

(19) World Intellectual Property Organization  
International Bureau(43) International Publication Date  
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number  
**WO 01/67891 A1**(51) International Patent Classification<sup>7</sup>: **A23L 1/10**[KR/KR]; 407-1001 Jugong APT., Deungchon-dong, Kangsu-gu, Seoul 157-030 (KR). **YUN, Jungmi** [KR/KR]; 63-28 Noyu2-dong, Kwangjin-gu, Seoul 143-845 (KR).

(21) International Application Number: PCT/KR01/00395

(22) International Filing Date: 14 March 2001 (14.03.2001)

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(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:

|            |                               |    |
|------------|-------------------------------|----|
| 2000/12911 | 14 March 2000 (14.03.2000)    | KR |
| 2000/28471 | 25 May 2000 (25.05.2000)      | KR |
| 2000/31492 | 8 June 2000 (08.06.2000)      | KR |
| 2000/67244 | 13 November 2000 (13.11.2000) | KR |

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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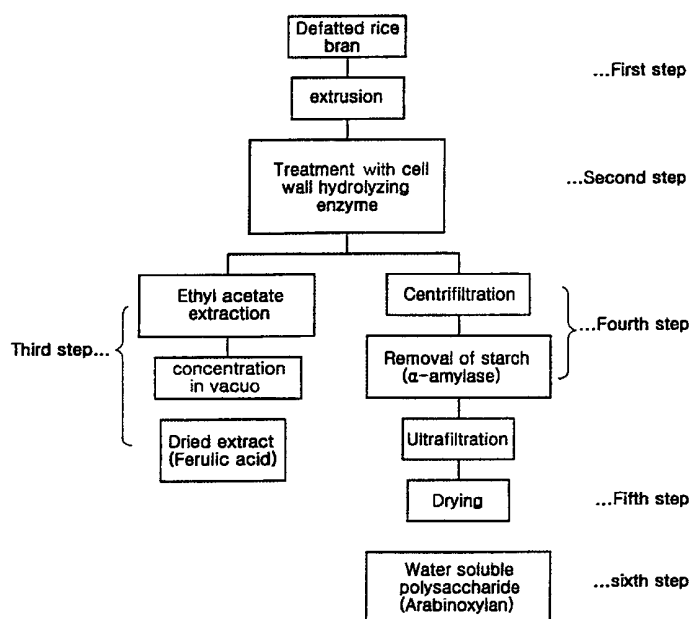
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Published:

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[Continued on next page]

(54) Title: BIOLOGICALLY ACTIVE MATERIALS FROM CEREALS AND PROCESS FOR PREPARATION THEREOF



(57) Abstract: The present invention relates to physiologically active materials separated from the cereals and manufacturing method thereof. Physiologically active materials such as ferulic acid and arabinoxylan present in cereal brans were separated by the extrusion process and the subsequent treatment with plant cell wall hydrolyzing enzymes. This combined process of extrusion and enzyme treatments for cereal brans, compared to the individual treatment, significantly increased the separation efficiency of physiologically active materials in cereal brans, ferulic acid and arabinoxylan, which inherently exist as insoluble materials in the cell wall of cereal bran.



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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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## BIOLOGICALLY ACTIVE MATERIALS FROM CEREALS AND PROCESS FOR PREPARATION THEREOF

### TECHNICAL FIELD

5 The present invention relates to biologically active materials separated from cereals and a process for preparation thereof. More particularly, the present invention relates to ferulic acid and arabinoxylan, both being of biological activity, separated from cereal bran and a process for separation thereof, which is a combined process of extrusion and the subsequent treatment with cell wall hydrolyzing enzymes for cereal bran.

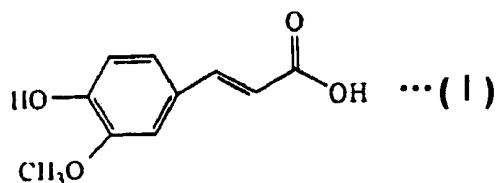
### 10 BACKGROUND ART

Cereal bran is a byproduct generated in the polishing process. The cereal bran contains a pericarp, a seed coat, an aleurone and the like, exclusive of the hull from the outer layer of cereals and occasionally contains a part of germ and endosperm as a result of the polishing process (Kulp, K. et al., Handbook of  
15 Cereal Science and Technology, Marcel Dekker, Inc., Switzerland, 2000).

The cell wall of cereal bran consists of macromolecules such as cellulose, hemicelluloses, lignin, glycoprotein, and the like. These molecules do not exist in free form, but mostly bind strongly to each other via covalent bonds, hydrogen bonds and ionic bonds, existing in insoluble form (Dey, P.M. and Brinson, K.  
20 Adv. Carbohydr. Chem. Biochem., 42: 265-382, 1986).

Aside from the above macromolecules, cereal bran contains vitamin and mineral, etc. in the cell wall. Particularly, having been lately characterized as functional active materials, phenolic compounds such as caffeic acid, sinapic acid, ferulic acid and coumaric acid are disclosed (Clifford, M.N. et al., J. Sci. Food.  
25 Agric., 79:373-378, 1999).

One of the most abundant phenolic compounds in cereal bran is the ferulic acid represented by the following formula I. It has been known until now that the ferulic acid has several physiological activities such as antioxidant, anticancer, anticholesterol, antibiotic, anti-mutation and anti-inflammatory, etc.  
5 (Castelluccio, C. et al., *Biochem. J.*, 316:691-694, 1996; Fernandez, M.A. et al., *J. Pharm. Pharmacol.*, 50:1183-1186, 1998; Saija, A. et al., *Int. J. Pharmacol.*, 199:39-47, 2000).



10

Ferulic acid does not exist in free form in the cell wall of the cereal bran, but forms an insoluble linkage that is bound to arabinoxylan, one of cell wall components, by ester linkage (Saulnier, L. and Thibault, J.F., *J. Sci. Food Agric.*, 79: 396-402, 1999).

