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PATENT SPECIFICATION
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COMPLETE SPECIFICATION

Method for Seasoning Foodstuff

- We, TAKEDA PHARMACEUTICAL INDUSTRIES, LTD., of 27, Doshomachi 2-chome, Higashiku, Osaka, Japan, a body corporate organised under the laws of Japan, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—
- 10 The present invention relates to a method for seasoning foodstuffs.
- 15 It has been known widely that monosodium glutamate or sodium succinate has an ability to bring out the natural flavour of food, and both of these salts have been used for improving the taste or flavour of food, not only in the home and restaurants, but also in the processing of such things as wine, meats and canned goods. Sodium inosinate has also been known as a substance having a similar activity to these substances, though it has never been actually used.
- 25 The rich flavour of soup-stock of any kind is considered to be caused by the presence of soluble components therein, particularly by the above mentioned chemical compounds. Hydrolysates of animal or vegetable proteins, obtained by the action of acids or protease of microorganisms, are also known and used as chemical condiments for foodstuffs. Chemical condiments of this kind generally contain various amino acids as their chief components.
- 35 Each of the chemical condiments described above may be used independently, or may sometimes be used together with two or more kinds of such chemical condiments. For example, 5'-inosinic acid may sometimes be used in combination with monosodium glutamate. It is also known that chemical condiments are sometimes used together with a natural condiment such as dried bonito (so-called *Katsuobushi*) to improve the taste or flavour of foodstuffs. In this case, the improvement in the taste or flavour is deemed to originate from the synergy between the chemical condiments and 5'-inosinic acid contained in dried bonito.
- 50 All of these chemical condiments are useful for the improvement of the taste or flavour of foodstuff to varying degrees. In other words, each of the compounds has its own speciality in seasoning foodstuff.
- 55 It has been found by the present inventor that the taste or flavour of foodstuff can be improved or enhanced by the addition of 5'-guanylic acid, 5'-uridylic acid or 5'-cytidylic acid or their derivatives, whereas other nucleotides such as adenylic acid and thymidylic acid, 2'- or 3'-guanylic acid or 2'- or 3'-uridylic acid or their derivatives have no such activity. Furthermore the enhanced taste or flavour is far better than that brought by the addition of 5'-inosinic acid and smaller amounts of such 5'-nucleotides are necessary.
- 60 The present invention provides a process for improving the taste or flavour of foodstuffs which comprises adding to the foodstuff 5'-guanylic acid, 5'-xanthylic acid, 5'-uridylic acid or 5'-cytidylic acid, or their derivatives.
- 65 Examples of foodstuffs which can be improved are soups, sauces, pastes, sausages, canned foods, salads, boiled foods, pickles, alcoholic drinks, vinegars, table salt, breads, fruit juices or fermented foodstuffs.
- 70 The 5'-nucleotides can, for example, be prepared by the process of our copending Applications Nos. 10016/59 and 23287/60 (Serial Nos. 912,790 and 933,826).
- 75 It is known that 5'-guanylic acid or 5'-uridylic acid or 5'-cytidylic acid can be obtained from a hydrolysate of nucleic acids or oligonucleotides. The hydrolysis can be effected by means of the enzyme system of snake venom or of a microorganism. The enzyme system of snake venom is difficult to obtain, and therefore it is rather preferred
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- [Price 4s. 6d.]

able to utilise that of microorganisms. The microorganisms capable of producing an enzyme system which can hydrolyse nucleic acid or oligonucleotides into the 5-nucleotides are distributed among those belonging to the classes *Schizomycetes* and *Ascomycetes*, more particularly microorganisms belonging to class *Fungi Imperfecti* (e.g. genus *Fusarium*, *Verticillium*, *Gliomastix* or *Helminthosporium*), order *Eubacteriales* (e.g. *Bacillus*), order *Actinomycetales* (e.g. genus *Streptomyces*), order *Sphaeriales* (e.g. genus *Anixiella*, *Botryosphaeria*, *Chaetomidium*, *Glomerella*, *Neurospora*, *Ophiobolus*, *Ophiostoma*, *Sordaria* or *Tilacclidium*) and order *Plectascales* (e.g. genus *Aspergillus*).

