powders between fermentation processes

10 [00144] 2-1. Production of glutamic acid-lysine fermented powder

[00145] Conventionally, a flavor containing glutamic acid and lysine was produced by individually fermenting a glutamic acid-producing microorganism and a lysine-producing microorganism and then mixing the amino acid-containing fermentation broths or dried products thereof at a suitable ratio. To compare the differences in taste between this conventional individual fermentation method and the method based on mixed fermentation of the glutamic acid-producing microorganism and the lysine-producing microorganism, the components of glutamic acid-lysine (GA-LYS) fermented

powders obtained by the production methods were compared.
(See FIG. 1).

[00146] The glutamic acid seed culture broth and lysine seed culture broth used here were prepared in the same manner as
5 in Example 1-1.

[00147] ① Individual fermentation (Preparation Example 1)

[00148] In the method of mixing individual dried products among the conventional methods, each of the glutamic acid seed culture broth and the lysine seed culture broth was
10 transferred into a 50-L fermenter and then individually subjected to main fermentation. Next, cells were separated from each fermentation broth, and then decolorization and filtration processes were performed. The filtrates were concentrated and dried to obtain dried products. The
15 obtained dried glutamic acid and lysine products were mixed together so that the ratio of glutamic acid to lysine was 1:1, thereby preparing fermented powder containing glutamic acid and lysine.

**[00149] ② Mixing of fermentation broths after individual
20 fermentation (Preparation Example 2)**

[00150] In the method of mixing individual fermentation broths among the conventional methods, each of the glutamic acid seed culture broth and the lysine seed culture broth was transferred into a 50-L fermenter and then individually
25 subjected to main fermentation. The fermentation broths

obtained in the main fermentation were mixed together so that the ratio of glutamic acid to lysine was 1: 1. Next, cells were separated from the fermentation broth mixture, and then decolorization and filtration processes were performed. The filtrate was concentrated and dried to obtain glutamic acid-lysine fermented powder.

[00151] ③ Mixed fermentation (Preparation Example 3)

[00152] In mixed fermentation, the glutamic acid seed culture broth and the lysine seed culture broth were inoculated at a ratio of 65: 35 in the same manner as in Example 1-2 so that the ratio of glutamic acid to lysine in the fermentation broth was about 1:1 in the same manner as in Preparation Examples 1 and 2, followed by mixed fermentation. Next, cells were separated from the fermentation broth, and decolorization and filtration processes were performed. The filtrate was concentrated and dried to obtain glutamic acid-lysine fermented powder.

[00153] 2-2. Comparison of components between glutamic acid-lysine fermented powders

[00154] Component analysis was performed on the glutamic acid-lysine fermented powder obtained through individual fermentation in each of Preparation Examples 1 and 2 and the glutamic acid-lysine fermented powder obtained through mixed fermentation in Preparation Example 3.

[00155] Amino acids (L-glutamic acid and L-lysine) were

measured by HPLC analysis (GA - 210 nm, UV detector, flow rate 0.9 ml/min; LYS - 214 nm, UV detector, flow rate 0.8 ml/min), and organic acids (citric acid, succinic acid, lactic acid, acetic acid, etc.) were measured by HPLC analysis (HPX-87H column, 214 nm, 25 min). Ions (Na, Mg, K, PO₄, SO₄, Cl, NH₄, etc.) were measured by an ion analyzer (Dionex IcS-1100, Thermo Scientific). The results are shown in Table 4 below.

[00156] [Table 4]

Item	Preparation Example 1	Preparation Example 2	Preparation Example 3
GA	22%	35%	43%
LYS	22%	35%	43%
AA/TS*	47%	72%	88%
Total nitrogen	9.0%	12.2%	11.8%
Organic acids	3.1%	2.6%	1.5%
Ions	8.1%	10.2%	2%
Ammonium	3.8%	2.5%	0.7%
*AA/TS: proportion of amino acids (GA+LYS) relative to total solid content			

10 **[00157]** Referring to Table 4 above, mixed fermentation (Preparation Example 3) showed a higher amino acid proportion than individual fermentation (Preparation Examples 1 and 2) and showed significant decreases in the contents of organic acids, ions and ammonium.

[00158] 2-3. Sensory comparison between individual fermentation and mixed fermentation

[00159] Sensory evaluation was performed on the glutamic acid-lysine fermented powders obtained through individual fermentation in Preparation Examples 1 and 2 and the glutamic acid-lysine fermented powder obtained through mixed fermentation in Preparation Example 3.

[00160] For sensory evaluation of the glutamic acid-lysine fermented powders, the umami taste, umami persistence, salty taste, sour taste, bitter taste and sweet taste of each powder itself or a sample obtained by diluting each powder in lukewarm water at a concentration of 1 to 5% were evaluated by a trained panel consisting of 10 to 15 persons. The results are shown in Table 5 below.

[00161] [Table 5]

Item	Preparation Example 1 (individual fermentation)	Preparation Example 2 (individual fermentation)	Preparation Example 3 (mixed fermentation)
Initial Umami taste	+++	++++	++++
Umami persistence	++	+++	++++
Salty taste	-	+	++
Sour taste	++	+	-

Bitter taste	++	+	-
Sweet taste	+	+	++

[00162] Referring to Table 5 above, due to the difference in components of the glutamic acid-lysine fermented powder as shown in Table 4 above, the sample prepared by the mixing process after individual fermentation showed a weak umami taste and an increased bitter taste and sour taste compared to the sample prepared by mixed fermentation. In addition, since the increase in by-products such as organic acids and the increase in ions as shown in Table 4 above also affect the sensory properties of the sample, the mixed fermentation process is more effective in terms of taste or process simplification than mixing after individual fermentation.

[00163] Example 3. Mixed fermentation of glutamic acid and arginine

[00164] 3-1. Seed culture

[00165] *Corynebacterium glutamicum* NFG6 (KCCM13164P) was used as a glutamic acid-producing microorganism, and *Corynebacterium glutamicum* NFA40 (KCCM13165P) that produces L-arginine (ARG) was used as an arginine-producing microorganism.

[00166] A glutamic acid seed culture broth of the glutamic acid-producing microorganism was prepared in the same manner as in Example 1-1.

