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(54) **Title**: MIXED AQUEOUS SOLUTION OF L-LYSINE AND L-THREONINE

(57) **Abstract**: It is an object of the present invention to provide a mixed aqueous solution of L-lysine and L-threonine industrially useful as an animal feed ingredient, which solution is stable and concentrated and has good handleability. The above problem has been solved by providing a mixed aqueous solution of L-lysine and L-threonine essentially consisting of L-lysine, L-threonine and water, which solution has 3300 cp or less of viscosity at 20°C, 10-13 of pH and 70 g/100 g of water or more of the total concentration of L-lysine and L-threonine in the mixed aqueous solution.

DESCRIPTION

Mixed Aqueous Solution of L-Lysine and L-Threonine

Technical Field

The present invention relates to a mixed aqueous solution of L-lysine and L-threonine essentially consisting of L-lysine, L-threonine and water and is used as a feed ingredient or the like.

Background Art

A L-lysine based aqueous solution is already known as a feed ingredient containing an amino acid (see Patent document No. 1). It is also known that by adding an acid ion such as a sulfate ion into the L-lysine base aqueous solution to increase the solubility of L-lysine therein, a stable L-lysine base aqueous solution in which no crystals precipitate can be obtained (see Patent document No. 2). In addition, it is known that by electrodialyzing the L-lysine base aqueous solution to remove counter anions, a highly pure L-lysine base aqueous solution can be obtained (see Patent document No. 3).

Furthermore, it is preferred that a product form containing an amino acid for feed use be a liquid, due to the fact that the liquid form is more convenient in handling during its addition into the feed and that the uniform mixing can be more easily attained. In fact, liquid form amino acids are widely used in feed industry now. If the feed is distributed in a liquid form, it is generally preferred that the content of amino acid be high, which means the lower content of water because of the following reasons: 1) Lower transportation cost 2) Lower risk of microbial development during the storage of a feed after mixing 3) More suitable in formulating high nutrient density feed. For example, the L-lysine base aqueous solution is distributed at the concentration slightly below the saturation

point to achieve the maximum concentration without the risk of the precipitation of crystals.

On the other hand, a liquid feed ingredient containing L-threonine is also desired, but has not been put into practical use yet.

[Patent document No. 1]

EP 111628 B.

[Patent document No. 2]

EP 1035109 B.

[Patent document No. 2]

FR 2822396 B.

Disclosure of Invention

Since L-threonine has low solubility unlike L-lysine, a solution containing only L-threonine tends to contain high amount of water, which result in high transportation cost and higher risk of microbial development. It is an object of the invention to provide a mixed aqueous solution of L-lysine and L-threonine with high total concentration of two amino acids, which can be prepared, sold, distributed, stored and used in stable conditions.

The present inventions comprise the following aspects:

(1) A mixed aqueous solution of L-lysine and L-threonine essentially consisting of L-lysine, L-threonine and water, which solution has 3300 cp or less of viscosity at 20°C, 10-13 of pH and 70 g/100 g of water or more of the total concentration of L-lysine and L-threonine in the mixed aqueous solution.

(2) The mixed aqueous solution of L-lysine and L-threonine of aspect (1), wherein the concentrations of L-lysine and L-threonine in the mixed aqueous solution are within the region delineated by the L-lysine line, L-threonine line, vertical axis and horizontal axis in the mutual solubility diagram of L-lysine and

L-threonine as measured at 20°C provided that said region does not include said L-lysine line, L-threonine line, vertical axis and horizontal axis.

(3) The mixed aqueous solution of L-lysine and L-threonine of aspect (1), wherein the concentrations of L-lysine and L-threonine in the mixed aqueous solution are within the region delineated by L-lysine line, L-threonine line, vertical axis and horizontal axis in the mutual solubility diagram of L-lysine and L-threonine as measured at -5°C provided that said region does not include said L-lysine line, L-threonine line, vertical axis and horizontal axis.

(4) The mixed aqueous solution of L-lysine and L-threonine of any one of aspects (1)-(3), which has 2000 cp or less of viscosity at 20°C.

(5) The mixed aqueous solution of L-lysine and L-threonine of any one of aspects (1)-(4), which has 100 g/100 g of water or more of the total concentration of L-lysine and L-threonine in the mixed aqueous solution.