15

Ferulic acid, though present in the cereal bran, has almost no physiological activity, because there are no enzyme systems which can break the ester linkage of ferulic acid and arabinoxylan in the human body. Therefore, very limited bioavailability can be obtained even if cereals are ingested (Saunders, R.M. et al., *Cereal Chem.*, 49:436-442, 1972; Annison, G. et al., *World's Poult. Sci. J.*, 47:232-242, 1991).  
20 Arabinoxylan, one of the hemicelluloses constituting the cell wall of cereal bran, is a sort of complex carbohydrate. It has recently been found that arabinoxylan has several physiological activities, such as immunomodulating effect, anti-diabetes, increasing resistance to infection, therapeutic aid for malignant tumors, water-soluble dietary fiber and the like, and

thus attract particular attention as a new functional food material (Ghoneum, M., Int. J. Immunother., 104(2): 89-99, 1998; Miyazaki, H. et al., Int. J. Immunopharmacol., 16(2): 163-170, 1994; Menon, P.V. et al., J. Nutr., 106(4): 555-562, 1976).

5           Like ferulic acid, arabinoxylan, however, does not exist in free form in the cell wall of cereal bran, but is present as an insoluble form bound with other cell wall components (Hatfield, R.D. et al., J. Sci. Food Agric., 79: 404-407, 1999). The absence of enzyme systems which can dissolve such insoluble materials in the human body makes the bioavailability of arabinoxylan very low.

10           Generally, food processing is carried out by steaming, roasting, drying, grinding and the like. Because these cause only physical changes of food, it is very difficult to separate the ferulic acid and arabinoxylan, which exist as insoluble materials in cereal bran, from the other cell wall components.

          In addition, the rigid structure of cereal cell walls rarely allows enzymes  
15 to infiltrate therein owing to its being highly dense, which makes it very difficult to separate insoluble materials in cereal bran by individual enzyme treatment processes.

          Because the extrusion process accompanies not only high temperature and pressure, but also a strong shearing force, a rigid structure of plant cell walls  
20 is efficiently disintegrated by the extrusion process (Hwang et al., J. Korean Soc. Food Nutr., 23(2):358-370, 1994).

          Therefore if enzyme treatment follows an extrusion process, it is possible to separate biologically active materials, since enzymes can readily infiltrate into the cell wall structure, which is already disintegrated by extrusion.

## 25   DISCLOSURE OF THE INVENTION

          Bearing the above-mentioned backgrounds in mind, the present inventors have accomplished the separation of ferulic acid and arabinoxylan by a combined

process, first comprising extrusion in order to physically break down the rigid structure of cereal bran derived from cereals such as rice, wheat, rye, corn, barley, oats and the like, and secondly the subsequent treatment with cell wall hydrolyzing enzymes to hydrolyze insoluble linkages.

5           Accordingly, it is an object of the present invention to provide biologically active materials such as ferulic acid and arabinoxylan, which are separated from cereals.

It is another object of the present invention to provide a method of separating biologically active materials from the above-mentioned cereal.

10           To accomplish the objects of the present invention, first, cereal bran derived from cereals such as rice, wheat, rye, corn, barley, oats and the like is injected into a twin screw extruder for extrusion, followed by hydrolyzing with cellulase or hemicellulase, such as arabinase, xylanase, mannase, glucanase and the like, to separate biologically active materials such as ferulic acid and  
15           arabinoxylan.

For the preparation of biologically active materials from cereal bran, first, cereal bran is physically damaged by extrusion from a twin screw extruder. The extrudate is dispersed in water and its cell wall is decomposed by treatment with hydrolyzing enzymes. Subsequently, the hydrolysate is extracted with ethyl  
20           acetate, followed by concentration in vacuo to give ferulic acid. Next, after the hydrolysate is filtered, the filtrate is added with an  $\alpha$ -amylase, such as TERMAMYL 120L(Novozymes A/S, Denmark), for hydrolyzing starch. After ultrafiltration, the residue is dried to dryness to give water-soluble polysaccharides, including arabinoxylan.

25           BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flow diagram briefly showing the preferred embodiment of the method to separate ferulic acid and arabinoxylan from defatted rice bran by the combined process of extrusion and subsequent enzyme treatment;

5 Fig. 2 is a capillary electropherogram showing the total amount of ferulic acid separated from defatted rice bran by NaOH treatment;

Fig. 3 is a capillary electropherogram showing the total amount of ferulic acid separated from defatted rice bran by the combined process of the extrusion and the subsequent enzyme treatment;

10 Fig. 4 is a capillary electropherogram showing the total amount of ferulic acid separated from defatted rice bran by the extrusion process alone;

Fig. 5 is a capillary electropherogram showing the total amount of ferulic acid separated from defatted rice bran by the enzyme treatment alone;

Fig. 6 is a graph showing the proportion of neutral sugars in water soluble polysaccharides separated from defatted rice bran by NaOH treatment;

15 Fig. 7 is a graph showing the proportion of neutral sugars in water soluble polysaccharides separated from defatted rice bran by the combined process of the extrusion and the subsequent enzyme treatment;

Fig. 8 is a graph showing the molecular weight distribution of water soluble polysaccharides separated from defatted rice bran by NaOH treatment; and;

20 Fig. 9 is a graph showing the molecular weight distribution of water soluble polysaccharides separated from defatted rice bran by the combined process of the extrusion and the subsequent enzyme treatment.

#### BEST MODES FOR CARRYING OUT THE INVENTION

25 The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings.

Referring Fig. 1, there is shown a process of preparing biologically active materials in accordance with the present invention. The cereal bran useful in the present invention is the bran layer generated in the polishing process of cereals such as rice, wheat, rye, corn, barley, oats, etc.

5 As seen in the process diagram of Fig. 1, the cereal bran is first extruded. In this regard, a useful extruder is a co-rotating intermeshed type twin-screw extruder (Buhler Brothers Co. DNDL-40, Switzerland) with an L/D ratio of 20:1. Preferably, the extrusion is conducted under conditions, including a screw rotation rate of 200~400 rpm, a feed rate of 20~50 kg/hr, a moisture content of  
10 15~40% and a temperature of 100~200 °C. Examples of the extruder to break down the solid cell wall structure of cereal bran include a single screw extruder and a twin screw extruder with preference for the twin screw extruder.

