

PATENT SPECIFICATION

DRAWINGS ATTACHED

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COMPLETE SPECIFICATION

Process for Recovering Glutamic Acid from Fermentation Broth

5 We, AJINOMOTO CO., INC., a corporation organized and existing under the laws of Japan and located at No. 7, Takaracho 1-Chome, Chuo-ku, 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:—

10 This invention relates to a process for recovering glutamic acid or its salts (hereinafter referred to as "glutamic acid") from fermentation broth.

15 Glutamic acid fermentation broth always contains difficulty filtrable cells and carbohydrates remaining after the fermentation. The cells become pseudo nuclei of glutamic acid crystals which deteriorate the crystal habit and intermix with crystallised glutamic acid to degrade its purity. The remnant carbohydrates also counteract the crystallisation and the growth of crystals to form such minute glutamic acid crystals of extremely inferior crystal habit that the separation of crystals is hard to carry out on an industrial scale. The "inferior crystal habit", as used in this specification, means the formation of minute crystals of needle-, spindle- or thin tabular shape of less than 10 μ or an amorphous deposit. Previously, the separation of both the cells and the remnant carbohydrates have been believed to be indispensable in the treatment of glutamic acid fermentation broth.

20 We have found that the principal cause of such obstruction of crystallisation by the bacterial cells lies in the heat-treatment for sterilisation and concentration of the broth after fermentation, the bacterial cells denatured by said heat-treatment are apt to become pseudo nuclei of glutamic acid crystals, the partly dispersed cells formed by said heat-treatment especially retard the growth of crystals and that, when the broth is heated, the sedimentation velocity of the cells increases to

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such an extent that their separation from glutamic acid crystals becomes extremely difficult.

25 The present invention provides a process for recovering glutamic acid from fermentation broth, which comprises the steps of (i) treating the fermentation broth (which has been neither sterilised nor concentrated by heating and which contains bacterial cells) with acid-treated and concentrated mother liquor obtained from a previous operation (ii) adjusting the pH to 2.5—3.5, (iii) subjecting the solution, still containing the cells in suspension, to crystallisation and separation of glutamic acid, (iv) treating the mother liquor—still containing the cells—with acid (to convert glutamic acid in the non-proper form to glutamic acid in the proper form, as hereinafter defined, and simultaneously to hydrolyse the protein of cells into glutamic acid and other amino acids), filtering off the huminous material, concentrating the solution and circulating the concentrated solution into fermentation broth for the next operation.

30 The term "glutamic acid in the proper form", as used herein, means glutamic acid, glutamates, (e.g. sodium glutamate) and inorganic salts thereof (e.g. glutamic acid hydrochloride), whereas "glutamic acid in the non-proper form" means compounds capable of being converted to glutamic acid in the proper form under the conditions of step (iv) of the invention (e.g. pyroglutamic acid, glutamine, glutamic acid combined in protein and glutamic acid amide).

35 The essential features of this invention are as follows:—

40 (1) The relations between the ratio of sedimentation velocities of the cells in a broth containing 4% of wet cells just after the fermentation and the several broths just after the heat-treatment for sterilisation under various conditions are shown in Table 1.

TABLE 1

	Before heat- treatment	After heat-treatment under the conditions of			Reference crystals
		80° C., 10 min.	100° C., 10 min.	115° C., 10 min.	
Ratio of sedimentation velocities	100	130	150	160	180

Thus the sedimentation velocity of the cells tends to approach that of glutamic acid crystals when the broth is heated. The difference between the sedimentation velocity of glutamic acid crystals and that of the cells in the heated broth is further decreased, if the broth is adjusted to approximately pH 3 which is necessary for crystallisation of glutamic acid. The accompanying drawing illustrates how the sedimentation velocities of cells and crystals approach each other when the fermentation broth, which was heated at 100° C. for 10 minutes, is acidified to pH 3. Each sample was poured into a cylinder. The result is shown in the accompanying drawing in which the ordinate denotes the extent to which the cells and crystals sedimented and the abscissa the period for which the broth was allowed to stand. The value "100" represents the solution and "O" represents total sedimentation. Bb and Ba are respectively the sedimentation curves of the cells before and after the heat-treatment, while Gb and Ga are the respective curves of sedimentation of glutamic acid crystals before and after the heat-treatment. It will be understood from the drawing that, when the fermentation broth is heated, the sedimenting property of crystallised glutamic acid is decreased (moreover, their crystal habit also becomes worse, as hereinafter described) while that of the bacterial cells is increased, and such mutual approach of both sedimentation properties brings further difficulty of separation of the cells and crystals. The heat-treatment for sterilisation of the broth after the completion of fermentation and before the crystallisation of glutamic acid should be therefore avoided.

