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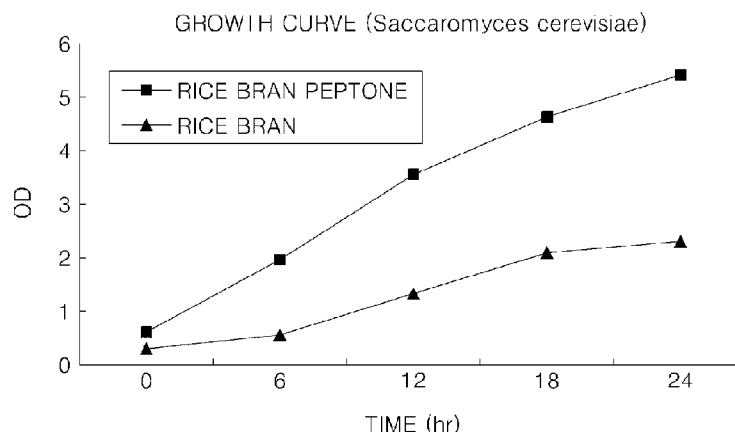
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(54) Title: COMPOSITION COMPRISING RICE BRAN PROTEIN HYDROLYSATE FOR USE IN CULTURING OF MICROORGANISMS

[Fig. 1]



(57) Abstract: The present invention relates to a composition for use in culturing microorganisms, comprising rice bran peptone.

Description

Title of Invention: COMPOSITION COMPRISING RICE BRAN PROTEIN HYDROLYSATE FOR USE IN CULTURING OF MICROORGANISMS

Technical Field

- [1] CROSS-REFERENCE TO RELATED PATENT APPLICATION
- [2] This application claims the benefit of Korean Patent Application No. 10-2010-0021026, filed on March 9, 2010, in the Korean Intellectual Property Office, the disclosure of which is incorporated herein in its entirety by reference.
- [3] The present invention relates to a composition comprising rice bran protein hydrolysate for use in culturing microorganisms.

Background Art

- [4] It has been known that rice bran, a by-product obtained in the process of rice pounding, has abundant nutrients. The nutritional composition of rice bran comprise 34 to 52% soluble saccharides, 15 to 20% crude fat, 11 to 15% crude protein, 7 to 11% crude fiber, 7 to 10% ash and 14% starch. In particular, the protein content of rice bran is higher than that of milk, and the protein of rice bran is vegetable protein, and is known to be hypoallergenic.
- [5] However, despite its high nutritional value, rice bran has not been efficiently used as a source of nutrition. Rice bran protein has a complex structure, and exists in a strongly aggregated state (Hamada, J. Protease solubilization of proteins in rice bran, Presented at the IFT Annual Meeting, Anaheim, CA, 1995; Abstract No. 68A-55). Moreover, it is difficult for individuals to absorb and use the protein because rice bran protein contains disulphide bond cross-links. One of the other factors which make it difficult to use rice bran protein is high molecular carbohydrates such as phytate (1.7%) and fiber (12%).
- [6] Attempts have been made to use rice bran in culture media for microorganisms as a nutritional source (Korean patent application publication No.2004-0041883, WO 2009/094199 A1, and Krishna Suresh Babu Naidu et al, African Journal of Biotechnology Vol. 4 (7), pp. 724-726). However, rice bran in culture media could not be efficiently used because its nutritional elements exist in low-bioavailable forms as described above and led to a long time required for cultivation. In addition, rice bran's water insolubility makes it difficult to prepare culture media comprising it and collect and purify useful materials after fermentation.
- [7] Accordingly, there is still a need for developing industrial application of excellent nutrients from rice bran.

Disclosure of Invention

Technical Problem

- [8] After a series of studies regarding methods of using abundant nutrients of rice bran, especially protein for culture media for microorganism, the present inventors have found improvement in growth of microorganism and fermentation productivity in culture media containing rice bran peptone obtained by hydrolysis of protein separated from rice bran and completed the present invention.
- [9] The present invention provides a composition containing rice bran peptone for use in culturing of microorganisms.

