

PATENT SPECIFICATION

NO DRAWINGS

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Date of Application and filing Complete Specification: Aug. 16, 1957.

No. 25986/57.

Complete Specification Published: April 6, 1961.



April

Index at acceptance:—Class 49, B6G.

International Classification:—A23I.

COMPLETE SPECIFICATION

A process for the Production of Seasoning Materials containing Glutamic Acid or a Derivative thereof as the Main Constituent

We, AJINOMOTO KABUSHIKI KAISHA and SANRAKU SHUZO KABUSHIKI KAISHA, corporations organized under the laws of Japan, both residing at No. 7, 1-chome, Takaracho, Chuoku, Tokyo, 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:—

This invention relates to a process for producing seasoning materials containing glutamic acid, which is an amino acid, or a derivative thereof, as the main constituent.

It is known that α -keto acids, such as α -ketoglutaric acid, oxalacetic acid and pyroglutonic acid, can be produced as intermediate products or final products in the metabolism of sugars by the action of various microorganisms, particularly those belonging to the genera *Pseudomonas*, *Aerobacter*, *Bacillus*, *Serratia*, *Lactobacillus*, *Acetobacter*, and *Gluconobacter*. However, these keto acids have not been utilized for commercial purpose because they are unstable biochemically (they are transformed in the metabolising process) and they are difficult to separate from the fermentation liquor.

It has also been widely known theoretically that α -keto acids combine with NH_3 (in the form of ammonium salts) in the metabolising process by the action of microorganisms to form α -amino acids, and synthesis of protein is effected, and it is now a well recognized theory that the growth of the microorganism body results from the presence of the sugar and the ammonium salts.

However, there have been very few instances, if any, in which study and investigation have been made as to the metabolism of ammonia occurring together with the metabolism of sugar by adding ammonium salts, urea,

nitrites or nitrites in an excess of the amount necessary for the growth of the microorganism, and no attempt to produce a seasoning material by such a process has previously been made.

We have attempted to utilize the theory of protein synthesis, that α -keto acids are transformed to α -amino-acids, for the preparation of glutamic acid, the most important constituent of the seasoning material contemplated in this invention, and we have found, in cultivating one or more microorganisms which have a particular propensity for producing α -ketoglutaric acid in a medium containing a sugar material such as saccharides and a nitrogen source such as ammonium salts, urea, nitrates and nitrites, that the growth and multiplication of the microorganism is not increased above a certain limit even with the addition of further nitrogen compound, but that the consumption of the nitrogen compound added to the medium is increased greatly with the further addition of the nitrogen compound. In other words, the added nitrogen compound is only partly consumed for the growth of the microorganism and the excess of the nitrogen compound is metabolised separately and amino acid is formed.

It is generally sufficient to have 0.1—0.2% of ammonium sulphate as the nitrogen source in a cultivation medium for the growth of the microorganisms or for the purpose of general fermentation, and it has been hitherto considered useless or harmful to use more than this amount.

According to the present invention there is provided a process for producing a seasoning material containing glutamic acid or a derivative thereof as the main constituent, which comprises preparing a cultivation medium from a sugar material and a nitrogen source, inoculating the medium with microorganisms of

[Price 3s. 6d.]



f/ one or more of the genera *Pseudomonas*, *Acetobacter*, *Aerobacter*, *Serratia*, *Escherichia*, *Bacillus*, *Gluconobacter*, *Acetogluconobacter*, *Gluconoacetobacter*, *Saccharomyces*, and *Aspergillus*, cultivating the microorganisms in the medium and thereby producing α -ketoglutaric acid, there being present an excess of the nitrogen source over the amount required for the cultivation of the microorganisms, which excess of the nitrogen source promotes the production of α -ketoglutaric acid and facilitates amination by the action of the microorganisms of the α -ketoglutaric acid to glutamic acid, and thereafter refining the glutamic acid by a conventional method.

The nitrogen compound is generally used in an amount of 0.5–8.0% by weight and in some cases it is present in a proportion as high as 15% in the cultivation medium.

The seasoning material containing glutamic acid may be refined by a conventional method such as filtration and concentration, or the glutamic acid may be separated and purified by treatment with hydrochloric acid or with ion exchange resins, and then with caustic soda to give monosodium glutamate.