[00167] For seed culture of the arginine-producing microorganism, the arginine-producing microorganism was inoculated into a 2-L flask containing 0.2 L of a seed culture medium, followed by primary culture for 16 to 18 hours at 30°C and 140 rpm (OD_{610} = 12 to 18). Next, 2 to 4% of the primary culture was inoculated into a 5-L jar fermenter, and 2 to 2.5 L of a seed culture medium was added thereto, followed by secondary culture at 32°C, pH 6.7, 600 rpm and an aeration rate of 1.0 vvm for 16 to 24 hours (OD_{610} = 20 to 60), thereby preparing an arginine seed culture broth. The composition of the seed culture medium used is shown in Table 6 below.

[00168] [Table 6]

	Composition
Seed culture medium for arginine- producing microorganism	1.5 to 4.5% glucose, 2 to 6% raw sugar, 2 to 3% yeast extract paste, 0.6% $(NH_4)_2SO_4$, 0.2% KH_2PO_4 , 0.2% K_2HPO_4 0.2%, 15 ppm $MnSO_4 \cdot 5H_2O$, 0.2 % $MgSO_4 \cdot 7H_2O$ 1 ppm thiamine HCl, 10 ppm $ZnSO_4 \cdot 7H_2O$, 0.3 ppm biotin, 15 ppm $FeSO_4 \cdot 7H_2O$, and 0.01% antifoaming agent for food

[00169] 3-2. Main fermentation

[00170] In order to examine the ratio of glutamic acid to arginine in the fermentation broth depending on the inoculums of the glutamic acid seed culture broth and the arginine seed culture broth, the glutamic acid seed culture

broth and the arginine seed culture broth were inoculated at various ratios and fermented.

[00171] In main fermentation, 14 to 18 L of a fermentation medium was added to a 50-L fermenter and inoculated with 1.2 to 1.8 L of the total seed culture broth at an inoculation ratio of 30 to 99.95 (glutamic acid seed culture broth): 70 to 0.05 (arginine seed culture broth), followed by mixed fermentation by fed-batch culture for 31 to 60 hours. The fermentation medium composition and fermentation conditions used are shown in Table 7 below.

[00172] [Table 7]

Fermentation medium composition	1.5 to 3% molasses, 2.5 to 4% glucose, 0.4 to 1% yeast extract paste, 0.05 to 0.3% (NH ₄) ₂ SO ₄ , 0.1 to 0.2% H ₃ PO ₄ , 0.05 to 0.12% betaine, and 0.005% antifoaming agent for food
Fermentation conditions	Temperature 32 to 37°C, pH 6.5 to 7.5, aeration rate 0.8 to 1.2 vvm, internal pressure 0.5 to 1.0 kg/cm ³ , agitation speed 320 to 350 rpm, and dissolved oxygen (DO) concentration 20 to 70%

[00173] This mixed fermentation was performed a total of three times, the average value was calculated, and the results are shown in Table 8 below.

[00174] [Table 8]

Inoculation ratio between	Fermentation time	GA: ARG ratio in
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seed culture broths (GA: ARG)		fermentation broth
99.95 : 0.05	31 hours	99.0 : 1
90 : 10	31 hours	12.2 : 1
80 : 20	34 hours	4.36 : 1
70 : 30	36 hours	2.41 : 1
60 : 40	38 hours	1.96 : 1
50 : 50	40 hours	1.57 : 1
40 : 60	44 hours	1.21 : 1
30 : 70	45 hours	1.04 : 1

[00175] Referring to Table 8 above, it was confirmed that, when the seed culture broths of the amino acid-producing microorganisms were inoculated at a ratio of 30 to 99.95 (glutamic acid-producing microorganism): 70 to 0.05 (arginine-producing microorganism), L-glutamic acid and L-arginine in the fermentation broth were produced at a ratio of 1.04 to 99: 1.

[00176] Example 4. Comparison of glutamic acid-arginine fermented powders between fermentation processes

[00177] 4-1. Production of glutamic acid-arginine fermented powder

[00178] Conventionally, a flavor containing glutamic acid and

arginine was produced by individually fermenting a glutamic acid-producing microorganism and an arginine-producing microorganism and then mixing the amino acid-containing fermentation broths or dried products thereof at a suitable ratio. To compare the differences in taste between this conventional individual fermentation method and the method based on mixed fermentation of the glutamic acid-producing microorganism and the arginine-producing microorganism, the components of glutamic acid-arginine (GA-ARG) fermented powders obtained by the production methods were compared (see FIG. 2).

[00179] The glutamic acid seed culture broth and arginine seed culture broth used here were prepared in the same manner as in Example 3-1.

15 **[00180] ① Individual fermentation (Preparation Example 4)**

[00181] In the method of mixing individual dried products among the conventional methods, each of the glutamic acid seed culture broth and the arginine seed culture broth was transferred into a 50-L fermenter and then individually subjected to main fermentation. Next, cells were separated from each fermentation broth, and then decolorization and filtration processes were performed. The filtrates were concentrated and dried to obtain dried products. The obtained dried glutamic acid and arginine products were mixed together so that the ratio of glutamic acid to

arginine was 1:1, thereby preparing fermented powder containing glutamic acid and arginine.

[00182] ② Mixing of fermentation broths after individual fermentation (Preparation Example 5)

5 **[00183]** In the method of mixing individual fermentation broths among the conventional methods, each of the glutamic acid seed culture broth and the arginine seed culture broth was transferred into a 50-L fermenter and then individually subjected to main fermentation. The fermentation broths
10 obtained in the main fermentation were mixed together so that the ratio of glutamic acid to arginine was 1: 1. Next, cells were separated from the fermentation broth mixture, and then decolorization and filtration processes were performed. The filtrate was concentrated and dried to
15 obtain glutamic acid-arginine fermented powder.

[00184] ③ Mixed fermentation (Preparation Example 6)

[00185] In mixed fermentation, the glutamic acid seed culture broth and the arginine seed culture broth were inoculated at a ratio of 30: 70 in the same manner as in Example 3-2
20 so that the ratio of glutamic acid to arginine in the fermentation broth was about 1:1 in the same manner as in Preparation Examples 4 and 5, followed by mixed fermentation. Next, cells were separated from the fermentation broth, and decolorization and filtration
25 processes were performed. The filtrate was concentrated and

dried to obtain glutamic acid-arginine fermented powder.