(6) The mixed aqueous solution of L-lysine and L-threonine of any one of aspects (1)-(5), which is prepared using solutions derived from a fermentation solution of L-lysine and/or L-threonine or a treated solution thereof.

(7) A feed ingredient wherein the mixed aqueous solution of L-lysine and L-threonine of any one of aspects (1)-(6) is formulated.

According to the present invention, a mixed aqueous solution of L-lysine and L-threonine can be put into practical use, which is stable and concentrated, easy to handle with low viscosity and thus can be applied to a compound feed.

Brief Description of Drawings

Figure 1 shows a mutual solubility diagram of L-lysine and L-threonine at 20°C and pH 11.3.

Figure 2 shows a mutual solubility diagram of L-lysine and L-threonine at -5°C and pH 12.

Figure 3 shows temperature dependences of a mutual solubility of L-lysine and L-threonine.

Best Mode for Carrying Out the Invention

L-lysine and L-threonine used in the present inventions as raw materials may be an aqueous solution containing each amino acid or each amino acid crystal, or alternatively a mixed solution of L-lysine and L-threonine or mixed crystal of L-lysine and L-threonine may be used. Generally, the origin of the above amino acid is not restricted to a specific one, but from the viewpoint of physiological safety or the like, it is preferred that the above amino acid be a raw material prepared using a fermentation method or an enzymatic method, which material is purified to be used. A raw material purity of L- lysine is preferably 95 % or more in dry matter weight, while a raw material purity of L- threonine is preferably 98.5 % or more in dry matter weight. In addition, these raw materials may contain minerals such as potassium, magnesium, calcium etc., but the total amount minerals is preferably 2400 ppm or less. This is because the risk of precipitations of minerals can be minimized. The mixed solution of L-lysine and L-threonine according to the present invention is generally used at the temperature range of -5 to 60°C. Those skilled in the art may determine said temperature for the product of the present invention considering a manufacturing temperature, temperature of the area where the product is sold and distributed, storage temperature and operating temperature to keep the mixed solution in the stable state in which insoluble substances such as crystal are not precipitated. If necessary, a storage tank with an insulating jacket may be used to avoid the precipitation. Generally, the mixed solution is distributed and used at from -5°C to a normal temperature, around 20°C, and it is not preferred to use it at -5°C or less from the viewpoint of the precipitation of crystal and it is not preferred to use

it at 60°C or more from the viewpoint of the generation of decomposed material by amino-carbonyl reaction, which reaction occurs especially if raw materials prepared using a fermentation method or an enzymatic method are used. In this connection, it has been confirmed that if the mixed solution saturated with L-lysine and L-threonine at -5°C is heated to a higher temperature, insoluble substances such as crystal are not precipitated.

As mentioned above, although the mixed solution of L-lysine and L-threonine according to the present invention is generally used at the range of -5 to 60°C, the mutual solubility value of the mixed solution saturated with both L-lysine and L-threonine increases with an increase of temperature. In this connection, the mutual solubility values at the temperature between -5°C and 20°C can be estimated from those at -5°C and 20°C as shown in Fig. 3. Since pH 11.3 (20°C) and pH 12 (-5°C) are very near each other, these pH values are considered the same values when preparing Fig. 3.

The range of pH of the mixed solution of L-lysine and L-threonine according to the present invention is restricted to from 10 to 13. pH of less than 10 is not preferred because of the low solubility of L-threonine and pH of more than 13 is not preferred because of the handling difficulty when using the mixed solution as a feed ingredient (i.e. it is designated as a hazardous material). Although a pH variation between 10 and 13 is accompanied by a mutual solubility variation and a viscosity variation, a mutual solubility diagram is easily prepared according to the method described in the present specification.

In order to control a pH value, if alkali is added to the solution, alkali metal or alkaline earth metal such as caustic soda, caustic potash or the like is used, and if acid is added to the solution, sulfuric acid, acetic acid or the like is used.

Then 70 g/100 g of water or more of the total concentration of L-lysine and L-threonine in the mixed aqueous solution result in a feed solution containing a high level of L-lysine and L-threonine.