In the next step, the extrudates are subjected to cell wall decomposition in the presence of a cell wall hydrolyzing enzyme system. In accordance with  
15 the present invention, the cell wall hydrolyzing enzyme system is comprised of at least two enzymes selected from the group consisting of cellulase, arabinase, xylanase, mannanase and glucanase, and they may be used separately or together.

The cell wall hydrolyzing enzymes useful in the present invention may be commercially available, for example, exemplified by CELLUCLAST  
20 (Novozymes A/S, Denmark), ECONASE HC400 (Econase Co., Finland), CEREMIX (Novozymes A/S, Denmark). In this invention the above-exemplified commercial enzymes without purification are more preferred because they contain various enzymes such as cellulase,  $\beta$ -glucanase, arabinase, xylanase, mannanase and the like. That is, these complex enzymes can effectively hydrolyze  
25 the cell walls of cereal, consisting of complex carbohydrates.

In accordance with the present invention, the hydrolyzing enzymes are preferably used in a weight ratio of 1:0.001-1:0.1 to the cereal bran extrudate used. The enzymatic hydrolysis is preferably conducted at 30 to 60 °C for 0.5 to 12 hours.

The present invention will be more apparent by the following embodiments and experiments thereof, but do not limit the scope of the present invention.

#### EXAMPLE 1

##### 5           Separation of Ferulic Acid from Rice Bran by the Combined Process of Extrusion and Subsequent Enzyme Treatment

Milled, defatted rice bran was injected into a corotating intermeshed type twin-screw extruder (Buhler Brothers Co. DNDL-40, Switzerland). The extrusion process was performed under the conditions of screw rotation rate of  
10   300rpm, feed rate of 30 kg/hr, moisture content of 30% and temperature of 150°C. 1kg of the rice bran extrudate thus obtained was dispersed in 20L of water. Then, 10 mL of enzyme, comprising CELLUCLAST (Novozymes A/S, Denmark), ECONASE HC 400 (Econase Co., Finland) and CEREMIX (Novozymes A/S, Denmark) in 1:1:1 weight ratio, was added to the dispersed  
15   solution, followed by conducting hydrolyzing reactions for 2 hours at a temperature of 60°C with stirring. From the hydrolysate, materials of interest were extracted with 20L of ethyl acetate. Then, the extract was concentrated in vacuo to dryness to leave a residue containing ferulic acid.

##### 20   EXPERIMENT 1: Production Yield of Ferulic Acid

0.1 g of the extract prepared in Example 1 was dissolved in 10L of a mixture of methanol and water in the volume proportion of 1:1, followed by measuring the ferulic acid level therein with the aid of a capillary electrophoresis

apparatus (Beckman Inc., USA) using a fused silica capillary column with a buffer solution of 0.1 M borate.

The sample was injected for 3 seconds under pressure at 25°C in an electric field of 25kV. Quantification of the ferulic acid was achieved using a pulsed diode array (PDA) detector.

In order to measure the total amount of the ferulic acid, 20L of 1N NaOH was added to 1Kg of rice bran which was then subjected to enzymatic hydrolysis for 1 hour at 25°C and dried to obtain ferulic acid. In the manner same as in Example 1, the ferulic acid was extracted and quantified using capillary electrophoresis.

Fig. 2 shows total ferulic acid separated from defatted rice bran by NaOH treatment under the above capillary electrophoresis conditions.

Fig. 3 shows ferulic acid separated from defatted rice bran by the combined process of extrusion and subsequent enzyme treatment under the above capillary electrophoresis conditions.

Fig. 4 shows ferulic acid separated from defatted rice bran by the extrusion process under the above capillary electrophoresis conditions.

Fig. 5 shows ferulic acid separated from defatted rice bran by the enzyme treatment alone, under the above capillary electrophoresis conditions.

The following table 1 shows an amount of the ferulic acid separated from rice bran and a relative yield on the basis of an amount of ferulic acid separated by NaOH treatment.

TABLE 1. Content of ferulic acid separated from rice bran and relative yield

| Sample                                     | Content of ferulic acid<br>in sample (% , w/w) | Relative yield of<br>ferulic acid (%) |
|--------------------------------------------|------------------------------------------------|---------------------------------------|
| NaOH Treatment                             | 0.27                                           | 100                                   |
| Extrusion &<br>subsequent enzyme treatment | 0.22                                           | 81.5                                  |
| Extrusion alone                            | 0                                              | 0                                     |
| Enzyme Treatment alone                     | 0.02                                           | 7.4                                   |

As seen in Table 1, ferulic acid, which was associated with other cell wall components, existing in insoluble form, was separated in free form at high yield by the combined process of extrusion and subsequent enzyme treatment. As much as about 81.5 % of the total ferulic acid was isolated in free form from insoluble form. As for the sole process of extrusion and enzyme treatment, respectively, they were very poor in separation yield of ferulic acid.

### EXAMPLE 2

#### Separation of Arabinoxylan from Rice Bran by the Combined Process of Extrusion and Subsequent Enzyme Treatment

Milled, defatted rice bran was injected into a corotating intermeshed type twin-screw extruder (Buhler Brothers Co. DNDL-40, Switzerland). The extrusion process was performed under the conditions of screw rotation rate of 300 rpm, feed rate of 30 kg/hr, moisture content of 30% and temperature of 150°C. 1kg of the rice bran extrudate thus obtained was dispersed in 20L of water. Then, 10 mL of enzyme, comprising CELLUCLAST (Novozymes A/S, Denmark), ECONASE HC 400 (Econase Co., Finland) and CEREMIX (Novozymes A/S, Denmark) in equal weight, was added to the dispersed solution, followed by conducting hydrolyzing reactions for 2 hours at a temperature of 60°C with stirring. After filtration through a centrifugal filter, the filtrate was added with 10 mL of TERMAMYL 120L (Novozymes A/S, Denmark) and its starch was hydrolyzed at 90°C for 1 hour. The resulting hydrolysate was filtered using an ultrafiltrator having a filter membrane with molecular weight cutoff 5,000. The ultrafiltrate was concentrated to dryness to give water-soluble polysaccharides including arabinoxylan.