As stated above, the excess of nitrogen compound is not used for the cultivation of the microorganisms as their nutritive, the growth of the microorganisms being suspended at a certain limit, but is used exclusively for the continuation of the production of α -ketoglutaric acid which is thereafter transformed into glutamic acid. The following test was carried out in order to ascertain the relation between the

quantity of added nitrogen material, the growth of the microorganism, and the amount of glutamic acid produced.

TEST 1

Cultivation media were prepared, each comprising glucose 8%, potassium dihydrogen phosphate (KH_2PO_4) 0.05%, potassium hydrogen phosphate (K_2HPO_4) 0.05%, magnesium sulphate dihydrate 0.03%, and 0.001% of each of sodium chloride, calcium chloride, manganese chloride and copper sulphate. To several cultivation media prepared as above was added ammonium sulphate in the following percentages 0.01, 0.03, 0.05, 0.1, 0.2, 0.3, 0.5, 1.0, 3.0, 5.0, 6.0 and 8.0. The prepared mediums were all sterilized by heating at 130° C. for 20 minutes before use. The microorganisms used were:

(a) *Aerobacter cloacae* and *Aerobacter aerogenes*,

(b) *Acetobacter aceti*, *Gluconobacter cerinus* and *Gluconobacter scleroideus*, and

(c) *Pseudomonas fluorescens* and *Serratia marcescens*.

Cultivation was carried out under aerated conditions for 48 hours at 32° C. and the medium was left to stand for 12 hours and was then decanted. The bacterial body was separated by centrifuging. The amount of medium employed in each test was 10 litres.

In the following table the mark “—” indicates that no trace of glutamic acid was detected by paper chromatography while the mark “±” indicates that a trace of glutamic acid was detected by paper chromatography.



55 (h)

60

65

70

Bacteria Ammonium sulphate (%)	Glutamic acid produced (% of the hydrochloride salt)			Bacterial body present (% based on dry weight)		
	(a)	(b)	(c)	(a)	(b)	(c)
0.01	—	—	—	0.017	0.019	0.008
0.03	—	—	—	0.050	0.062	0.031
0.05	±	—	—	0.071	0.081	0.068
0.1	0.03	—	±	0.113	0.134	0.101
0.2	0.12	±	0.04	0.165	0.153	0.114
0.3	0.24	0.19	0.13	0.159	0.160	0.125
0.5	0.75	0.81	0.53	0.174	0.168	0.122
1.0	1.07	1.12	0.92	0.146	0.167	0.110
3.0	1.15	1.24	1.08	0.157	0.169	0.113
5.0	1.23	1.29	1.16	0.160	0.176	0.104
6.0	1.20	1.36	1.2	0.145	0.152	0.098
8.0	0.98	1.19	1.05	0.119	0.135	0.076

It can be seen from the above table, that the growth and multiplication of bacteria was substantially suspended when the amount of ammonium sulphate present had reached a certain value, and that the production of glutamic acid did not generally commence until the amount of ammonium sulphate present reached that value, and that the production of glutamic acid then increased as the amount of ammonium sulphate employed was increased.

The following test was carried out in order to determine the relation between the quantities of α - ketoglutaric acid and glutamic acid produced in a certain period of time.

TEST 2

Several cultivation media were prepared, similar to those employed in Test 1, except that saccharified starch liquor at the rate of reduced sugar 5.4% was used in place of the glucose used in Test 1, and ammonium sul-

phate was added in the amount shown in the following table. The microorganisms used were:

A. *Aerobacter cloacea* and *Serratia marcescens*,

B. *Acetobacter aceti*, *Acetogluconobacter dioxyacetomicus* and *Saccharomyces cerevisiae*, and

C. *Pseudomonas fluorescens* and *Pseudomonas ovalis*.

Cultivation was carried out with 20 litre portions of the cultivation medium at 32° C. with shaking and standing and samples were taken from time to time.

The bacterial body was suspended in water by subjecting the medium to centrifuging, and the amount of the bacterial body was estimated by a photonephelometer, the value set forth in the following table having been adjusted on the basis of the dried weight of the bacterial body.