As described below, the present inventors have newly found that in the mixed solution of L-lysine and L-threonine according to the present invention, the viscosity thereof changes significantly depending on whether or not a L-lysine concentration is higher than a L-lysine line. In said mixed solution, from the viewpoint of workability and the like it is preferred that the viscosity thereof at 20°C be 3300 cp or less, more preferably 3000 cp or less. In addition, from the viewpoint of distribution, storage and the like it is preferred that the viscosity thereof at -5°C be 3300 cp or less, more preferably 3000 cp or less. That is, by keeping the viscosity of the mixed solution 3000 cp or less, stability of the mixed solution by distribution and storage can more easily be increased. Therefore, it is understood that 3300 cp or less is preferred, 3000 cp or less is more preferred.

As an example of a mutual solubility diagram at room temperature, for example as shown in Fig. 1 regarding Example 1, a L-lysine line can be specified as Y=0.895X+228 and a L-threonine line can be specified as Y=2.06X-173 at 20°C and pH 11.3.

As an example of a mixed solution within the scope of the present invention, a mixed solution containing 191 g/100 g of water of L-lysine and 40 g/100 g of water of L-threonine, which are slightly lesser concentrations than the L-lysine line, do not precipitate any crystals, has 2519 cp of viscosity and is a stable solution having an excellent handleability.

On the other hand, as an example of a mixed solution outside of the scope of the present invention, a mixed solution containing 154 g/100 g of water of L-lysine and 95 g/100 g of water of L-threonine, which are higher

concentrations than the L-lysine line, precipitates crystals containing L-lysine which set in a gel form, has a viscosity reaching up to 11081 cp and is a completely uncontrollable liquid.

As an example of a mutual solubility diagram at a lower temperature, for example as shown in Fig. 2 regarding Example 2, a L-lysine line can be specified as Y=-1.94X+215 and a L-threonine line can be specified as Y=1.99X-147 at -5°C and pH 12.

As an example of a mixed solution within the scope of the present invention, a mixed solution containing 60 g/100 g of water of L-lysine and 76 g/100 g of water of L-threonine, which are slightly lesser concentrations than the L-lysine line, do not precipitate any crystals, has 2361 cp of viscosity and is a stable solution having an excellent handleability at -5°C.

On the other hand, as an example of a mixed solution outside of the scope of the present invention, a mixed solution containing 110 g/100 g of water of L-lysine and 60 g/100 g of water of L-threonine, which are higher concentrations than the L-lysine line, precipitates crystals containing L-lysine which set in a gel form, has a viscosity reaching up to 6469 cp and is a completely uncontrollable liquid.

In the right region of the L-threonine line in which the solution contains a large amount of L-threonine, crystals containing L-threonine precipitate on the bottom of the solution.

Furthermore, in the present invention, from the viewpoint of handleability such as enabling the solution to spray when the solution is mixed with a feed, it is preferred that the viscosity of the mixed solution be 2000 cp or less at a specific temperature between -5°C and 60°C, for example 60°C, in particular 20°C.

In addition, from the viewpoint of reducing the content of water, that is: 1) Lower transportation cost 2) Lower risk of microbial development during the storage of a feed after mixing 3) More suitable in formulating high nutrient density feed, it is preferred that the total concentration of L-lysine and L-threonine be 100 g/100 g of water or more in the mixed aqueous solution.

For example, the above mutual solubility diagram used in the present inventions can be determined as follows.

By using a method in which L-threonine is added into a saturated solution of L-lysine at a predetermined pH and temperature or L- lysine is added into a saturated solution of L-threonine at a predetermined pH and temperature, or a method comprising concentrating or cooling a mixed aqueous solution of L-lysine and L-threonine having a variety of mix ratios prepared at a predetermined pH and temperature to precipitate crystals, the resulting crystals are separated from the mother liquor and then a solubility diagram is plotted setting the concentration of L-lysine in the mother liquor on the horizontal axis and the concentration of L-threonine in the mother liquor on the vertical axis. A line obtained using linear approximation of saturation points at which gelatinous L-lysine is precipitated is defined as a "L-lysine line" and a line obtained using linear approximation of saturation points at which needle-shaped L-threonine is precipitated is defined as a "L-threonine line" in the mutual solubility diagram. Data of the mutual solubility at pH 11.3 and 20°C, and at pH 12 and -5°C are shown in the following tables.