25

EXPERIMENT 2: Content of Arabinoxylan in the Water Soluble Polysaccharides

1.2 mL of 2N sulfuric acid was added to 1 mg of the water soluble polysaccharides prepared in Example 2 and then the resulting solution was heated for 3 hours at 100°C to produce neutral sugars. The content of arabinoxylan was determined as the sum of the contents of arabinose and xylose. Quantification of the neutral sugars resorted to Bio-LC (Dionex DX-500, USA) using Carbopac™  
5 PA1 with isocratic eluent of 22.6 mM NaOH solution and a regeneration buffer of 200 mM NaOH solution. The injection volume was 50µL while the flow rate of isocratic eluent was 0.3 mL/min with detection of the eluate by means of an electro-chemical detector (ECO).

10 For comparison, water soluble polysaccharides were prepared from rice bran by NaOH treatment. To this end, first, 1 kg of rice bran was added with 20 L of 1N NaOH, and the pH of the solution was adjusted to 6 with 1N HCl. Then, starch removal and ultrafiltration were conducted in the manner same as Example 2 to give water soluble polysaccharides.

15 Fig. 6 is a graph showing the distribution of neutral sugars in the water soluble polysaccharides separated from defatted rice bran by NaOH treatment as measured under the above conditions for Bio-LC.

Fig. 7 is a graph showing the distribution of neutral sugars in the water soluble polysaccharides separated from defatted rice bran by the combined  
20 process of the extrusion and subsequent enzyme treatment as measured under the above conditions for Bio-LC.

The following table 2 shows a yield of water soluble polysaccharides separated from rice bran by each process and the distribution of neutral sugars by each process.

25

Table 2. Yield of water soluble polysaccharides separated from rice bran and the distribution of neutral sugars.

| Sample                          | Yield<br>(%, w/w) | Amount of neutral sugars (mole%) |        |           |         |         |
|---------------------------------|-------------------|----------------------------------|--------|-----------|---------|---------|
|                                 |                   | arabinose                        | xylose | galactose | glucose | mannose |
| NaOH Treatment                  | 15.9              | 21.1                             | 31.6   | 4.1       | 43.2    | 0.0     |
| Extrusion &<br>enzyme treatment | 16.2              | 33.6                             | 56.9   | 3.3       | 6.2     | 0.0     |
| Extrusion alone                 | 2.2               | 23.6                             | 20.1   | 12.0      | 30.5    | 13.8    |
| Enzyme treatment alone          | 1.6               | 28.1                             | 30.2   | 11.2      | 20.9    | 9.6     |

As shown in Table 2, the combined process is almost similar in production yield to NaOH treatment. In contrast, the extrusion or the enzyme treatment, when being adopted alone, showed a very low yield for water soluble polysaccharides.

Particularly when using the combined process, the total amount of arabinose and xylose was measured to amount to 90.5 mole%, which was much higher than the total amount obtained by NaOH treatment, that is, 52.7 mole%. This means that the purity of arabinoxylan produced by the combined process is very high, compared with NaOH treatment.

Therefore, compared with each individual process, the combined process greatly increases the production yield of water soluble polysaccharides and amount of arabinoxylan.

### EXPERIMENT 3: Molecular weight of water soluble polysaccharides

0.01g of the water soluble polysaccharides prepared in Example 2 was dispersed in 10 mL of distilled water, and measured for molecular weight distribution by means of gel permeation chromatography (GPC) (Alliance 2690, Waters, USA) using a column filled with a combination of Ultrahydrogel linear and Ultrahydrogel 500. As an isocratic eluent, 0.1N NaNO<sub>3</sub> was used with a flow rate of 1.0mL/min. The injection volume was 100μL and the detector was a refractive index detector. The column was maintained at 45°C and the detector was done at 35°C.

For comparison, water soluble polysaccharides were prepared from rice bran by NaOH treatment. To this end, first, 1 kg of rice bran was added with 20 L of 1N NaOH, and the pH of the solution was adjusted to 6 with 1N HCl. Then, starch removal and ultrafiltration were conducted in the manner same as  
5 Example 2 to give water soluble polysaccharides.

1 kg of rice bran is added to 20 L of 1 N NaOH and pH of water solution is adjusted to pH 6 using 1N HCl. And then in the manner same as above example 2, starch is removed and the resultant is ultrafiltrated, so alkali soluble polysaccharide is prepared.

10 Fig. 8 is a graph showing the molecular weight distribution of water soluble polysaccharides separated from defatted rice bran by NaOH treatment as measured under the above conditions for GPC.

Fig. 9 is a graph showing the molecular weight distribution of water soluble polysaccharides separated from defatted rice bran by the combined  
15 process of extrusion and subsequent enzyme treatment as measured under the above conditions for GPC.

As seen in the graphs, the water soluble polysaccharides prepared by alkali treatment have a broad range of molecular weight distribution ranging from from about 5,000 to 1,170,000, while the arabinoxylan prepared by the combined  
20 process has a mean molecular weight of about 5,000 with a uniform molecular weight distribution.

### EXAMPLE 3

#### Separation of Biologically active Materials from Wheat Bran

25

Biologically active materials were separated from wheat bran by the combined process of extrusion and subsequent enzyme treatment as in Examples 1 and 2. The separated ferulic acid was found to amount to 84.6 % of the total ferulic acid present in wheat bran as measured in the same manner as in

Experiment 1. As for the separated arabinoxylan, its amount was measured to be 15.2 % of the total weight of the wheat bran, according to the same manner as in Experiment 2.