However, if the fermentation broth is left as it is, without sterilising it, after-fermentation takes place and the glutamic acid content decreases, or the broth spoils and obstruction

occurs in the crystal separation step. Steps must therefore be taken to avoid this. It has been found that, if the pH of the broth is adjusted to 2.5—3.5 immediately after the fermentation is completed, instead of the heat-treatment, the after-fermentation and putrefaction of the broth can be avoided and favourable crystallisation can be carried out. For example, acidification of a fermentation broth of pH 6.8 with hydrochloric acid to pH 3.0 and standing at the room temperature brought no deterioration which obstructed the separation of glutamic acid crystals, even after 5 days. Since the separation of glutamic acid crystals and the acid treatment of its mother liquor are usually carried out within 3 days after the pH adjustment, it is found that such a pH adjustment instead of the heat-treatment for sterilisation is sufficient to suppress the after-fermentation and the putrefaction. This is the first criterion of the present invention.

(2) If the fermentation broth is heated to raise the concentration of glutamic acid and so increase the yield of crystals, the concentration of the cells is inevitably raised and the broth is denatured on account of heating. In consequence of this, the crystal habit of the obtained glutamic acid crystals becomes remarkably inferior and separation of the cells and glutamic acid is found to be impossible. A fermentation broth containing 3% of wet cells, 5% of glutamic acid and 0.8% of the remnant carbohydrates, for example, was concentrated under reduced pressure at 60° C. to $\frac{1}{5}$ volume and the resulting broth was mixed with the unconcentrated broth, in different ratios as shown in Table 2, and then subjected to crystallisation. Table 2 illustrates the fact that the crystal habit becomes worse as the concentration progresses.



sterilising

TABLE 2

Unconc. broth	100	90	50	0
Conc. broth	0	10	50	100
Ratio of crystal habit of glutamic acid	12	30	53	57
Purity of crystals (%)	97.5	95	90	88
Yield of crystals (%)	65	68	72	75

5 "Ratio of crystal habit of glutamic acid crystals" means the ratio of the quantity of retained mother liquor to that of glutamic acid crystallised under the given conditions. For example, "50" means that the ratio of the quantity of the mother liquor contained in a cake of separated wet crystals to the quantity of the cake is 1:2.

10 Many experiments proved that the smaller the ratio is, the better the crystal habit is and that the industrial separation of crystals is difficult when the ratio is larger than 30.

15 Since the concentration of the fermentation broth increases the yield of crystals of glutamic acid but inevitably degrades the crystal habit, and accordingly decreases the purity of the crystals obtained, the crystallisation step and the separation step should be carried out without previously concentrating the broth, in such a state that the cells are still suspended in the broth. This means that somewhat lower yield of crystals are obtained in this stage in order later to separate glutamic acid of superior habit, and higher purity in an ultimately higher yield. This is the second criterion of this invention.

20 (3) The somewhat lower yield of crystals in the first operation is more than compensated for by the second and further operations by circulating the mother liquor after treating as mentioned below. According to the present invention, the mother liquor containing the cells in a suspended state is first concentrated and subjected to an acid hydrolysing step wherein glutamic acids in non-proper form (such as glutamine and pyroglutamic acid) are converted to glutamic acid proper. At the same time, proteins constituting the bacterial cells are simultaneously hydrolysed into various

kinds of amino acids and, moreover, the residual carbohydrates are changed into huminous substance. The huminous substance is separated from the solution together with the partly remaining cells.

45 The resulting filtrate no longer contains many impurities which deteriorate the crystal habit of glutamic acid, and can be concentrated to contain more than 20% of glutamic acid.

50 According to this invention, the concentrated solution, which contains an inorganic acid-used for said hydrolysis — together with glutamic acid, is circulated to the fermentation broth for the next operation. In this way, the pH of the broth is adjusted and, at the same time, the concentration of glutamic acid in said broth and the purity of the latter are both increased. Thus the solution containing mineral acid serves for pH adjustment of the broth. The above-stated is the third characteristic of this invention.

55 In consequence of the circulation of the purified acidic solution of glutamic acid, there is obtained a yield increase of (a) 2—4% due to the hydrolysis of the cells, (b) 7—10% due to conversion of glutamine and pyroglutamic acid and moreover (c) 3—5% due to the increase of the concentration of the fermentation broth, (used as a raw material in the next operation). Thus the total yield amounts to more than 95% based on the total content of glutamic acid including glutamine, pyroglutamic acid and glutamic acid derived from the hydrolysis of the cells, and at the same time the purity is also increased to over 95%.