Table 1: the mutual solubility of L-lysine and L-threonine at pH 11.3 and 20°C

No.	Lys [g/100 g of water]	Thr [g/100 g of water]	Remarks
1	241	0	Lys line
2	224	0	
3	191	40	
4	143	73	
5	154	95	
6	129	106	
7	113	137	
8	0	81	Thr line
9	0	87	
10	61	117	
11	64	118	
12	85	122	
13	88	125	

Table 2: the mutual solubility of L-lysine and L-threonine at pH 12 and -5°C

No.	Lys [g/100 g of water]	Thr [g/100 g of water]	Remarks
1	213	0	Lys line
2	110	60	
3	60	76	
4	0	74	Thr line
5	38	93	

In accordance with the above mutual solubility diagram, it is preferred from the viewpoint of handleability that the total concentration of L-lysine and L-threonine be 70 g/100 g of water or more, and more preferable 100 g/100 g of water or more.

The viscosity was measured using a rotational viscometer (Rheomat RM 180, Metller Toledo) and the measure system DIN 53019 regarding saturated solutions of L-lysine containing L-threonine in the predetermined concentrations, saturated solutions of L-threonine containing L-lysine in the predetermined concentrations and mixed solutions of L-lysine and L-threonine at the predetermined concentrations. Data of the mixed solutions at pH 11.3 and 20°C, and pH 12 and -5°C are shown in the following tables.

Table 3: the viscosity of the mixed solutions of L-lysine and L-threonine at pH 11.3 and 20°C

No.	Lys [g/100 g of water]	Thr [g/100 g of water]	Viscosity [cp]
1	224	0	614
2	191	40	2519
3	143	73	49
4	154	95	11081
5	129	106	2216
6	113	137	3762
7	0	81	27
8	0	87	30
9	61	117	592
10	85	122	1160
11	88	125	1488
13	53	54	63
14	53	53	64
15	53	53	74
17	40	78	94
18	39	85	110
19	121	52	312
20	120	51	329
21	122	61	484
22	158	0	156
23	199	0	386
24	254	0	1054

Table 4: the viscosity of the mixed solutions of L-lysine and L-threonine at pH 12 and -5°C

No.	Lys [g/100 g of water]	Thr [g/100 g of water]	Viscosity [cp]
1	213	0	10774
2	110	60	6469
3	60	76	2361
4	0	74	139
5	38	93	2826
6	55	55	415
7	86	43	772
8	164	22	3260

Thus, since the mutual solubility can be identified, the mixed aqueous solution of L-lysine and L-threonine having the viscosity of 3300 cp or less can be obtained if pH is determined to be 10 to 13, the total concentration of L-lysine and L-threonine is determined to be 70 g/100 g of water or more and the concentration of L-threonine is determined to be the specific concentration within the area of the mutual solubility.

The mixed aqueous solution of L-lysine and L-threonine according to the present invention can be used as an animal feed ingredient.

A solution prepared using an appropriate mix ratio of L-lysine and L-threonine (for example, 25 wt.% of L-threonine and 25 wt.% of L-lysine) is usually added in the amount of about 1 to 5 kg into one ton of animal feed using a spray nozzle. Because two kinds of required amino acids are accurately and

easily added into animal feed with a single mixing, it is expected to be more useful as an animal feed ingredient than an aqueous solution or granulated dry crystals, containing L-lysine or L-threonine alone

The present invention will be explained in more detail with reference to the following specific Examples in which the analysis of L-lysine and L-threonine was made by AOAC official method 999.13 (AOAC Official Methods of Analysis (2005) - Animal feed, Chapter 4, p20-24).

Examples

Example 1: Example at 20°C and pH 11.3

A 763.69 g quantity of 50% L-lysine solution and a 50.12 g quantity of L-threonine crystal, which were obtained from a commercial source (Ajinomoto Eurolysine S.A.S., (50% L-lysine; Lot 6256),(L-threonine; Lot 6255)), were mixed in a 1 l glass beaker. A 61.83 g quantity of 50% caustic soda (solid sodium hydroxide; Merck KGaA, reagent grade (purity >99%)) was then added to adjust pH at 11 at room temperature. This solution was concentrated to about 1.6 fold by using a rotary evaporator (pressure: 100mbar, water bath temperature: 60°C). As a result, precipitation of crystals was observed. The slurry was stirred overnight at room temperature (20°C), and so saturated solution does not become the status of super saturated. Then, saturated solution and crystals were separated at 20 °C by centrifugation at 4000 rpm for 30 min (J2-21M/E-Beckman, rotor JA-14). Viscosity and the contents of L-lysine, L-threonine, sodium, and water in saturated solution were measured by analysis under the following conditions:

L-lysine content: Amino Acid Analyzer AMINOTAC JLC-500/V (JEOL)

L-threonine content: Amino Acid Analyzer AMINOTAC JLC-500/V (JEOL)

Viscosity: a rotational viscometer (Rheomat RM 180, Metller Toledo) and the

measure system DIN 53019

Sodium content: Ion Chromatography DX320 (DIONEX)

Water content: Drying in the oven at 105°C overnight

Then, L-lysine and L-threonine content in the saturated solution were plotted in the graph. There are three regions with crystals precipitated in the saturated solution, the region of containing L-lysine alone, the region of containing both L-lysine and L-threonine, the region of containing L-threonine alone. And if separated crystals corresponding to the saturated solution were determined as L-lysine, these plots were defined as "L-lysine line" on a straight line. And if separated crystals corresponding to the saturated solution were determined as L-threonine, these plots were defined as "L-threonine line" on a straight line.

Example 2: Example at -5°C and pH 12

A 514.20 g quantity of a L-lysine solution, which had L-lysine concentration of 61.46% and pH of which was adjusted at 10.98 at 20°C , and a 441.79g quantity of a L-threonine solution, which had L-threonine concentration of 42.93% and pH of which was adjusted at 11.02 at 20°C , were mixed in a 1 l glass beaker. The 61.46% L-lysine solution was obtained from a commercial source (Ajinomoto Eurolysine S.A.S., (50% L-lysine; Lot 6256)) after evaporation using a rotary evaporator (pressure: 100mbar, water bath temperature: 60°C), and the 42.93% L-threonine solution was also obtained from a commercial one (Ajinomoto Eurolysine S.A.S., (L-threonine; Lot 7158). The resulting solution mixed from l-lysine and l-threonine was stirred at room temperature overnight and cooled down to -5°C. The solution was maintained at -5°C under agitation for 48 hours, and then under static condition overnight. As a result, precipitation of crystals was observed. Then, saturated solution and crystals were separated at -5°C by centrifugation at 4000 rpm for 30 min (J2-21M/E-Beckman, rotor

JA-14). Viscosity and the contents of L-lysine, L-threonine, sodium, and water in saturated solution were measured by analysis under the following conditions:

L-lysine content: Amino Acid Analyzer AMINOTAC JLC-500/V (JEOL)

L-threonine content: Amino Acid Analyzer AMINOTAC JLC-500/V (JEOL)

Viscosity: a rotational viscometer (Rheomat RM 180, Metller Toledo) and the measure system DIN 53019

Sodium content: Ion Chromatography DX320 (DIONEX)

Water content: Drying in the oven at 105°C overnight

Then, L-lysine and L-threonine contents in the saturated solution were plotted in the graph. There are three regions with crystals precipitated in the saturated solution, the region of containing L-lysine alone, the region of containing both L-lysine and L-threonine, the region of containing L-threonine alone. If separated crystals corresponding to the saturated solution were determined as L-lysine by gel, these plots were defined as "L-lysine line" on a straight line. And if separated crystals corresponding to the saturated solution were determined as L-threonine by needle-like crystal, these plots were defined as "L-threonine line" on a straight line.

Industrial Applicability

The present invention is a mixed aqueous solution of L-lysine and L-threonine which is stable and concentrated and which has good handleability and thus can be used as an animal feed ingredient.

CLAIMS

1. A mixed aqueous solution of L-lysine and L-threonine essentially consisting of L-lysine, L-threonine and water, which solution has 3300 cp or less of viscosity at 20°C, 10-13 of pH and 70 g/100 g of water or more of the total concentration of L-lysine and L-threonine in the mixed aqueous solution.

2. The mixed aqueous solution of L-lysine and L-threonine of Claim 1, wherein the concentrations of L-lysine and L-threonine in the mixed aqueous solution are within the region delineated by L-lysine line, L-threonine line, vertical axis and horizontal axis in the mutual solubility diagram of L-lysine and L-threonine as measured at 20°C provided that said region does not include said L-lysine line, L-threonine line, vertical axis and horizontal axis.

3. The mixed aqueous solution of L-lysine and L-threonine of Claim 1, wherein the concentrations of L-lysine and L-threonine in the mixed aqueous solution are within the region delineated by L-lysine line, L-threonine line, vertical axis and horizontal axis in the mutual solubility diagram of L-lysine and L-threonine as measured at -5°C provided that said region does not include said L-lysine line, L-threonine line, vertical axis and horizontal axis.