5                    EXAMPLE 4

Separation of Biologically active Materials from Rye Bran

                    Biologically active materials were separated from rye bran by the combined process of extrusion and subsequent enzyme treatment as in Examples  
10    1 and 2. The separated ferulic acid was found to amount to 78.5 % of the total ferulic acid present in rye bran as measured in the same manner as in Experiment 1. As for the separated arabinoxylan, its amount was measured to be 13.5 % of the total weight of the rye bran, according to the same manner as in Experiment 2.

15                   EXAMPLE 5

Separation of Biologically active Materials from Corn Bran

                    Biologically active materials were separated from corn bran by the combined process of extrusion and subsequent enzyme treatment as in Examples  
20    1 and 2. The separated ferulic acid was found to amount to 78.5 % of the total ferulic acid present in corn bran as measured in the same manner as in Experiment 1. As for the separated arabinoxylan, its amount was measured to be 13.5 % of the total weight of the corn bran, according to the same manner as in Experiment 2.

25

EXAMPLE 6

Separation of Biologically active Materials from Barley Bran

Biologically active materials were separated from barley bran by the combined process of extrusion and subsequent enzyme treatment as in Examples 1 and 2. The separated ferulic acid was found to amount to 78.5 % of the total ferulic acid present in barley bran as measured in the same manner as in  
5 Experiment 1. As for the separated arabinoxylan, its amount was measured to be 11.9 % of the total weight of the barley bran, according to the same manner as in Experiment 2.

#### EXAMPLE 7

##### 10 Separation of Biologically active Materials from Oat Bran

Biologically active materials were separated from oat bran by the combined process of extrusion and subsequent enzyme treatment as in Examples 1 and 2. The separated ferulic acid was found to amount to 69.2 % of the total  
15 ferulic acid present in oat bran as measured in the same manner as in Experiment 1. As for the separated arabinoxylan, its amount was measured to be 15.2 % of the total weight of the oat bran, according to the same manner as in Experiment 2.

#### INDUSTRIAL APPLICABILITY

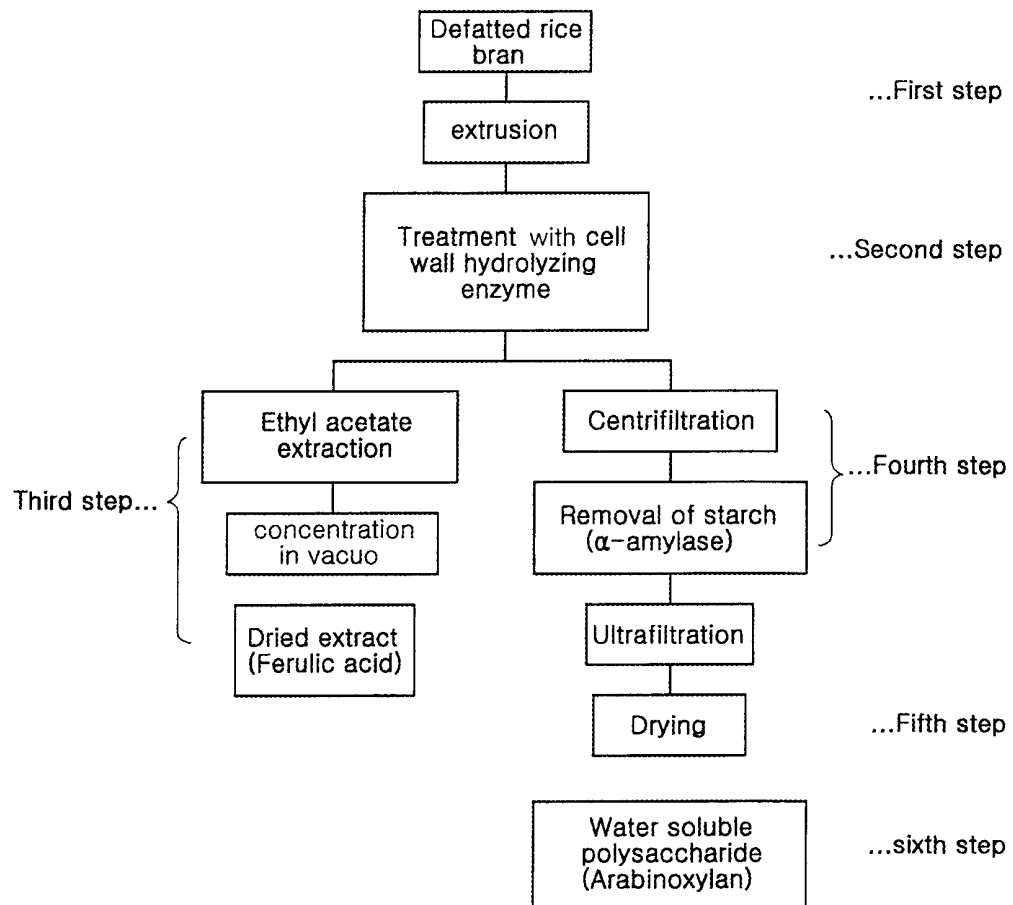
20 Compared to the extrusion process or the enzyme treatment process alone, as described hereinbefore, the combined process of extrusion and subsequent enzyme treatment for cereal bran remarkably increases the separation efficiency of the biologically active materials, ferulic acid and arabinoxylan, so that the present invention will be very useful in the food processing industry.

## CLAIMS

1. A method for preparing biologically active materials from cereal bran by using a combined process of extrusion and subsequent treatment with cell wall hydrolyzing enzymes.
- 5           2. The method as set forth in claim 1 wherein said cereal bran is selected from the group consisting of rice bran, wheat bran, rye bran, corn bran, barley bran and oat bran.
- 10           3. The method as set forth in claim 1, wherein said extrusion is carried out at 200-400 rpm, a feed rate of 20-50kg/h, a moisture content of 15-40% and a temperature of 100-200°C.
- 15           4. The method as set forth in claim 1, wherein said cell wall hydrolyzing enzymes are cellulase and hemicellulase selected from the group consisting of arabinase, xylase, mannase and glucanase, or a mixture thereof.
5. Biologically active materials, derived from cereal bran, comprising ferulic acid and water soluble arabinoxylan in free form prepared by the method of claim 1.