Content		Glutamic acid (%)				α -ketoglutaric acid (%)				Bacterial body (%) based on dry weight			
Microorganism		A	B	C		A	B	C		A	B	C	
Ammonium Sulphate (%)		2	2	2	0.2	2	2	2	0.2	2	2	2	0.2
Time	15 hours	0.02	±	±	—	0.01	0.02	±	0.01	0.04	0.05	0.02	0.03
	30 "	0.25	0.17	0.30	±	0.23	0.31	0.12	0.18	0.12	0.10	0.11	0.13
	50 "	0.96	0.24	0.71	0.01	0.72	0.98	0.03	0.74	0.14	0.14	0.15	0.15
	75 "	1.53	1.33	1.08	0.01	0.18	0.20	0.01	0.95	0.14	0.15	0.16	0.15
	100 "	1.60	1.52	1.45	0.02	0.02	0.01	±	1.02	0.14	0.15	0.16	0.16
	125 "	1.61	1.53	1.47	0.03	±	—	±	1.02	0.13	0.15	0.15	0.16

It can be seen from the above that glutamic acid was only produced in negligible amounts when 0.2% of ammonium sulphate was present in the cultivation medium, whereas when 2.0% of ammonium sulphate was present, the production of glutamic acid and the production of α -ketoglutaric acid increased for up to 50—75 hours of cultivation, and thereafter the production of glutamic acid increased, with the consumption of α -ketoglutaric acid, the concentration of which correspondingly decreased. Thus the yield of glutamic acid is remarkably increased when ammonium sulphate is present in the larger amount.

It can be seen from the table that the growth of the microorganism, i.e. the increase of bacterial body, was substantially the same irrespective of whether the quantity of ammonium sulphate present was 2% or 0.2%, but that the amount of glutamic acid produced, when 2% of ammonium sulphate is present, was greatly increased, since the major portion of the α -ketoglutaric acid produced was transformed into glutamic acid. In the table, the values for the production of α -ketoglutaric acid represent the amounts remaining after the transformation to glutamic acid and do not represent the actual values of α -ketoglutaric acid produced. However, taking the quantity of glutamic acid produced from the α -ketoglutaric acid into account, it may be seen that the total production of α -ketoglutaric acid is greater by about 50—60% in the case in which 2% ammonium sulphate was present compared with the case in which 0.2% ammonium sulphate was present, and this increase in the total production of α -ketoglutaric acid contributes to the increase of the production of glutamic acid.

The following Examples illustrate the invention:

EXAMPLE 1

10% of ammonium sulphate were added to an aqueous solution containing 10% of sugar molasses, and the mixed liquor was inoculated with *Aerobacter cloacae* and *Pseudomonas fluorescens* and cultivated for 7 days at 33° C. A seasoning material of good taste was obtained after refining the cultivated liquor by filtration and concentration.

EXAMPLE 2

10 litres of a cultivation medium containing 5% of urea and 8% of sucrose, together with 0.1% potassium dihydrogen phosphate (KH_2PO_4) and 0.05% of magnesium sulphate as nutritive was prepared. The quantity of urea present in this medium corresponds to about 2.3% of nitrogen. *Aerobacter cloacae* and *Gluconoacetobacter roseus* were inoculated into the cultivation medium and cultivated under aerated condition at 30° C. for 72 hours. The cultivated liquor was concentrated without removing the bacterial body, and then treated with concentrated hydrochloric acid. Glutamic acid was separated as the hydrochloride salt

according to conventional methods and 105.6 g. of the product was obtained.

When the medium containing the bacterial bodies was separately examined, it was estimated that it contained only 18.5 g. of bacterial bodies, calculated on the basis of the dried weight, and thus the glutamic acid was not produced as a result of the presence of the bacterial bodies, but was produced biochemically in the medium from the excess urea.

EXAMPLE 3

A cultivation medium was prepared by adding 1500 g. of ammonium sulphate (containing 318 g of nitrogen), and 500 g. of calcium carbonate to 10 l. of a wort solution (containing 10% by weight of sugar). To this cultivation medium a piece of cheese (weighing about 1 g.) was added, and cultivated at 33° C. In the resulting liquor, the presence of *Pseudomonas fluorescens*, *Aerobacter cloacae*, *Aerobacter aerogenes*, *Saccharomyces cerevisiae*, *Saccharomyces ellipsoideus*, *Aspergillus niger*, *Bacillus subtilis* and *Bacillus coagulans* was detected.