4. The mixed aqueous solution of L-lysine and L-threonine of any one of Claims 1-3, which has 2000 cp or less of viscosity at 20°C.

5. The mixed aqueous solution of L-lysine and L-threonine of any one of Claims 1-4, which has 100 g/100 g of water or more of the total concentration of L-lysine and L-threonine in the mixed aqueous solution.

6. The mixed aqueous solution of L-lysine and L-threonine of any one of Claims 1-5, which is prepared using solutions derived from a fermentation solution of L-lysine and/or L-threonine or a treated solution thereof.

7. A feed ingredient wherein the mixed aqueous solution of L-lysine and L-threonine of any one of Claims 1-6 is formulated.

FIG.1

Mutual solubility diagram of L-lysine and L-threonine at 20°C and pH 11.3.

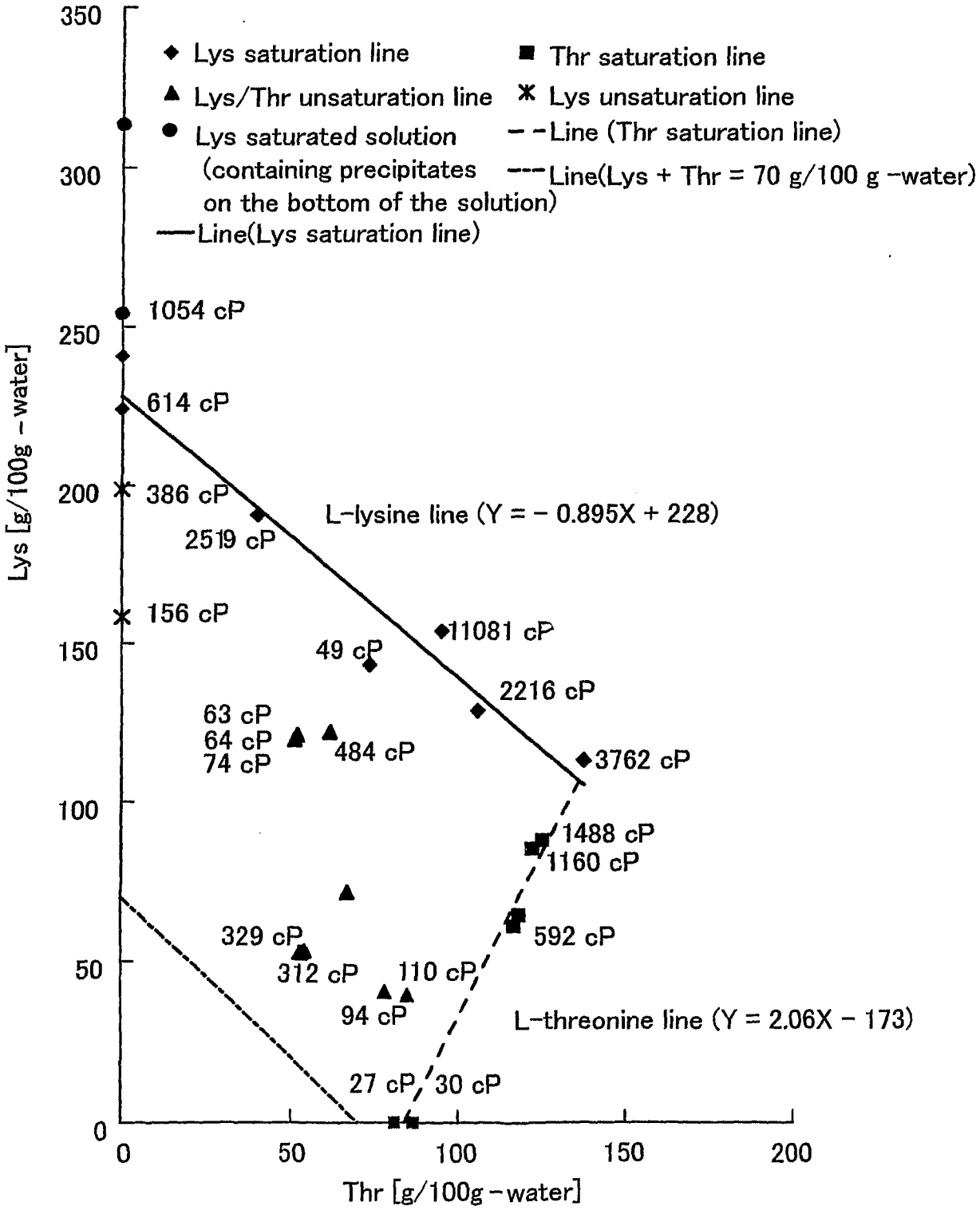


FIG.2

Mutual solubility diagram of L-lysine and L-threonine at -5°C and pH 12.