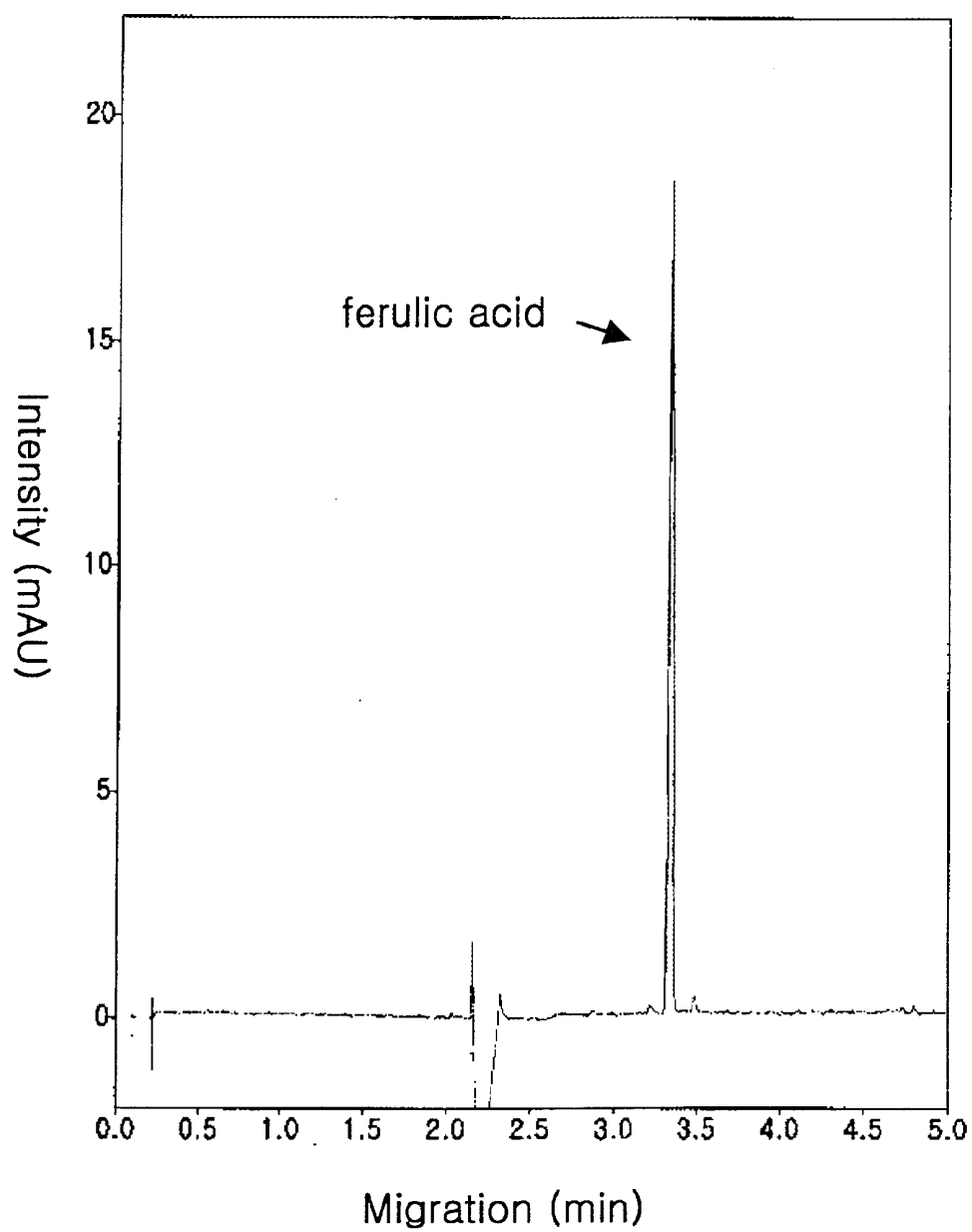
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FIG 1.



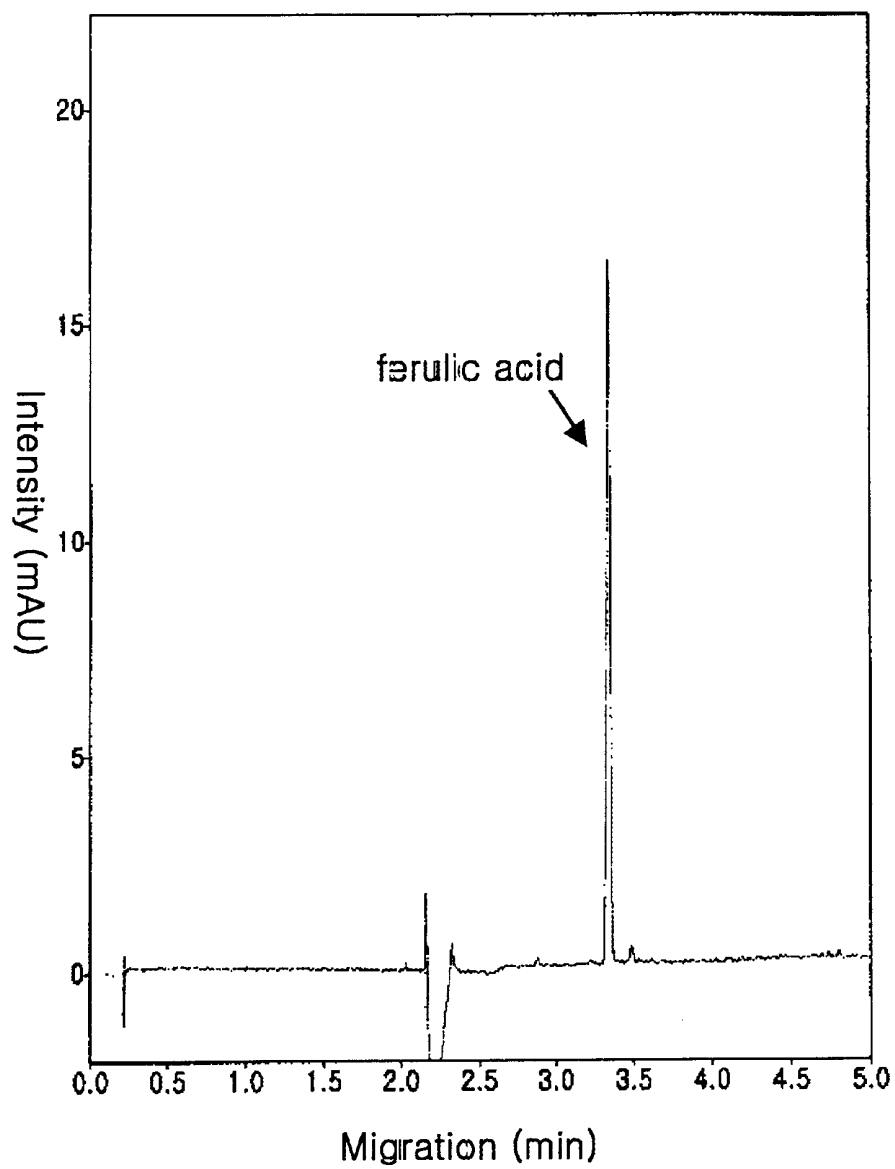
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FIG. 2