Solution to Problem

- [10] DETAILED DESCRIPTION OF THE INVENTION

[11]

- [12] The present invention provides a composition comprising rice bran peptone for use in culturing of microorganisms.

- [13] The term "composition for use in culturing of microorganisms" used herein refers to culture media that provide an environment for growth of microorganisms including nutrients required for the growth. Generally, compositions for use in culturing of microorganism comprise a carbon source, a nitrogen source and various minerals. Some microorganisms use carbon dioxide as a carbon source, and atmospheric nitrogen as a nitrogen source, but generally saccharides or organic acids are required as a carbon source, and inorganic or organic nitrogen compounds are required as a nitrogen source as well as various minerals. In addition, some microorganisms require vitamins or trace elements. A nitrogen source is used for protein synthesis. Some microorganisms can use inorganic nitrogen such as ammonium salt and nitrate and others require organic nitrogen such as amino acid and peptone. Depending on the type of microorganisms and the purpose of cultivation, one of ordinary skill in the art may select an appropriate composition for use in culturing of microorganisms.

- [14] The term "rice bran peptone" used herein refers to hydrolysates of protein separated from rice bran. Breakdown of rice bran protein may be conducted by a method which comprises, but is not limited to, physical treatments such as treatments with acid, base, enzyme, high pressure, heat and pulverization, and the breakdown may be partial. Rice bran peptone is obtained by a partial breakdown of rice bran protein, and is a mixture containing free amino acids, peptides composed of several to tens of amino acids or unbroken water-soluble protein.

- [15] The term "rice bran protein" used herein refers to protein separated from rice bran.

- [16] Rice bran is an agricultural by-product obtained in the process of pounding rice, and contains an abundant amount of lipids, vitamins and proteins. As shown in Table 1 below, rice bran may be used as an economical and excellent nitrogen source for use in

culturing of microorganisms because it has higher protein content than rice and is obtained as a by-product. However, the amount of freed and eluted amount of peptides and amino acids obtained from rice bran even after its treatment with proteases is very small because protein in rice bran exists in a strongly aggregated state with other nutrients.

[17] Table 1

[Table 1]

Nutrients	Composition			Unit
	Rice	Rice bran	Rice bran protein	
Crude protein	6.6-7.5	12.0-15.6	60-80	%N x 6.25
Crude lipid	0.3-0.5	15.0-19.7	2.3-6.0	%
Carbohydrate	76.7-78.4	34.1-52.3	10.0-15.5	%
Crude ash	0.3-0.8	6.6-9.9	3.5-11.3	%

[18] In an attempt to efficiently use proteins of rice bran as a nitrogen source, rice bran peptone may be obtained and used after protein is separated from rice bran and treated with an acid, base, enzyme, high pressure, heat or pulverization. Rice bran peptone may be used as an excellent nitrogen source for use in culturing of microorganism since it contains peptides, protein hydrolysate and various amino acids including lysine, an essential amino acid.

[19] Rice bran peptone may replace animal peptone such as in meat or casein peptone, which are used as a nitrogen source of culture media for microorganisms and be used as an excellent nitrogen source for use in culturing of microorganisms because it is hypoallergenic compared to vegetable peptone such as bean peptone or wheat peptone, and does not have the problems associated with genetically modified organism (GMO).

[20] According to an embodiment of the present invention, rice bran peptone may be prepared from the rice bran protein obtained by a process, which comprises; preparing fat-free rice bran by extracting rice bran with n-hexane as much as 2 to 4 times the weight of rice bran to remove rice bran oils; adding water as much as 4 to 8 times the weight of the fat-free rice bran to hydrate it; and treating the hydrated fat-free rice bran with a 1 to 5N KOH solution to extract rice bran protein.

[21] According to an embodiment of the present invention, rice bran peptone may be obtained by breaking down the protein separated from rice bran with an acid or base treatment.