[00186] 4-2. Comparison of components between glutamic acid-arginine fermented powders

[00187] Component analysis was performed on the glutamic acid-arginine fermented powder obtained through individual fermentation in each of Preparation Examples 4 and 5 and the glutamic acid-arginine fermented powder obtained through mixed fermentation in Preparation Example 6.

[00188] Amino acids (L-glutamic acid and L-arginine) were measured by HPLC analysis (GA - 210 nm, UV detector, flow rate 0.9 ml/min; ARG - 195 nm, UV detector, flow rate 1 ml/min). Organic acid and ions were measured in the same manner as in Example 2-2. The results are shown in Table 9 below.

[00189] [Table 9]

Item	Preparation Example 4	Preparation Example 5	Preparation Example 6
GA	22%	35%	42%
ARG	22%	34%	42%
AA/TS*	48%	71%	86%
Total nitrogen	9.4%	12.0%	11.7%
Organic acids	3.6%	2.5%	1.8%
Ions	8.3%	9.9%	2.1%

Ammonium	4.0%	2.6%	0.9%
*AA/TS: proportion of amino acids (GA+ ARG) relative to total solid content			

[00190] Referring to Table 9 above, mixed fermentation (Preparation Example 6) showed a higher amino acid proportion than individual fermentation (Preparation Examples 4 and 5) and showed significant decreases in the contents of organic acids, ions and ammonium.

[00191] 4-3. Sensory comparison between individual fermentation and mixed fermentation

[00192] Sensory evaluation was performed on the glutamic acid-arginine fermented powders obtained through individual fermentation in Preparation Examples 4 and 5 and the glutamic acid-arginine fermented powder obtained through mixed fermentation in Preparation Example 6.

[00193] Sensory evaluation was performed in the same manner as in Example 2-3. The results are shown in Table 10 below.

[00194] [Table 10]

Item	Preparation Example 4 (individual fermentation)	Preparation Example 5 (individual fermentation)	Preparation Example 6 (mixed fermentation)
Initial Umami taste	++	+++	++++
Umami	+	++	++++

persistence			
Salty taste	-	+	++
Sour taste	+	+	-
Bitter taste	++	++	-
Sweet taste	-	-	-

[00195] Referring to Table 10 above, due to the difference in components of the glutamic acid-arginine fermented powder as shown in Table 9 above, the sample prepared by the mixing process after individual fermentation showed a weak
5 umami taste and an increased bitter taste compared to the sample prepared by mixed fermentation. In addition, since the increase in by-products such as organic acids and the increase in ions as shown in Table 9 above also affect the sensory properties of the sample, the mixed fermentation
10 process is more effective in terms of taste or process simplification than mixing after individual fermentation.

[00196] Example 5. Mixed fermentation of glutamic acid and inosinic acid

[00197] 5-1. Seed culture

15 **[00198]** *Corynebacterium glutamicum* NFG6 (KCCM13164P) was used as a glutamic acid-producing microorganism, and *Corynebacterium ammoniagenes* NFI545 (KCCM13162P) that produces IMP was used as an inosinic acid-producing microorganism.

20 **[00199]** A glutamic acid seed culture broth of the glutamic

acid-producing microorganism was prepared in the same manner as in Example 1-1.

[00200] For seed culture of the inosinic acid-producing microorganism, the inosinic acid-producing microorganism was inoculated into a 2-L flask containing 0.3 L of a seed culture medium, followed by primary culture for 20 to 24 hours at 31°C and 150 rpm (OD_{610} = 15 to 20). Next, 1% of the primary culture was inoculated into a 5-L jar fermenter, and 2 to 2.5 L of a seed culture medium was added thereto, followed by secondary culture at 31°C, pH 7.1, 600 rpm and an aeration rate of 1.0 vvm for 21 to 24 hours (OD_{610} = 20 to 40), thereby preparing an inosinic acid seed culture broth. The composition of the seed culture medium used is shown in Table 11 below.

[00201] [Table 11]

	Composition
Seed culture medium for inosinic acid-producing microorganism	4 to 6% glucose, 2 to 4% yeast extract paste, 0.3% $(NH_4)_2SO_4$, 0.2% KH_2PO_4 , 0.2% K_2HPO_4 , 200 to 300 ppm adenine, 200 to 300 ppm guanine, 0.15% $MgSO_4 \cdot 7H_2O$, 10 ppm nicotinic acid, 100 ppm Ca-pantothenate, 15 ppm cysteine, 1 ppm thiamine HCl, 5 ppm $ZnSO_4 \cdot 7H_2O$, 10 ppm $MnSO_4 \cdot 5H_2O$, 0.1 ppm biotin, 15 ppm $FeSO_4 \cdot 7H_2O$, and 0.01% antifoaming agent for food

[00202] 5-2. Main fermentation

[00203] In order to examine the ratio of glutamic acid to inosinic acid in the fermentation broth depending on the inoculums of the glutamic acid seed culture broth and the inosinic acid seed culture broth, the glutamic acid seed culture broth and the inosinic acid seed culture broth were inoculated at various ratios and fermented.

[00204] In main fermentation, 14 to 18 L of a fermentation medium was added to a 50-L fermenter and inoculated with 1.2 to 1.8 L of the total seed culture broth at an inoculation ratio of 0.05 to 99.95 (glutamic acid seed culture broth): 99.95 to 0.05 (inosinic acid seed culture broth), followed by mixed fermentation by fed-batch culture for 30 to 90 hours. The fermentation medium composition and fermentation conditions used here are shown in Table 12 below.

[00205] [Table 12]

Fermentation medium composition	1 to 3% molasses, 5 to 7% raw sugar, 2 to 3% yeast extract paste, 0.6 to 1.2% H_3PO_4 , 0.05 to 0.1% betaine, 100 to 200 ppm adenine, 50 to 150 ppm guanine, 0.2 to 0.5% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 to 100 ppm Ca-pantothenate, 5 to 15 ppm vitamin B3, 5 to 20 ppm thiamine HCl, 0.4 to 0.8% NaOH, 5 to 10 ppm FeSO_4 , 10 to 20 ppm MnSO_4 , 10 to 20 ppm ZnSO_4 , and 0.005% antifoaming agent for food
Fermentation	Temperature 31 to 32°C, pH 6.5 to 7.5, aeration

conditions	rate 0.8 to 1.2 vvm, internal pressure 0.6 to 1.0 kg/cm ³ , agitation speed 320 to 350 rpm, and dissolved oxygen (DO) concentration 20 to 70%
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[00206] This mixed fermentation was performed a total of three times, the average value was calculated, and the results are shown in Table 13 below.