Nucleic acids or oligonucleotides can be hydrolysed by the enzyme system of such a microorganism as above. The hydrolysis may be effected by (1) incubation of the microorganism on a nucleic acid- and/or oligonucleotide-containing medium, (2) reaction between nucleic acid and/or oligonucleotide and filtered broth of the cultures of the microorganism, or (3) reaction between nucleic acid or oligonucleotide, and a purified enzyme. Of these, the second process is the most preferable for an industrial purpose. In this, the microorganism is incubated by aerobic submerged culture in an aqueous medium at an adequate temperature for sufficient time to accumulate the desired enzyme system, the culture is filtered to obtain a crude enzymatic solution, the enzymatic solution is added to nucleic acid or oligonucleotides, then the mixture is kept standing at an adequate temperature for sufficient time to complete the desired hydrolysis. When the enzymatic solution is contaminated with phosphomonoesterase, the desired 5'-ribonucleotides are further hydrolysed into corresponding ribonucleotides, so that the addition of a phosphomonoesterase-inhibitor such as arsenates, phosphates or cyanates, to the reaction mixture is necessary. Thus, in the reaction mixture, the desired 5'-guanylic acid and/or 5'-uridylic acid is produced together with other nucleotides such as 5'-adenylic acid and 5'-thymidylic acid which have no desired seasoning activity. The reaction mixture contains, as mentioned above, other inactive nucleotides, and further contains various impurities originating from culture process. Of course, 5'-guanylic acid or 5'-uridylic acid or 5'-cytidylic acid to be used for the purpose of this invention need not be in a pure state, but it may be used even in a considerably impure state so long as it is not contaminated by a component or components having unpleasant or undesirable odour or taste or by toxic or harmful components.

The purification of the 5'-ribonucleotides for use in the present invention may be conducted by means of a strongly acidic

cation exchange resin. Such a resin can adsorb 5'-guanylic acid, 5'-uridylic acid, and 5'-cytidylic acid, and the eluate therefrom with an aqueous solvent contains 5'-guanylic acid and/or 5'-uridylic acid and/or 5'-cytidylic acid. On the other hand, the reaction mixture can be treated with a weakly acid ion exchange resin to remove impurities by adsorption, and the matter left unadsorbed contains 5'-guanylic acid, 5'-uridylic acid and 5'-cytidylic acid. From these solutions, crude 5'-guanylic acid, 5'-uridylic acid and 5'-cytidylic acid can be collected by concentration separately or in combination, the solution may be neutralised to obtain alkali metal salts of 5'-guanylic acid or 5'-uridylic acid or 5'-cytidylic acid. Alternatively, the reaction mixture may be processed by means of electrophoresis using an ion exchange resin membrane to purify 5'-guanylic acid, 5'-uridylic acid or 5'-cytidylic acid.

Further purification may be conducted by adsorption or partition chromatography on an adequate adsorbent such as active charcoal, silica gel or cellulose powder.

5'-guanylic acid, 5'-uridylic acid or 5'-cytidylic acid can be used for the purpose of this invention as its free acid, alkali metal salt, alkaline earth metal salt, ammonium salt, histidine salt, acid amide, for instance. As stated above, 5'-guanylic acid, 5'-xanthylic acid, 5'-uridylic acid or 5'-cytidylic acid, or salts thereof can be utilised containing impurities having no unpleasant odour or taste or toxicity or harmfulness. As impurities which may be allowed to be contained are inorganic salts, nucleotides or nucleosides such as adenylic acid, thymidylic acid and inosinic acid. Thus a crude hydrolysis of nucleic acid or of oligonucleotide from which undesirable components are removed may also be used in the present invention.

Although 5'-xanthylic acid has never been detected as a component of natural nucleic acid, it can be derived from 5'-guanylic acid. The semi-synthesis of 5'-xanthylic acid from 5'-guanylic acid may for example be effected as follows:— 5'-guanylic acid is first converted into its 2-diazonium salt by means of nitrite and an acid, then the diazonium salt is hydrolysed with an acid to form a hydroxyl group. 5'-xanthylic acid seems to be a little weaker than 5'-guanylic acid in the activity of improving the taste or flavour of foodstuff. But the taste or flavour so improved or enhanced with 5'-xanthylic acid is reasonably good.