[22] According to an embodiment of the present invention, rice bran peptone may be prepared by a process, which comprises; mixing the protein separated from rice bran with an equal amount of 30-40% (w/w) HCl by weight, adding to the mixture water

equivalent to 1/3 of the volume, and performing acid hydrolysis of the mixture at 105°C for over 30 hours by using steam; alkalifying the resulting mixture up to pH 10 by using 50% (w/w) sodium hydroxide at 80°C after cooling; reverse neutralizing the alkalified mixture up to pH 4.9 by using 30-40% (w/w) HCl after cooling, and removing solid contents by centrifugation and filtration with a filter press to obtain a liquid phase; adjusting pH of the liquid phase to 5.9 to 6.1 and desalinizing the resultant liquid phase through electrolysis; and grinding the resultant after performing ultrafiltration through an ultrafiltration membrane.

- [23] According to an embodiment of the present invention, rice bran peptone may be prepared by a process, which comprises; performing alkali hydrolysis of the separated from rice bran at 100°C for over 10 hours by adding 12% (w/w) sodium hydroxide; neutralizing the hydrolysates by using 5% HCl (w/w) after cooling, and removing solid contents by centrifugation and filtration with a filter press to obtain a liquid phase; desalinizing the liquid phase through electrolysis; and grinding the resultant after ultrafiltering through an ultrafiltration membrane.
- [24] According to an embodiment of the present invention, rice bran peptone may be obtained by an enzymatic treatment of protein separated from rice bran.
- [25] According to an embodiment of the present invention, rice bran peptone may be obtained by treating protein separated from rice bran with carbohydrases or proteases or a mixture thereof. Available carbohydrases comprise but are not limited to, amylase, cellulase, pectinase, hemicellulase, viscoenzyme L and xylase. Proteases may be classified as endo-type or exo-type enzymes, and they can be used in combination, if necessary. Depending on the degree of hydrolysis, enzymatic reactions may be completed in a single step or multiple steps. Upon completion of the enzymatic process, enzymes are inactivated, and unreacted solid contents are separated if necessary, and then rice bran peptone paste is obtained by concentrating a resultant reaction mixture, or rice bran peptone powder may be obtained by concentrating and drying the reaction mixture. A degree of hydrolysis of protein ($\text{DH \%} = \frac{\text{nitrogen in a form of free amino acids}}{\text{total nitrogen}} \times 100$) may vary depending on type and amount of enzymes used and reaction time. The higher the degree of hydrolysis, the lower the average molecular weight of the hydrolysates decreases and the hydrolysates may be used in a variety of fermentation culture media according to the degree of hydrolysis.
- [26] According to an embodiment of the present invention, rice bran peptone may be obtained by physical treatments such as treatments with high pressure, heat or pulverization.
- [27] According to an embodiment of the present invention, rice bran peptone may be used in the form of powder which is obtained by concentrating and drying a resultant reaction mixture obtained by the treatments of rice bran protein with an acid, base or

enzyme, or in the form of a paste obtained by concentrating the resultant reaction mixture.

Advantageous Effects of Invention

- [28] The composition comprising rice bran protein hydrolysate for use in culturing microorganisms disclosed by the present invention provides an excellent nitrogen source resulting in improvement in growth rate of microorganisms and fermentation productivity, and when applied to food, obtained cultures may be used as an excellent GMO-free natural material which is allergy-free.

Brief Description of Drawings

- [29] The above and other features and advantages of the present invention will become more apparent by describing in detail exemplary embodiments thereof with reference to the attached drawings in which:
- [30] FIG. 1 is a graph showing growth of *Sacchromyces cerevisiae* in culture media for microorganisms, according to an embodiment of the present invention, and in culture media comprising rice bran;
- [31] FIG. 2 is a graph showing growth of *Candida tropicalis* in culture media for microorganisms, according to an embodiment of the present invention, and in culture media comprising rice bran hydrolysates;
- [32] FIG. 3 is a graph showing growth of *Lactobacillus plantarum* in culture media for microorganisms, according to an embodiment of the present invention, and in culture media comprising rice peptone;
- [33] FIG. 4 is a graph showing a pH change accompanied by growth of *Lactobacillus plantarum* in culture media for microorganisms, according to an embodiment of the present invention, and in culture media comprising rice peptone; and
- [34] FIG. 5 is a graph showing growth of *Bacillus licheniformis* in culture media for microorganisms, according to an embodiment of the present invention, and in culture media comprising Bactopeptone.