[00207] [Table 13]

Inoculation ratio between seed culture broths (GA: IMP)	Fermentation time	GA: IMP ratio in fermentation broth
99.95 : 0.05	33 hours	99.8 : 1
80 : 20	42 hours	51.3 : 1
70 : 30	48 hours	31.2 : 1
50 : 50	60 hours	16.8 : 1
20 : 80	83 hours	1.04 : 1
0.05 : 99.95	90 hours	0.02 : 1

5 **[00208]** Referring to Table 13 above, it was confirmed that, when the seed culture broths of the microorganisms were inoculated at a ratio of 0.05 to 99.95 (glutamic acid-producing microorganism): 99.95 to 0.05 (inosinic acid-producing microorganism), L-glutamic acid and IMP in the
10 fermentation broth were produced at a ratio of 0.02 to 99.8:

1.

**[00209] Example 6. Comparison of glutamic acid-inosinic acid
fermented powders between fermentation processes**

**[00210] 6-1. Production of glutamic acid-inosinic acid
5 fermented powder**

[00211] Conventionally, a flavor containing glutamic acid and
inosinic acid was produced by individually fermenting a
glutamic acid-producing microorganism and an inosinic acid-
producing microorganism and then mixing the fermentation
10 broths or dried products thereof at a suitable ratio. To
compare the differences in taste between this conventional
individual fermentation method and the method based on
mixed fermentation of the glutamic acid-producing
microorganism and the inosinic acid-producing microorganism,
15 the components of glutamic acid-inosinic acid (GA-IMP)
fermented powders obtained by the production methods were
compared (see FIG. 3).

[00212] The glutamic acid seed culture broth and inosinic
acid seed culture broth used here were prepared in the same
20 manner as in Example 5-1.

[00213] ① Individual fermentation (Preparation Example 7)

[00214] In the method of mixing individual dried products
among the conventional methods, each of the glutamic acid
seed culture broth and the inosinic acid seed culture broth
25 was transferred into a 50-L fermenter and then individually

subjected to main fermentation. Next, cells were separated from each fermentation broth, and then decolorization and filtration processes were performed. The filtrates were concentrated and dried to obtain dried products. The
5 obtained dried glutamic acid and isosinic acid products were mixed together so that the ratio of glutamic acid to inosinic acid was 1:1, thereby preparing fermented powder containing glutamic acid and inosinic acid.

**[00215] ② Mixing of fermentation broths after individual
10 fermentation (Preparation Example 8)**

[00216] In the method of mixing individual fermentation broths among the conventional methods, each of the glutamic acid seed culture broth and the inosinic acid seed culture broth was transferred into a 50-L fermenter and then
15 individually subjected to main fermentation. The fermentation broths obtained in the main fermentation were mixed together so that the ratio of glutamic acid to inosinic acid was 1: 1. Next, cells were separated from the fermentation broth mixture, and then decolorization and
20 filtration processes were performed. The filtrate was concentrated and dried to obtain glutamic acid-inosinic acid fermented powder.

[00217] ③ Mixed fermentation (Preparation Example 9)

[00218] In mixed fermentation, the glutamic acid seed culture
25 broth and the inosinic acid seed culture broth were

inoculated at a ratio of 20: 80 in the same manner as in Example 5-2 so that the ratio of glutamic acid to inosinic acid in the fermentation broth was about 1:1 in the same manner as in Preparation Examples 7 and 8, followed by mixed fermentation. Next, cells were separated from the fermentation broth, and decolorization and filtration processes were performed. The filtrate was concentrated and dried to obtain glutamic acid-inosinic acid fermented powder.

[00219] 6-2. Comparison of components between glutamic acid-inosinic acid fermented powders

[00220] Component analysis was performed on the glutamic acid-inosinic acid fermented powder obtained through individual fermentation in each of Preparation Examples 7 and 8 and the glutamic acid-inosinic acid fermented powder obtained through mixed fermentation in Preparation Example 9.

[00221] L-glutamic acid and IMP were measured by HPLC analysis (GA - 210 nm, UV detector, flow rate 0.9 ml/min; IMP - 254 nm, UV detector, flow rate 0.9 ml/min). Organic acid and ions were measured in the same manner as in Example 2-2. The results are shown in Table 14 below.

[00222] [Table 14]

Item	Preparation Example 7	Preparation Example 8	Preparation Example 9

GA	22%	31%	37%
IMP	20%	31%	36%
(GA+IMP) / TS*	42%	62%	73%
Total nitrogen	8.6%	9.7%	10.5%
Organic acids	4.0%	3.3%	3.1%
Ions	10.4%	8.8%	4.2%
Ammonium	4.0%	3.0%	1.7%
* (GA+IMP) / TS: Proportion of product relative to total solid content			

[00223] 6-3. Sensory comparison between individual fermentation and mixed fermentation

[00224] Sensory evaluation was performed on the glutamic acid-inosinic acid fermented powders obtained through individual fermentation in Preparation Examples 7 and 8 and the glutamic acid-inosinic acid fermented powder obtained through mixed fermentation in Preparation Example 9.

[00225] Sensory evaluation was performed in the same manner as in Example 2-3. The results are shown in Table 15 below.

10 **[00226]** [Table 15]

Item	Preparation Example 7 (individual fermentation)	Preparation Example 8 (individual fermentation)	Preparation Example 9 (mixed fermentation)
Initial Umami taste	+++	++++	++++
Umami	+	+++	++++

persistence			
Kokumi	+	++	+++
Salty taste	+	++	++
Sour taste	++	++	+
Bitter taste	++	+	-
Sweet taste	+	+	++

[00227] Referring to Table 15 above, due to the difference in components of the glutamic acid-inosinic acid fermented powder as in Table 14 above, the sample prepared by the mixing process after individual fermentation showed a weak
5 umami taste and umami persistence and an increased bitter taste compared to the sample prepared by mixed fermentation. In addition, since the increase in by-products such as organic acids and the increase in ions as shown in Table 14 above also affect the sensory properties of the sample, the
10 mixed fermentation process is more effective in terms of taste or process simplification than mixing after individual fermentation.