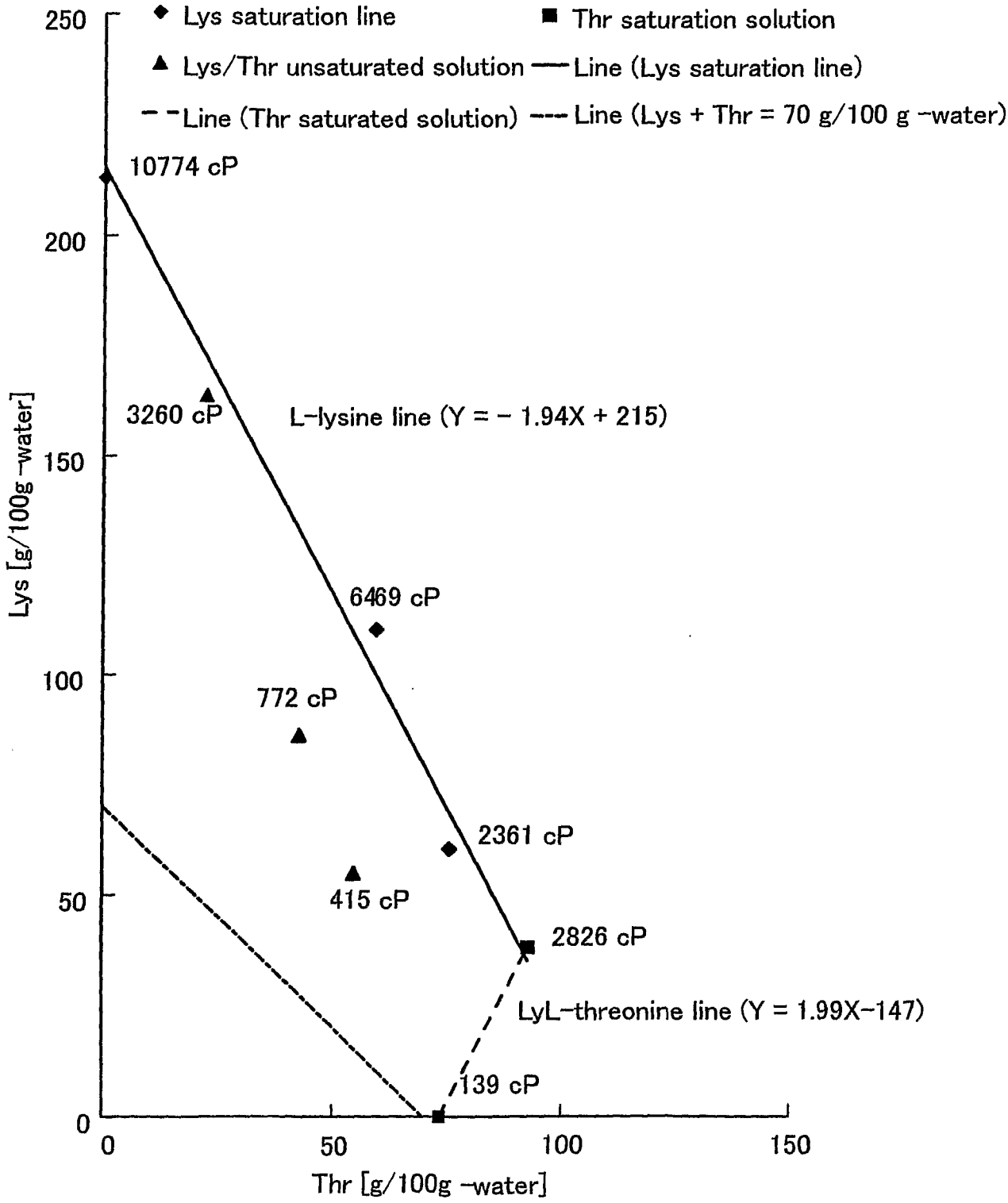
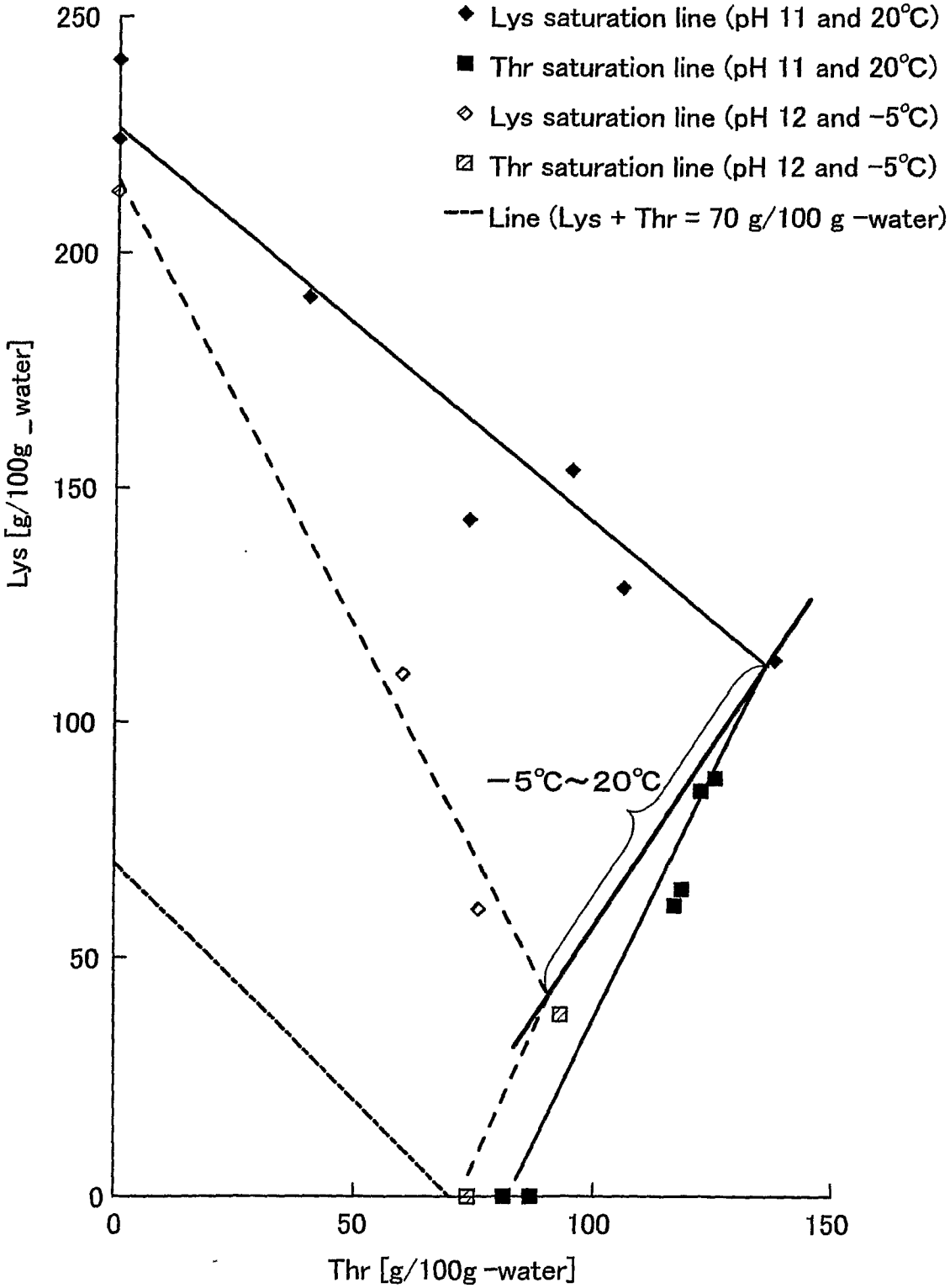


FIG.3

Temperature dependences of a mutual solubility of L-lysine and L-threonine.



INTERNATIONAL SEARCH REPORT

International application No
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A. CLASSIFICATION OF SUBJECT MATTER
INV. A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, FSTA, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 162 442 A (LOTTER HERMANN [DE] ET AL) 19 December 2000 (2000-12-19) examples 1-4	1-7
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☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 October 2008	24/10/2008

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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