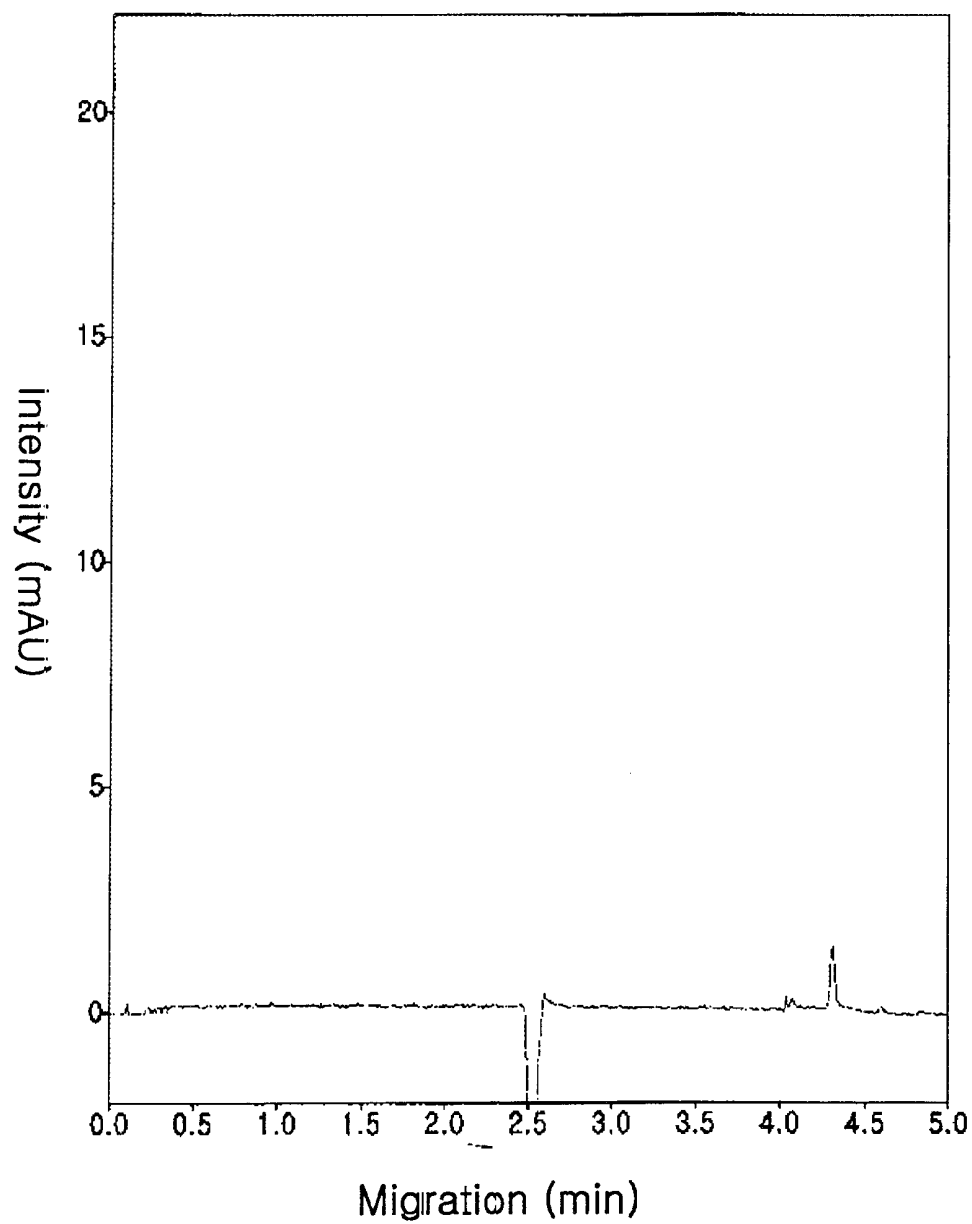
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FIG. 3