[00228] Example 7. Mixed fermentation of inosinic acid and lysine

15 **[00229] 7-1. Seed culture**

[00230] *Corynebacterium ammoniagenes* NFI545 (KCCM13162P) was used as an inosinic acid-producing microorganism, and *Corynebacterium glutamicum* NFL21 (KCCM13163P) was used as a lysine-producing microorganism.

[00231] An inosinic acid seed culture broth of the inosinic acid-producing microorganism and a lysine seed culture broth of the lysine-producing microorganism were prepared in the same manner as in Examples 5-1 and 1-1, respectively.

5 **[00232] 7-2. Main fermentation**

[00233] In order to examine the ratio of inosinic acid to lysine in the fermentation broth depending on the inoculums of the inosinic acid seed culture broth and the lysine seed culture broth, the inosinic acid seed culture broth and the
10 lysine seed culture broth were inoculated at various ratios and fermented.

[00234] In main fermentation, 14 to 18 L of a fermentation medium was added to a 50-L fermenter and inoculated with 1.2 to 1.8 L of the total seed culture broth at an
15 inoculation ratio of 0.05 to 99.95 (inosinic acid seed culture broth): 99.95 to 0.05 (lysine seed culture broth), followed by mixed fermentation by fed-batch culture for 45 to 90 hours. The fermentation medium composition and fermentation conditions used here are shown in Table 16
20 below.

[00235] [Table 16]

Fermentation medium composition	1 to 3% molasses, 5 to 7% raw sugar, 2 to 3% yeast extract paste, 0.6 to 1.2% H ₃ PO ₄ , 0.05 to 0.1% betaine, 100 to 200 ppm adenine, 50 to 150 ppm guanine, 0.2 to
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	0.5% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 to 100 ppm Ca-pantothenate, 5 to 15 ppm vitamin B3, 5 to 20 ppm thiamine HCl, 0.4% $(\text{NH}_4)_2\text{SO}_4$, 0.4 to 0.8% NaOH, 5 to 10 ppm FeSO_4 , 10 to 20 ppm MnSO_4 , 10 to 20 ppm ZnSO_4 , and 0.005% antifoaming agent for food
Fermentation conditions	Temperature 31 to 32°C, pH 6.5 to 7.5, aeration rate 0.8 to 1.2 vvm, internal pressure 0.6 to 1.0 kg/cm ³ , agitation speed 320 to 350 rpm, and dissolved oxygen (DO) concentration 20 to 70%

[00236] This mixed fermentation was performed a total of three times, the average value was calculated, and the results are shown in Table 17 below.

[00237] [Table 17]

Inoculation ratio between seed culture broths (IMP: LYS)	Fermentation time	IMP: LYS ratio in fermentation ratio
99.95 : 0.05	90 hours	92.3 : 1
80 : 20	73 hours	1.01 : 1
50 : 50	61 hours	0.05 : 1
20 : 80	48 hours	0.02 : 1
0.05 : 99.95	45 hours	0.01 : 1

5 **[00238]** Referring to Table 17 above, it was confirmed that,

when the seed culture broths of the microorganisms were inoculated at a ratio of 0.05 to 99.95 (inosinic acid-producing microorganism): 99.95 to 0.05 (lysine-producing microorganism), IMP and lysine in the fermentation broth
5 were produced at a ratio of 0.01 to 92: 1.

[00239] Example 8. Comparison of inosinic acid-lysine fermented powders between fermentation processes

[00240] 8-1. Production of inosinic acid-lysine fermented powder

10 **[00241]** Conventionally, a flavor containing inosinic acid and lysine was produced by individually fermenting an inosinic acid-producing microorganism and a lysine-producing microorganism and then mixing the fermentation broths or dried products thereof at a suitable ratio. To compare the
15 differences in taste between this conventional individual fermentation method and the method based on mixed fermentation of the inosinic acid-producing microorganism and the lysine-producing microorganism, the components of inosinic acid-lysine (IMP-LYS) fermented powders obtained
20 by the production methods were compared (see FIG. 4).

[00242] The inosinic acid seed culture broth and lysine seed culture broth used here were prepared in the same manner as in Example 7-1.

[00243] ① Individual fermentation (Preparation Example 10)

25 **[00244]** In the method of mixing individual dried products

among the conventional methods, each of the inosinic acid seed culture broth and the lysine seed culture broth was transferred into a 50-L fermenter and then individually subjected to main fermentation. Next, cells were separated
5 from each fermentation broth, and then decolorization and filtration processes were performed. The filtrates were concentrated and dried to obtain dried products. The obtained dried inosinic acid and lysine products were mixed together so that the ratio of inosinic acid to lysine was
10 1:1, thereby preparing fermented powder containing inosinic acid and lysine.

[00245] ② Mixing of fermentation broths after individual fermentation (Preparation Example 11)

[00246] In the method of mixing individual fermentation
15 broths among the conventional methods, each of the inosinic acid seed culture broth and the lysine seed culture broth was transferred into a 50-L fermenter and then individually subjected to main fermentation. The fermentation broths obtained in the main fermentation were mixed together so
20 that the ratio of inosinic acid to lysine was 1: 1. Next, cells were separated from the fermentation culture broth, and then decolorization and filtration processes were performed. The filtrate was concentrated and dried to obtain inosinic acid-lysine fermented powder.

[00247] ③ Mixed fermentation (Preparation Example 12)

[00248] In mixed fermentation, the inosinic acid seed culture broth and the lysine seed culture broth were inoculated at a ratio of 80: 20 in the same manner as in Example 7-2 so that the ratio of inosinic acid to lysine in the fermentation broth was about 1:1 in the same manner as in Preparation Examples 10 and 11, followed by mixed fermentation. Next, cells were separated from the fermentation broth, and decolorization and filtration processes were performed. The filtrate was concentrated and dried to obtain inosinic acid-lysine fermented powder.

[00249] 8-2. Comparison of components between lysine-inosinic acid fermented powders

[00250] Component analysis was performed on the inosinic acid-lysine fermented powder obtained through individual fermentation in each of Preparation Examples 10 and 11 and the inosinic acid-lysine fermented powder obtained through mixed fermentation in Preparation Example 12.