Mode for the Invention

- [35] Hereinafter, the present invention will be described in more detail. However, these examples are not intended to limit the scope of the one or more embodiments of the present invention.

[36]

[37] Example 1: Separation of rice bran protein

- [38] To separate protein from rice bran, n-hexane as much as 2 to 4 times the weight of rice bran or rice bran pellets was added to rice bran or bran pellets to prepare fat-free rice bran. Afterwards, the n-hexane was removed and fat-free rice bran was obtained, and water as much as 4 to 8 times the weight of the fat-free rice bran was added to the

fat-free rice bran and hydrated for 30 to 60 minutes with agitation to obtain a homogeneous dispersion of the fat-free rice bran. A 1 to 5N NaOH solution was slowly added on the above dispersion at 20 to 30°C to adjust and maintain the final pH at 8 to 10, thereby extracting proteins therefrom for 30 to 60 minutes. A solution of extracted proteins was centrifuged at 5000 g or more at room temperature to separate a supernatant from precipitates. Protein was precipitated and separated by adding 1 to 3N HCl at 10 to 30°C to the separated supernatant to adjust a pH to be 3 to 5. Precipitated protein was collected and washed by water and neutralized before use.

[39]

[40] Example 2: Preparation of rice bran peptone by acid hydrolysis of rice bran protein

[41] Rice bran protein obtained from Example 1 was mixed with 35% (w/w) HCl on the same weight ratio basis, and then, acid hydrolyzed by 105°C steam for 30 hours or more by adding water equivalent to 1/3 of the volume. After cooling off, the protein was alkalized up to pH 10 by using sodium hydroxide at 80°C. After cooling off again, the alkalized protein was reverse-neutralized up to pH 4.9 by using HCl, and solid contents were removed by centrifugation and filtration with a filter press. The pH of an obtained liquid phase was adjusted to between 5.9 and 6.1 and desalinized through electrolysis to finish at 0.7 s/m. Then, rice bran peptone was obtained by ultrafiltering the resultant through ultrafiltration membrane and grinding.

[42]

[43] Example 3: Preparation of rice bran peptone by enzyme treatment

[44] Rice bran protein obtained from Example 1 was dissolved in distilled water to prepare 20% (w/w) solution, and 1% (weight ratio based on the solid contents of rice bran protein) of amylase (Novozymes) was added to the solution for reaction at 90°C for 1 hour. Then, the reaction mixture was cooled off to 60°C, and 2% (weight ratio based on the solid contents of rice bran protein) of collupulin (DSM), a proteolytic enzyme, was added to the reaction mixture for reaction over 6 hours. After completing the reaction, the resulting reaction mixture was cooled off to 50°C, and 1% (weight ratio based on the solid contents of rice bran protein) of Flavourzyme (Novozymes), a proteolytic enzyme, was added to the reaction mixture for reaction over 6 hours. Upon completion of the above reaction, the resulting reaction mixture was heated at 90°C for 30 minutes to inactivate enzymes, and unreacted solid contents were removed by centrifugation. Rice bran peptone powder was obtained by concentrating and drying the resultant reaction mixture.

[45] Rice bran peptone having a desirable degree of hydrolysis of protein ($\text{DH \%} = \frac{\text{nitrogen in a form of free amino acids}}{\text{total nitrogen}} \times 100$) may be obtained by selecting the type and the amount of an enzyme used a reaction time.