The content of the nitrogen of the ammonium compound was estimated from time to time during 15 days cultivation and it was found that the content of nitrogen was minimum on the 13th day. By suspending cultivation at this minimum point, removing calcium carbonate and calcium sulphate produced, concentrating the cultivated liquor, removing calcium ions and excess ammonium ions by adding sodium carbonate, neutralizing the liquor by adding hydrochloric acid, and concentrating the neutralized liquor, a material of good taste suitable for seasoning was obtained. In this operation, 67.4% of the nitrogen of the ammonium compound was consumed and the proportion of nitrogen in the amino-compound obtained corresponded to 34% of the total amount of nitrogen originally added, about half of the total amount of nitrogen metabolised (67.4%) being assimilated in the amino-compound. Hence a major portion of the excess nitrogen originally added is metabolised, and a major portion of the metabolised nitrogen is accumulated as amino acid in the cultivated liquor.

EXAMPLE 4

Aerobacter aerogenes was inoculated into a cultivation medium containing 5% of glucose, 0.1% of potassium hydrogen phosphate (K_2HPO_4), 0.05% of magnesium sulphate, and 2% of sodium nitrate as a nitrogen source, and was cultivated at 30° C. for 5 days. The cultivated medium was then analyzed and was found to contain 1.50% of glutamic acid (about 30% of sugar). For the purpose of comparison, a further cultivation medium was prepared, containing 1.5% of ammonium chloride in place of sodium nitrate (the corresponding amount of nitrogen being the same in both cases, namely 0.33%). The same microorganism was inoculated into the second medium

and a similar cultivation was carried out, the yield of glutamic acid being 1.48%.

EXAMPLE 5

5 A cultivated medium was prepared by adding 0.1% of potassium hydrogen phosphate (K_2HPO_4), 0.04% of magnesium sulphate and 2% of sodium nitrate to saccharified starch liquor (5% sugar), and was inoculated with *Pseudomonas fluorescens* and then cultivated as described in Example 4. On the fifth day of cultivation the yield of glutamic acid (25% of sugar) was 1.25%.

EXAMPLE 6

15 A cultivation medium was prepared by adding 0.75% of ammonium chloride and 1% of sodium nitrate as nitrogen sources to a liquor containing 5% of sucrose, 0.1% of potassium dihydrogen phosphate (KH_2PO_4) and 0.05% of magnesium sulphate, and was inoculated with *Serratia marcescens* and *Bacillus subtilis* and then cultivated at 33° C. for 5 days. After completion of fermentation 1.70% of glutamic acid (34% of sugar) was produced in the cultivated liquor.

WHAT WE CLAIM IS:—

25 1. A process for producing a seasoning material containing glutamic acid or a derivative thereof as the main constituent, which comprises preparing a cultivation medium from a sugar material and a nitrogen source, inoculating the medium with microorganisms of one or more of the genera *Pseudomonas*, *Acetobacter*, *Aerobacter*, *Serratia*, *Escherichia*, *Bacillus*, *Gluconobacter*, *Acetogluconobacter*, *Gluconoactobacter*, *Saccharomyces*, and *Aspergil-*

lus, cultivating the microorganisms in the medium and thereby producing α -ketoglutaric acid, there being present an excess of the nitrogen source over the amount required for the cultivation of the microorganisms, which excess of the nitrogen source promotes the production of α -ketoglutaric acid and facilitates amination by the action of the microorganisms of the α -ketoglutaric acid to glutamic acid, and thereafter refining the glutamic acid by a conventional method.

2. A process according to Claim 1, in which the nitrogen source is an ammonium salt, ammonia, urea, a nitrate or a nitrite, or two or more thereof.

3. A process according to Claim 1 or 2, in which cultivation takes place at a temperature between 10 and 50° C.

4. A process according to any preceding claim, in which the nitrogen source contains between 0.5% and 8% by weight, based on the weight of the cultivation medium, of nitrogen.

5. A process for producing a seasoning material containing glutamic acid or a derivative thereof as the main constituent, substantially as described in any of the foregoing Examples.

6. A seasoning material containing glutamic acid or a derivative thereof as the main constituent, whenever produced by a process claimed in any preceding claim.

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