[00251] IMP and L-lysine were measured by HPLC analysis (IMP - 254 nm, UV detector, flow rate 0.9 ml/min; LYS - 214 nm, UV detector, flow rate 0.8 ml/min). Organic acid and ions were measured in the same manner as in Example 2-2. The results are shown in Table 18 below.

[00252] [Table 18]

Item	Preparation Example 10	Preparation Example 11	Preparation Example 12
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LYS	26%	32%	38%
IMP	24%	30%	33%
(LYS+IMP) /TS*	45%	62%	71%
Total nitrogen	9.7%	11.3%	12.5%
Organic acids	5.0%	4.1%	3.7%
Ions	9.2%	8.2%	5.6%
Ammonium	3.9%	3.5%	1.9%
*(LYS+IMP) /TS: proportion of product relative to total solid			

[00253] Referring to Table 18 above, mixed fermentation (Preparation Example 12) showed a higher LYS+IMP proportion than individual fermentation (Preparation Examples 10 and 11) and showed significant decreases in the contents of organic acids, ions and ammonium.

[00254] 8-3. Sensory comparison between individual fermentation and mixed fermentation

[00255] Sensory evaluation was performed on the inosinic acid-lysine fermented powders obtained through individual fermentation in Preparation Examples 10 and 11 and the inosinic acid-lysine fermented powder obtained through mixed fermentation in Preparation Example 12.

[00256] Sensory evaluation was performed in the same manner as in Example 2-3. The results are shown in Table 19 below.

[00257] [Table 19]

Item	Preparation Example 10	Preparation Example 11	Preparation Example 12
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	(individual fermentation)	(individual fermentation)	(mixed fermentation)
Initial Umami taste	+	++	++
Umami persistence	+	+++	++++
Kokumi	+	++	+++
Salty taste	+	++	++
Sour taste	++	++	+
Bitter taste	++	++	-
Sweet taste	+	+	++

[00258] Referring to Table 19 above, due to the difference in components of the inosinic acid-lysine fermented powder as shown in Table 18 above, the sample prepared by the mixing process after individual fermentation showed weak umami persistence and an increased bitter taste compared to the sample prepared by mixed fermentation. In addition, since the increase in by-products such as organic acids and the increase in ions as shown in Table 18 above also affect the sensory properties of the sample, the mixed fermentation process is more effective in terms of taste or process simplification than mixing after individual fermentation.

[00259] **Example 9. Mixed fermentation of inosinic acid and arginine**

[00260] **7-1. Seed culture**

[00261] *Corynebacterium ammoniagenes* NFI545 (KCCM13162P) was used as an inosinic acid-producing microorganism, and *Corynebacterium glutamicum* NFA40 (KCCM13165P) was used as an arginine-producing microorganism.

5 **[00262]** An inosinic acid seed culture broth of the inosinic acid-producing microorganism and an arginine seed culture broth of the arginine-producing microorganism were prepared in the same manner as in Examples 5-1 and 3-1, respectively.

[00263] 9-2. Main fermentation

10 **[00264]** In order to examine the ratio of inosinic acid to arginine in the fermentation broth depending on the inoculums of the inosinic acid seed culture broth and the arginine seed culture broth, the inosinic acid seed culture broth and the arginine seed culture broth were inoculated
15 at various ratios and fermented.

[00265] In main fermentation, 14 to 18 L of a fermentation medium was added to a 50-L fermenter and inoculated with 1.2 to 1.8 L of the total seed culture broth at an inoculation ratio of 65 to 99.95 (inosinic acid seed
20 culture broth): 35 to 0.05 (arginine seed culture broth), followed by mixed fermentation by fed-batch culture for 45 to 80 hours. The fermentation medium composition and fermentation conditions used here are shown in Table 20 below.

25 **[00266]** [Table 20]

Fermentation medium composition	1 to 3% molasses, 5 to 7% raw sugar, 2 to 3% yeast extract paste, 0.6 to 1.2% H_3PO_4 , 0.05 to 0.1% betaine, 100 to 200 ppm adenine, 50 to 150 ppm guanine, 0.2 to 0.5% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 50 to 100 ppm Ca-pantothenate, 5 to 15 ppm vitamin B3, 5 to 20 ppm thiamine HCl, 0.4% $(\text{NH}_4)_2\text{SO}_4$, 0.4 to 0.8% NaOH, 5 to 10 ppm FeSO_4 , 10 to 20 ppm MnSO_4 , 10 to 20 ppm ZnSO_4 , and 0.005% antifoaming agent for food
Fermentation conditions	Temperature 31 to 32°C, pH 6.5 to 7.5, aeration rate 0.8 to 1.2 vvm, internal pressure 0.6 to 1.0 kg/cm ³ , agitation speed 320 to 350 rpm, and dissolved oxygen (DO) concentration 20 to 70%

[00267] This mixed fermentation was performed a total of three times, the average value was calculated, and the results are shown in Table 21 below.

[00268] [Table 21]

Inoculation ratio between seed culture broths (IMP: ARG)	Fermentation time	IMP: ARG ratio in fermentation broth
99.95 : 0.05	80 hours	95.1 : 1
80 : 20	46 hours	5.3 : 1
70 : 30	45 hours	2.45 : 1
65 : 35	45 hours	1.04 : 1

[00269] Referring to Table 21 above, it was confirmed that, when the seed culture broths of the microorganisms were inoculated at a ratio of 65 to 99.95 (inosinic acid-producing microorganism): 35 to 0.05 (arginine-producing microorganism), IMP and L-arginine in the fermentation broth were produced at a ratio of 1.04 to 95.1: 1.

[00270] Example 10. Comparison of inosinic acid-arginine fermented powders between fermentation processes

[00271] 10-1. Production of inosinic acid-arginine fermented powder

[00272] Conventionally, a flavor containing inosinic acid and arginine was produced by individually fermenting an inosinic acid-producing microorganism and an arginine-producing microorganism and then mixing the fermentation broths or dried products thereof at a suitable ratio. To compare the differences in taste between this conventional individual fermentation method and the method based on mixed fermentation of the inosinic acid-producing microorganism and the arginine-producing microorganism, the components of inosinic acid-arginine (IMP-ARG) fermented powders obtained by the production methods were compared (see FIG. 5).