[46]

[47] Example 4: Cultivation of microorganisms in rice bran-added culture media and rice bran peptone-added culture media

[48] *Saccharomyces cerevisiae* (KCCM 11350) was inoculated into a 100 ml of culture media containing rice bran or rice bran peptone prepared in Example 2 as a nitrogen source (0.4% w/w of dextrose, 1.0% w/w of rice bran or acid-hydrolyzed rice bran peptone) and shaking-cultured at 200 rpm and 30°C. Samples were collected at specific time points (0, 6, 12, 18, 24 hours) of cultivation, and centrifugated for 10 seconds. An optical density (OD) (600 nm) of a supernatant was measured and a graph curve was obtained. The results are shown in FIG. 1.

[49] As shown in FIG. 1, the growth of microorganisms in the culture media containing rice bran peptone which was obtained from acid hydrolysis was higher than that in the culture media containing rice bran. It is considered that when rice bran that is not hydrolyzed is added to a culture medium, only some water-soluble nutrients may be used by microorganisms and high molecular nutrients in aggregated form could not be efficiently used by microorganism.

[50]

[51] Example 5: Cultivation of microorganisms in rice bran hydrolysates-added culture media and rice bran peptone-added culture media

[52] 100 g of rice bran was dissolved in 900 g of warm water of 60 °C and 1 g of alpha amylase was added to the rice bran solution with agitation for 1 hour. Then, the solution was autoclaved at 121°C for 30 minutes, and cooled off to 55°C and 0.2 g of glucoamylase was added to the solution and reacted for 3 hours. After the reaction, 2 g of protease was added to the solution and reacted for 2 hours to prepare rice bran hydrolysates for a comparative experiment. Rice bran peptone prepared as described in Example 3 was used.

[53] *Candida tropicalis* (KCCM 50075) was inoculated into 100 ml of culture media containing rice bran hydrolysates or rice bran peptone as sources of organic nitrogen (1.0% w/w of dextrose, 0.5% w/w of rice bran peptone obtained from rice bran or rice bran hydrolysates obtained from enzymatic hydrolysis, 0.3 % w/w of yeast extract, 0.3 % w/w of malt extract), and shaking cultured at 200 rpm and 30°C. Samples were collected at specific time points (0, 6, 12, 18, 24 hours) of cultivation, and centrifuged at 800 rpm for 10 seconds. The OD value (600 nm) of the supernatant was measured and a growth curve was obtained. The results are shown in FIG. 2.

[54] As shown in FIG. 2, the growth of microorganisms in culture media having rice bran peptone which was obtained from enzymatic hydrolysis was higher than that in culture media having rice bran hydrolysates. Since the protein contents in rice bran hydrolysates is considerably low (15% or below), the amount of peptide and amino acids released from the hydrolysates is also limited even with the treatment by proteolytic

enzymes. On the other hand, the contents of free amino acid and peptides in rice bran peptone is 4-5 times higher than that of rice bran hydrolysates because rice bran peptone is obtained by treating protein separated from rice bran with enzymes and thus has of the protein contents of 60% or more. Accordingly, it was confirmed that the growth of microorganisms is promoted when culture media with rice bran peptone, which has a high contents of peptide and amino acid available for microorganisms, are added to the composition for use in culturing of microorganisms.

[55]

[56] Example 6: Cultivation of microorganisms in rice peptone-added culture media and rice bran peptone-added culture media

[57] *Lactobacillus plantarum* (KCCM 11322) was inoculated into 100 ml culture media containing rice peptone (produced by Kerry bioscience) which is commercially available or rice bran peptone prepared in Example 3 as sources of organic nitrogen (2.5% w/w of rice peptone or rice bran peptone obtained from enzymatic hydrolysis, 2% w/w of dextrose, 0.1% w/w of polysorbate 80, 0.2% w/w of ammonium citrate, 0.5% w/w of sodium acetate, 0.005% w/w of magnesium sulfate, 0.005% w/w of manganese sulfate, and 0.2% w/w of dibasic potassium phosphate), and shaking-cultured at 200 rpm and 30°C. Samples were collected at specific time points (0, 6, 12, 18, 24 hours) of cultivation, and centrifuged at 800 rpm for 10 seconds. The OD value (640 nm) of the supernatant was measured and a growth curve was obtained, and the pH was measured. FIGS. 3 and 4 show the growth and pH change during the cultivation process.