These 5'-nucleotides used are stable against heat, and therefore it is convenient to isolate or to collect them from the hydrolysate of nucleic acid and to cook or to store the foodstuffs to which the chemical condiment or condiments are added.

The chemical condiments of this inven-

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tion may be added to improve or enhance the taste or flavour of foods during or after the cooking. In other words, they can be added to raw foodstuffs or already cooked foodstuffs.

The quantity of the chemical condiments to be added to foodstuff can easily be determined according to their form, kind and other conditions of foodstuff. However, a slight excess of the condiments hardly gives any unpleasant taste to the foodstuff prepared. The 5'-nucleotides of the present invention are preferably used in a concentration of around 0.007%, and for a liquid foodstuff, from 0.1 to 0.001 per cent by weight is suitable.

Among these 5'-ribonucleotides, the ability of improving or enhancing the flavour of foodstuffs of 5'-guanylic acid is the strongest, and of the other 5'-ribonucleotides, 5'-xanthylic acid, 5'-uridylic acid and 5'-cytidylic acid, are rather weaker. 5'-xanthylic acid, 5'-uridylic acid and 5'-cytidylic acid, however, play an important role that these support the action of 5'-guanylic acid on foodstuffs. For example, if the taste or flavour of a foodstuff to which 5'-guanylic

acid is added is compared with that of the same foodstuff to which 5'-uridylic acid and 5'-cytidylic acid are added together with 5'-guanylic acid (the ratio of these three components in moles is 1:1:1), the latter foodstuff is more tasty than the foodstuff simply flavoured with 5'-guanylic acid only. Namely, it seems that the use of a mixture of these 5'-ribonucleotides gives a better taste or flavour to foodstuffs than the use of only one.

It is noticeable that the taste of 5'-guanylic acid is sensitive in smaller portions compared with 5'-inosinic acid which is known as a chemical condiment. For example, the stimulus thresholds in water of respective sodium salts of 5'-guanylic acid and 5'-inosinic acid are measured as 0.01 per cent and 0.05 per cent. That is, the stimulus threshold of the former is about one-fifth of that of the latter.

The adequate concentrations of sodium salt of 5'-guanylic acid to be added to several kinds of foodstuffs are listed as follows in comparison with those of respective sodium salts of 5'-inosinic acid and glutamic acid.

Foodstuffs	5'-guanylic acid	Sodium salt of 5'-inosinic acid	Glutamic acid
Soy - - - -	0.03%	0.045%	0.3%
Soups - - - -	0.03	0.045	0.1
Bean paste soup - - - -	0.05	0.075	0.1
Vinegared dish - - - -	0.1	0.15	—
Sake - - - -	0.007	0.011	—

The taste or flavour of the 5'-nucleotides of the present invention resembles that of 5'-inosinic acid, but the effectiveness is several times as strong as that of 5'-inosinic acid. Therefore, in most cases, the quantity of 5'-guanylic acid to be added to foodstuff may be much less than that of 5'-inosinic acid.

The effectiveness of the chemical condiments of this invention seems to be enhanced when they are used together with known chemical condiments such as monosodium glutamate, 5'-inosinic acid and sodium succinate. And, the taste or flavour of foodstuff improved or enhanced with known condiments containing amino acids can further be made better by the addition of the chemical condiments of this invention.

The addition of the 5'-nucleotides of the present invention to fermented foodstuff should be effected taking into consideration the existence of phosphomonotesterase therein. The enzyme has an ability to hydrolyse 5'-guanylic acid, etc. into corresponding ineffective nucleosides. The addition, therefore, should be carried out after removing or destroying such an enzyme.