[00273] The inosinic acid seed culture broth and arginine seed culture broth used here were prepared in the same manner as in Example 9-1.

[00274] ① Individual fermentation (Preparation Example 13)

[00275] In the method of mixing individual dried products among the conventional methods, each of the inosinic acid seed culture broth and the arginine seed culture broth was transferred into a 50-L fermenter and then individually subjected to main fermentation. Next, cells were separated from each fermentation broth, and then decolorization and filtration processes were performed. The filtrates were concentrated and dried to obtain dried products. The obtained dried inosinic acid and arginine products were mixed together so that the ratio of inosinic acid to arginine was 1:1, thereby preparing fermented powder containing inosinic acid and arginine.

[00276] ② Mixing of fermentation broths after individual fermentation (Preparation Example 14)

[00277] In the method of mixing individual fermentation broths among the conventional methods, each of the inosinic acid seed culture broth and the arginine seed culture broth was transferred into a 50-L fermenter and then individually subjected to main fermentation. The fermentation broths obtained in the main fermentation were mixed together so that the ratio of inosinic acid to arginine was 1: 1. Next, cells were separated from the fermentation broth mixture, and then decolorization and filtration processes were performed. The filtrate was concentrated and dried to

obtain inosinic acid-arginine fermented powder.

[00278] ③ Mixed fermentation (Preparation Example 15)

[00279] In mixed fermentation, the inosinic acid seed culture broth and the arginine seed culture broth were inoculated
5 at a ratio of 65: 35 in the same manner as in Example 9-2 so that the ratio of inosinic acid to arginine in the fermentation broth was about 1:1 in the same manner as in Preparation Examples 13 and 14, followed by mixed fermentation. Next, cells were separated from the
10 fermentation broth, and decolorization and filtration processes were performed. The filtrate was concentrated and dried to obtain inosinic acid-arginine fermented powder.

[00280] 10-2. Comparison of components between inosinic acid-arginine fermented powders

15 **[00281]** Component analysis was performed on the inosinic acid-arginine fermented powder obtained through individual fermentation in each of Preparation Examples 13 and 14 and the inosinic acid-arginine fermented powder obtained through mixed fermentation in Preparation Example 15.

20 **[00282]** L-arginine and IMP were measured by HPLC analysis (ARG - 195 nm, UV detector, flow rate 1 ml/min; IMP - 254 nm, UV detector, flow rate 0.9 ml/min). Organic acid and ions were measured in the same manner as in Example 2-2. The results are shown in Table 22 below.

25 **[00283]** [Table 22]

Item	Preparation Example 13	Preparation Example 14	Preparation Example 15
ARG	24%	31%	34%
IMP	24%	31%	33%
(ARG+IMP) / TS*	48%	62%	76%
Total nitrogen	9.7%	11.3%	14.1%
Organic acids	4.7%	3.8%	2.5%
Ions	7.5%	6.2%	3.8%
Ammonium	3.1%	2.5%	0.8%
*(ARG+IMP) / TS: Proportion of product relative to total solid content			

[00284] 10-3. Sensory comparison between individual fermentation and mixed fermentation

[00285] Sensory evaluation was performed on the inosinic acid-arginine fermented powders obtained through individual fermentation in Preparation Examples 13 and 14 and the inosinic acid-arginine fermented powder obtained through mixed fermentation in Preparation Example 15.

[00286] Sensory evaluation was performed in the same manner as in Example 2-3. The results are shown in Table 23 below.

10 **[00287]** [Table 23]

Item	Preparation Example 13 (individual fermentation)	Preparation Example 14 (individual fermentation)	Preparation Example 15 (mixed fermentation)
Initial Umami	+	++	++

taste			
Umami persistence	+	+++	++++
Kokumi	+	++	+++
Salty taste	+	++	++
Sour taste	++	++	+
Bitter taste	++	++	-
Sweet taste	-	-	-

[00288] Referring to Table 23 above, due to the difference in components of the inosinic acid-arginine fermented powder as shown in Table 22 above, the sample prepared by the mixing process after individual fermentation showed weak
5 umami persistence and an increased bitter taste compared to the sample prepared by mixed fermentation. In addition, since the increase in by-products such as organic acids and the increase in ions as shown in Table 22 above also affect the sensory properties of the sample, the mixed
10 fermentation process is more effective in terms of taste or process simplification than mixing after individual fermentation.

[00289] So far, the present invention has been described with reference to the preferred embodiments. Those of ordinary
15 skill in the art to which the present invention pertains will appreciate that the present invention may be embodied in modified forms without departing from the essential

characteristics of the present invention. Therefore, the disclosed embodiments should be considered from an illustrative point of view, not from a restrictive point of view. The scope of the present invention is defined by the
5 claims rather than the foregoing description, and all differences within the scope equivalent thereto should be construed as being included in the scope of the present invention.

[00290] [Accession Numbers]

10 **[00291]** Depository authority: Korean Culture Center of Microorganisms (KCCM)

[00292] Accession number: KCCM13162P

[00293] Deposit date: April 21, 2022

[00294]

15 **[00295]** Depository authority: Korean Culture Center of Microorganisms (KCCM)

[00296] Accession number: KCCM13163P

[00297] Deposit date: April 21, 2022

[00298]

20 **[00299]** Depository authority: Korean Culture Center of Microorganisms (KCCM)

[00300] Accession number: KCCM13164P

[00301] Deposit date: April 21, 2022

[00302]

25 **[00303]** Depository authority: Korean Culture Center of

Microorganisms (KCCM)

[00304] Accession number: KCCM13165P

[00305] Deposit date: April 21, 2022

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: **DAESANG**

120, Changgyeonggung-ro,
Jongno-gu,
Seoul, 03130,
Republic of Korea

RECEIPT IN THE CASE OF AN ORIGINAL
DEPOSIT issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Corynebacterium ammoniagenes</i> NFI545	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCMI3162P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on April. 21. 2022 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Culture Center of Microorganism Address: Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03461 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: April. 21. 2022.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.
Form BP/4 (single page)

The above translation proves that there is no difference from the content of the original text.