[58] As shown in FIG. 3, the growth of microorganisms in culture media containing rice bran peptone was higher than that in culture media containing rice peptone. Also, as shown in FIG. 4, the pH of the culture media containing rice bran peptone which was obtained from enzymatic hydrolysis decreased more than that of culture media containing rice bran. Since the decrease in pH indicates a higher contents of lactic acid, it seems that *Lactobacillus plantarum* cultured in rice bran peptone-added culture media produced more lactic acid, the product of fermentation.

[59] This result shows that rice bran peptone may be used as an excellent nitrogen source compared to rice peptone since rice bran peptone has higher contents of protein and amino acids than rice peptone does. In addition, various minerals contained in rice bran peptone seem to serve as a growth factor in culturing microorganisms.

[60]

[61] Example 7: Cultivation of microorganisms in animal peptone-added culture media and rice bran peptone-added culture media

[62] In an attempt to compare animal peptone (Bactopeptone, Difco) commercially available with rice bran peptone prepared in Example 3, *Bacillus licheniformis* (KCCM

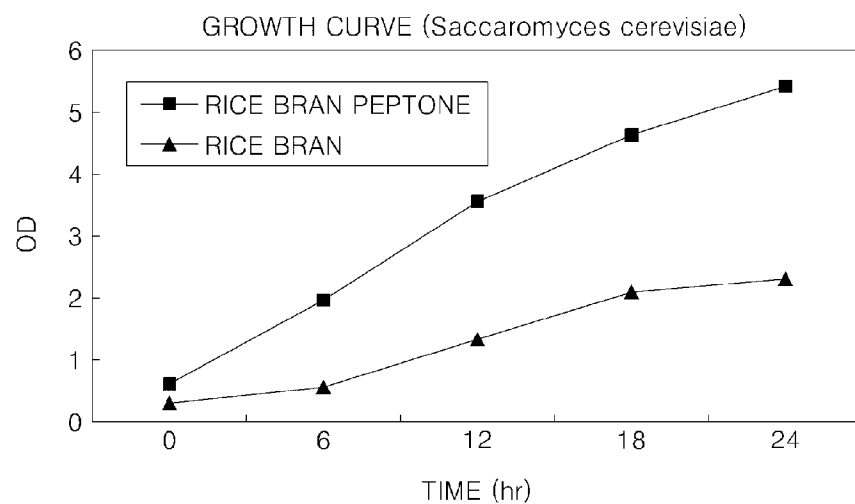
35409) was inoculated into 100 ml of culture media containing bactopectone or rice bran peptone as nitrogen sources(0.3% w/w of beef extract, 0.5% w/w of bactopectone or rice bran peptone obtained from enzymatic hydrolysis), and shaking-cultured at 200 rpm and 30°C. Samples were collected at specific time points (0, 6, 12, 18, 24 hours) of cultivation, and centrifuged at 800 rpm for 10 seconds. The OD value (600 nm) of the supernatant was measured and a growth curve was obtained. The results are shown in FIG. 5.

- [63] As shown in FIG. 5, the growth of microorganisms in culture media containing rice bran peptone was higher than that in culture media containing bactopectone, an animal peptone. This result shows that it is possible to replace existing animal peptone with rice bran peptone, a vegetable source as a component in a composition for use in culturing microorganisms.
- [64] While the present invention has been particularly shown and described with reference to exemplary embodiments thereof, it will be understood by those of ordinary skill in the art that various changes in form and details may be made therein without departing from the spirit and scope of the present invention as defined by the following claims.