The following examples further illustrate the present invention:—

EXAMPLE 1

A mixture composed of 4 kilograms of brewery yeast and 32 litres of 3% salt solution containing 0.16 per cent of sodium hydroxide is heated at 98° C. for 20 minutes to obtain an aqueous extract composed of nucleic acids. The extract is hydrolysed with a filtered broth of a strain of *Streptomyces aureus* (Waksman et Curtis) Waksman et Henrici, IFO 3303 identical with ATCC 13357 (IFO and ATCC are deposit number of the strain at Institute for Fermentation, Osaka, Japan and American Type Culture Collection, Washington, D.C., U.S.A., respectively), which contains phosphodiesterase and adenylic deaminase. The hydrolysate is passed through a layer of active charcoal to adsorb nucleotides. The active charcoal is eluted with ammoniacal water, then the eluate is concentrated to dryness to obtain a powdery condiment composed of about 50 per cent each of 5'-guanylic acid and 5'-inosinic acid.

Raw soya was brewed as follows:—

A mixture of boiled soybean and crushed wheat (about 50:50 by weight) is mixed well with melted rice and an equal amount of saline solution (about Bé 19°) is added. The mixture is kept in a large container

with occasional agitation for about 1 year at room temperature or for 3 to 4 months at higher temperature, whereby proteins and carbohydrates in the materials are degraded by the enzymes in the malted rice. Then the mush is filtered using a filter press, and heated at 60—70° C. for 30 minutes to destroy the enzymes in the product. (Malted rice is a crude culture of *Aspergillus* moulds on boiled rice). 2 grams of the condiment prepared as above are dissolved in 1 litre of the raw soya to bring out the better taste or flavour of the soya.

EXAMPLE 2

Refined *sake* which has been separated from lees is heated to destroy enzymatic impurities. To 1 liter of the so treated *sake* there is added 0.14 gram of the chemical condiment obtained in Example 1 to enhance the flavour of the refined *sake*.

EXAMPLE 3

150 grams of potato, 100 grams of onion and 50 grams of carrot are cut into small cubes. They are then boiled with 500 millilitres of water. Then, the mixture is allowed to simmer until the vegetables become macerated. The soup is diluted with hot water to 1 litre, and about 8 grams of table salt are added. To this vegetable soup, there are added 0.1 gram of the chemical condiment obtained in Example 1 and 0.5 gram of monosodium glutamate, whereupon the taste or flavour of the soup is improved.

EXAMPLE 4

To 1 litre of the vegetable soup prepared as in Example 3 are added 700 milligrams of sodium 5'-xanthylate and the mixture is cooked whereby the flavour of the vegetable soup is improved.

EXAMPLE 5

To 1 litre of the vegetable soup prepared as in Example 3 are added 400 milligrams of sodium 5'-guanylate and the mixture is cooked to improve the flavour of the soup.

EXAMPLE 6

To 1 litre of the vegetable soup prepared as in Example 3 there are added 400 milligrams of sodium 5'-guanylate and 200 milligrams of sodium 5'-uridylic acid, the mixture is cooked, whereupon the taste or flavour of the soup is improved.

WHAT WE CLAIM IS:—

1. A process for enhancing the flavour of a foodstuff, which comprises adding to the foodstuff at least one of the following, namely 5'-guanylic acid, 5'-uridylic acid, 5'-cytidylic acid or 5'-xanthylic acid or a non-toxic amide or salt thereof.

2. A process as claimed in Claim 1, wherein sodium glutamate is also added.

3. A process as claimed in Claims 1 or 2, wherein the 5'-nucleotide is added as the sodium salt.

4. A process as claimed in Claims 1 to 3, wherein the 5'-nucleotide is added in an amount of from 0.1 to 0.001% by weight of the foodstuff, in the case of a liquid foodstuff.

5. A process as claimed in Claims 1 to 4, substantially as described with reference to any of the examples.

6. Foodstuffs when treated by a process as claimed in any of the preceding claims.

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PATENTS ACT, 1949

SPECIFICATION NO. 933,827

Reference has been directed, in pursuance of Section 9, subsection (1) of the Patents Act, 1949, to Patent No. 898,335.

THE PATENT OFFICE,

ERRATUM

SPECIFICATION NO. 933,827

AMENDMENT NO. 1

Page 1, line 15, for "if food" read "of food"

THE PATENT OFFICE,
12th January, 1966

D 60482/15



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