Jun 14, 2022

Patent Attorney Hyuk Park



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: **DAESANG**

120, Changgyeonggung-ro,
Jongno-gu,
Seoul, 03130,
Republic of Korea

RECEIPT IN THE CASE OF AN ORIGINAL
DEPOSIT issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Corynebacterium glutamicum</i> NFL21	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCMI3163P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on April. 21. 2022 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Culture Center of Microorganism Address: Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03461 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  International depositary Date: April. 21. 2022.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.
Form BP/4 (single page)

The above translation proves that there is no difference from the content of the original text.

Jun 14, 2022

Patent Attorney Hyuk Park



BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

To: **DAESANG**

120, Changgyeonggung-ro,
Jongno-gu,
Seoul, 03130,
Republic of Korea

RECEIPT IN THE CASE OF AN ORIGINAL
DEPOSIT issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Corynebacterium glutamicum</i> NFG6	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCMI3164P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I above, which was received by it on April. 21. 2022 (date of the original deposit). ¹	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on (date of the original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: Korean Culture Center of Microorganism Address: Yurim B/D 45, Hongjeonae-2ga-gil Seodaemun-gu SEOUL 03461 Republic of Korea	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  International Depositary Authority Date: April. 21. 2022.

¹ Where Rule 6.4(d) applies, such date is the date on which the status of international depositary authority was acquired.
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RECEIPT IN THE CASE OF AN ORIGINAL
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INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Corynebacterium glutamicum</i> NFA40	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: KCCMI3165P
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I above was accompanied by: ☐ a scientific description ☐ a proposed taxonomic designation (Mark with a cross where applicable)	
III. RECEIPT AND ACCEPTANCE	
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Jun 14, 2022

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WHAT IS CLAIMED IS:

1. A method for producing a flavor, the method comprising a step of inoculating a fermentation medium with
5 a first microorganism and a second microorganism and then producing a fermentation broth containing amino acid, nucleic acid and/or organic acid by fermentation of the microorganisms,

wherein the first microorganism and the second
10 microorganism produce different products and each produces one selected from the group consisting of amino acid, nucleic acid and organic acid.

2. The method of claim 1, wherein the amino acid is
15 at least one selected from the group consisting of L-glutamic acid, L-alanine, L-valine, L-leucine, L-isoleucine, L-proline, L-phenylalanine, L-tryptophan, L-methionine, L-glycine, L-serine, L-threonine, L-cysteine, L-asparagine, L-glutamine, L-aspartic acid, L-lysine, L-arginine, and L-
20 histidine.

3. The method of claim 1, wherein the nucleic acid is at least one selected from the group consisting of inosinic acid, guanylic acid, xanthylic acid, and salts

thereof.

4. The method of claim 1, wherein the organic acid is at least one selected from the group consisting of
5 succinic acid, malic acid, citric acid, acetic acid, lactic acid, fumaric acid, tartaric acid, ascorbic acid, gluconic acid, and salts thereof.

5. The method of claim 1, wherein the first
10 microorganism is a glutamic acid-producing microorganism, and the second microorganism is a lysine-producing microorganism, an arginine-producing microorganism, a histidine-producing microorganism, a tryptophan-producing microorganism, a glycine-producing microorganism, an
15 alanine-producing microorganism, a succinic acid-producing microorganism, a lactic acid-producing microorganism, a guanylic acid-producing microorganism, or an inosinic acid-producing microorganism.

20 6. The method of claim 1, wherein the first microorganism is an inosinic acid-producing microorganism, and the second microorganism is a lysine-producing microorganism, an arginine-producing microorganism, a histidine-producing microorganism, a tryptophan-producing

microorganism, a glycine-producing microorganism, an alanine-producing microorganism, a succinic acid-producing microorganism, a lactic acid-producing microorganism, or a guanylic acid-producing microorganism.

5

7. The method of claim 1, wherein the step comprises further inoculating the fermentation medium with a third microorganism, which produces a product different from products produced from the first microorganism and the
10 second microorganism and produces one selected from the group consisting of amino acid, nucleic acid and organic acid.

8. The method of claim 1 or 7, wherein the first
15 microorganism is a glutamic acid-producing microorganism, the second microorganism is a guanylic acid-producing microorganism, the third microorganism is an inosinic acid-producing microorganism.

20 9. The method of claim 1 or 7, wherein the first microorganism, the second microorganism and the third microorganism are microorganisms of the genus *Corynebacterium*.

10. The method of claim 1 or 7, wherein the first microorganism, the second microorganism and the third microorganism are in a seed culture broth state obtained by individual culture or co-culture.

5

11. The method of claim 1 or 7, wherein the step comprises adjusting the inoculum of each microorganism in order to control the ratio between the products of the microorganisms in the fermentation broth.

10

12. The method of claim 1 or 7, wherein the fermentation broth contains the products of the microorganisms in an amount of 3 to 90 wt% relative to the total solid content of the fermentation broth.

15

13. A method for producing a flavor containing L-glutamic acid and L-lysine, the method comprising a step of inoculating a fermentation medium with a glutamic acid-producing microorganism and a lysine-producing microorganism and then producing a fermentation broth containing L-glutamic acid and L-lysine by fermentation of the microorganisms.

20

14. The method of claim 13, wherein the glutamic acid-producing microorganism and the lysine-producing microorganism are microorganisms of the genus *Corynebacterium*.

5

15. The method of claim 13, wherein the glutamic acid-producing microorganism and the lysine-producing microorganism are in a seed culture broth state obtained by individual culture or co-culture.

10

16. The method of claim 13, wherein the step comprises adjusting the inoculum of each of the glutamic acid-producing microorganism and the lysine-producing microorganism in order to control the ratio of L-glutamic acid to L-lysine in the fermentation broth.

15

17. The method of claim 13, wherein the fermentation broth contains amino acids, including L-glutamic acid and L-lysine, in an amount of 3 to 90 wt% relative to the total solid content of the fermentation broth.

20

18. A flavor produced by the method of claim 1 or 7.

19. A flavor containing L-glutamic acid and L-lysine,
produced by the method of claim 13.

20. The flavor of claim 19, containing L-glutamic
5 acid and L-lysine in an amount of 3 to 90 wt% relative to
the total solid content of the flavor.

21. A food composition containing the flavor of
claim 18.

10

22. A food composition containing the flavor
containing L-glutamic acid and L-lysine according to claim
19.

15