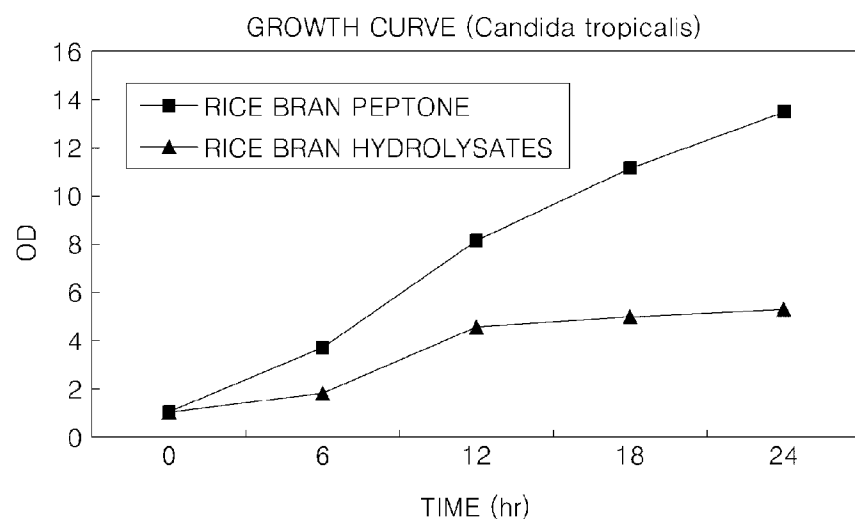
Claims

- [Claim 1] A composition for use in culturing microorganisms, comprising rice bran peptone.
- [Claim 2] The composition of claim 1, wherein the rice bran peptone is obtained by treating protein separated from rice bran with acids or bases.
- [Claim 3] The composition of claim 1, wherein the rice bran peptone is obtained by treating protein separated from rice bran with enzyme.
- [Claim 4] The composition of claim 1, wherein the rice bran peptone is obtained by treating protein separated from rice bran with heat, high-pressure or pulverization.

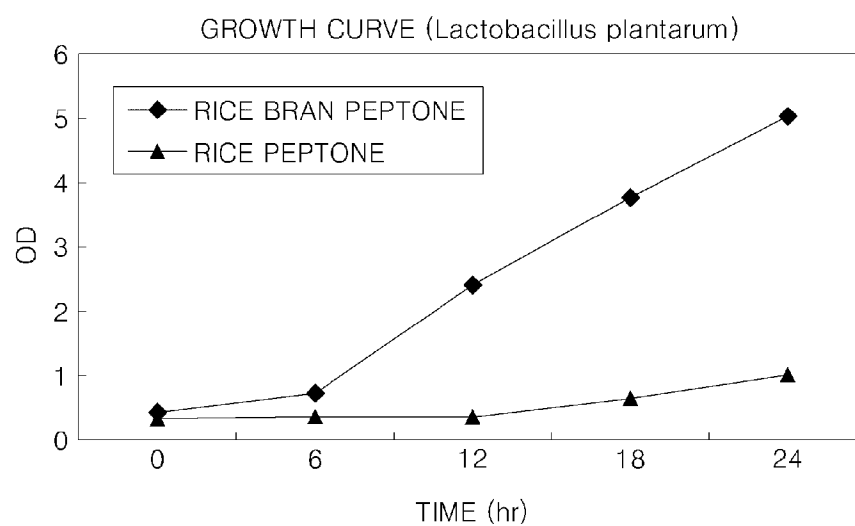
[Fig. 1]