70 The composition of the amino acids of the cells in the glutamic acid fermentation broth is for example as follows:



crystal

Glutamic acid	10.26%	Aspartic acid	7.61%
Glycine	4.25%	Alanine	6.69%
Valine	5.87%	Leucine	6.28%
Isoleucine	4.15%	Proline	2.51%
Tyrosine	2.15%	Tryptophan	0.64%
Serine	3.44%	Threonine	3.64%
Methionine	1.46%	Cystine	0.13%
Arginine	5.07%	Histidine	1.44%
Lysine	4.30%		

The waste liquor after the separation of glutamic acid in the process according to this invention contains large amounts of all the essential amino acids as shown in the foregoing table and may be partly utilised as seasoning liquor after simple neutralisation.

The following Example further illustrates the present invention:

EXAMPLE

10 Kg of a glutamic acid fermentation broth containing 1.5% of the cells (by weight in dried state), 4.8% of glutamic acid in the proper form (glutamic acid proper), 0.5% of glutamic acid in the non-proper form (such as glutamine and pyroglutamic acid) and 0.8% of remnant sugar was gradually treated with 1 kg of acid-treated and concentrated mother liquor obtained in a previous operation, and which contained 25% of glutamic acid and 20% of hydrochloric acid. Its pH was thereby adjusted to 3.0. It was cooled to 17° C. and agitated for 30 hours. The solution, in which glutamic acid had crystallised and was suspended together with the cells, was allowed to stand for 30 minutes in order for the glutamic acid crystals to sediment out. Glutamic acid crystals were separated by decantation. The crystals obtained were washed twice with 300 cc of water and then centrifuged. 520 G (converted into 100% purity) of glutamic acid crystals of 97.5% purity was obtained.

The mother liquor containing the bacterial cells in a suspended state was acidified to pH 1.0 and concentrated *in vacuo* to 2 kg. It was treated with 500 g of concentrated hydrochloric acid and heated for 3 hours at 110° C. Glutamic acid in the non-proper form and the cells were hydrolysed to glutamic acid proper. The remnant sugar was also changed by said acid treatment into huminous substance (130 g as the dried material) which was filtered off. The filtrate was concentrated to 1.3 kg. The greater part of the filtrate was circulated to the

fermentation broth as raw material in the next operation. Glutamic acid was recovered from the remaining part of the filtrate, by conventional treatments. A solution containing 3% of glutamic acid and 2.5% of amino-nitrogen was obtained. The solution was made alkaline and ammonia was evaporated off. After adjusting the pH to 5.0—5.2, 1 kg of delicious seasoning which contained 2.0% of amino-nitrogen and 2.5% of glutamic acid was obtained.

From the raw material, 10 kg of a fermentation broth, there was obtained 480 g of glutamic acid in the proper form, 50 g in the non-proper form and 15 g as a constituent of the cell protein, *i.e.*, a total of 545 g of glutamic acid. Accordingly, the above 520 g (converted into 100% purity) of glutamic acid crystals obtained of 97.5% purity corresponded to a yield of 95%. If based on the amount of glutamic acid proper *i.e.* in the proper form only, it corresponded to a yield of 108%.

WHAT WE CLAIM IS:—

1. A process for recovering glutamic acid or salts thereof from fermentation broth, which comprises the steps of (i) treating the fermentation broth (which has been neither sterilised nor concentrated by heating and which still contains bacterial cells) with acid-treated and concentrated mother liquor obtained from a previous operation, (ii) adjusting the pH to 2.5—3.5, (iii) subjecting the solution still containing the cells in suspension to crystallisation and separation of glutamic acid or salts thereof, (iv) treating the mother liquor still containing the cells with acid (to convert glutamic acid in the non-proper form into glutamic acid in the proper form as hereinbefore defined and simultaneously to hydrolyse the protein of cells into glutamic and other amino acids), filtering off the huminous material, concentrating the solution and circulating the concentrated solution into fermentation broth for the next operation.

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2. A process as claimed in claim 1, substantially as herein described with reference to the Example.

5 3. Glutamic acid and salts thereof when recovered by a process as claimed in either of the preceding claims.,

ELKINGTON & FIFE, ^{JA}
Consulting Chemists and Chartered Patent Agents,
Bank Chambers, 329, High Holborn,
London, W.C.1.
Agents for the Applicants.

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