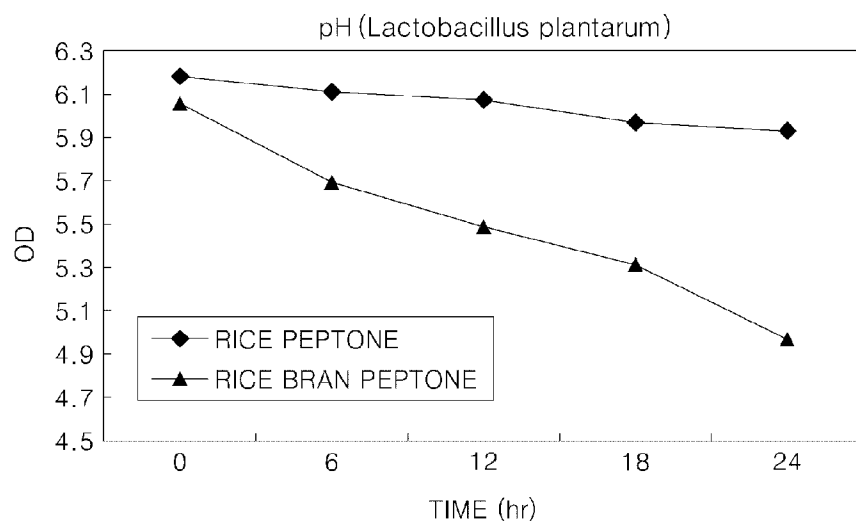
[Fig. 2]



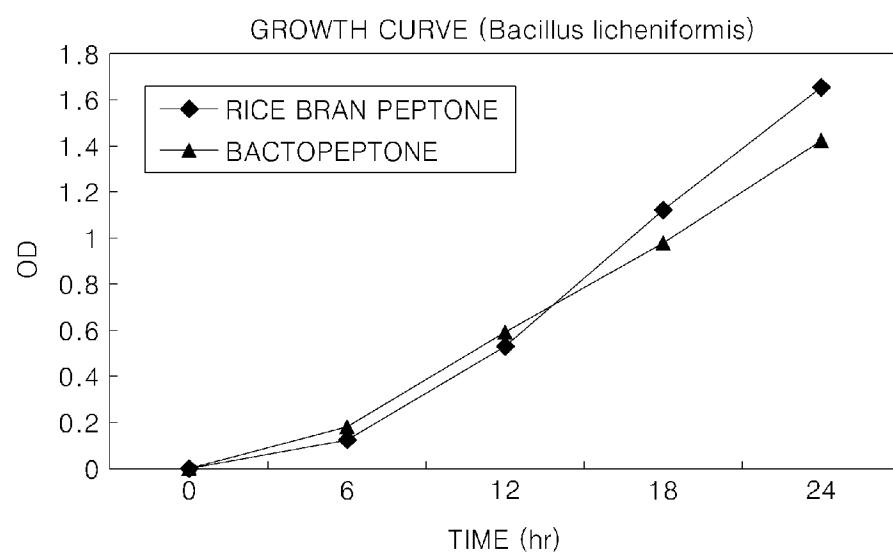
[Fig. 3]



[Fig. 4]



[Fig. 5]



A. CLASSIFICATION OF SUBJECT MATTER*C12N 1/20(2006.01)i, C07K 14/415(2006.01)i, C07K 1/14(2006.01)i*

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)

C12N 1/20

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal), Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEREWATTHANAWUT, I. et al. 'Extraction of protein and amino acids from deoiled rice bran by subcritical water hydrolysis' Bioresource Technology. Vol. 99(3), pp. 555-561 (22 February 2007) See the abstract; p.555, right column, line 8~ p.556, left column, line 11; Figs. 1, 2 and 6.	1-4
A	WATCHARARUJI, K. et al. 'Value-added subcritical water hydrolysate from rice bran and soybean meal' Bioresource Technology. Vol. 99(14), pp. 6207-6213 (24 January 2008) See the abstract.	1-4
A	APINUNJARUPONG, S. et al. 'Preparation and some functional properties of rice bran protein concentrate at different degree of hydrolysis using bromelain and alkaline extraction' Preparative Biochemistry and Biotechnology. Vol. 39(2), pp. 183-193 (2009) See the abstract.	1-4
A	WANG, M. et al. 'Preparation and functional properties of rice bran protein isolate' Journal of Agricultural and Food Chemistry. Vol. 47(2), p. 411-416 (February 1999) See the abstract.	1-4



Further documents are listed in the continuation of Box C.



See patent family annex.

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26 JULY 2011 (26.07.2011)

Date of mailing of the international search report

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