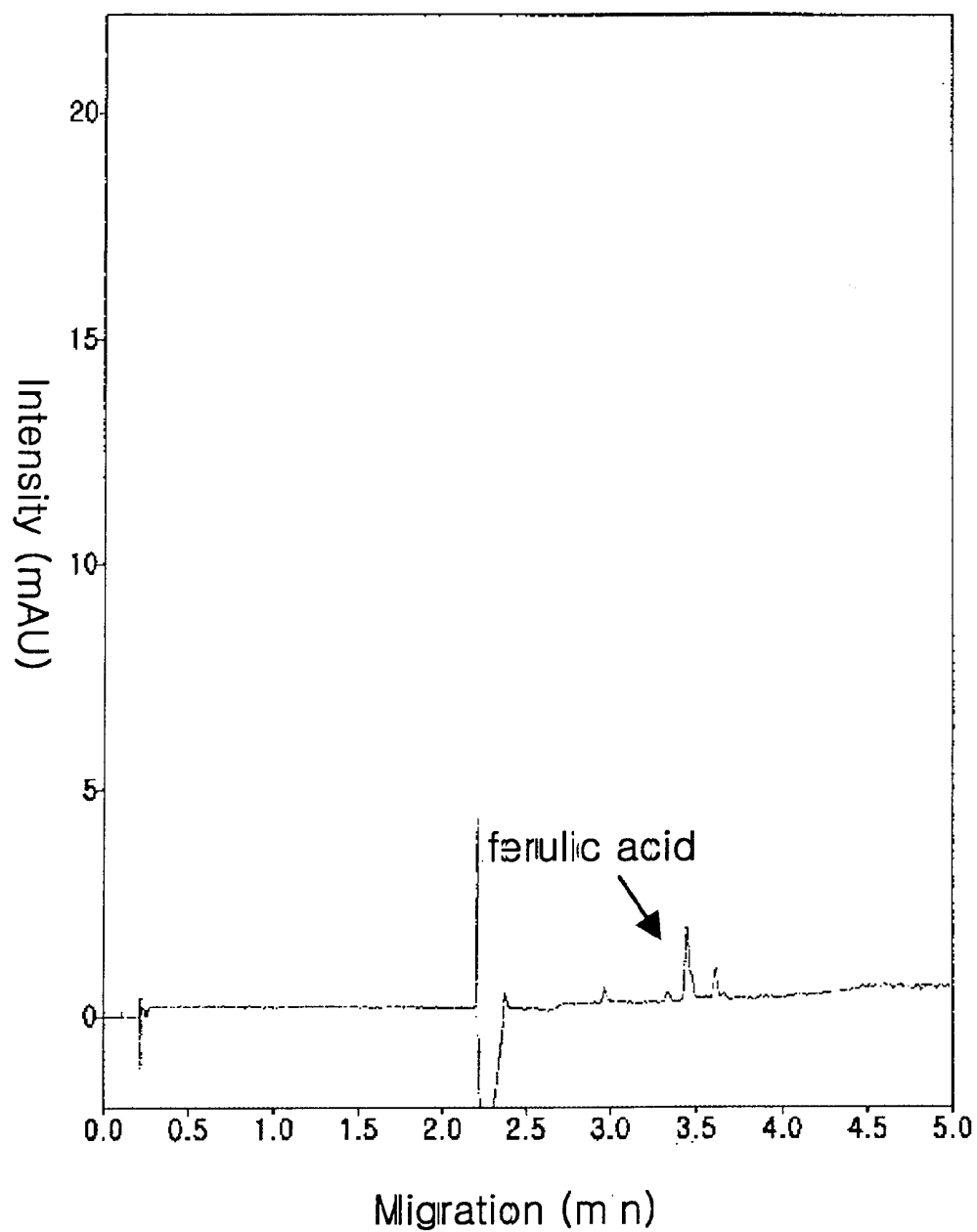
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FIG. 4



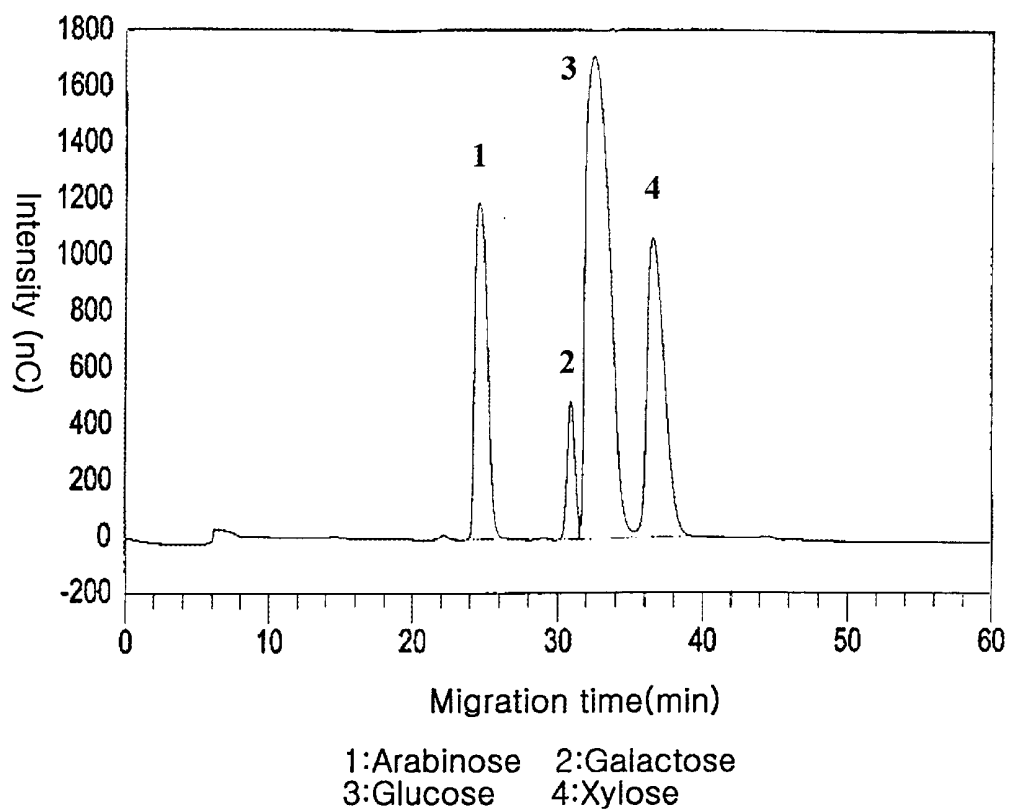
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FIG. 5



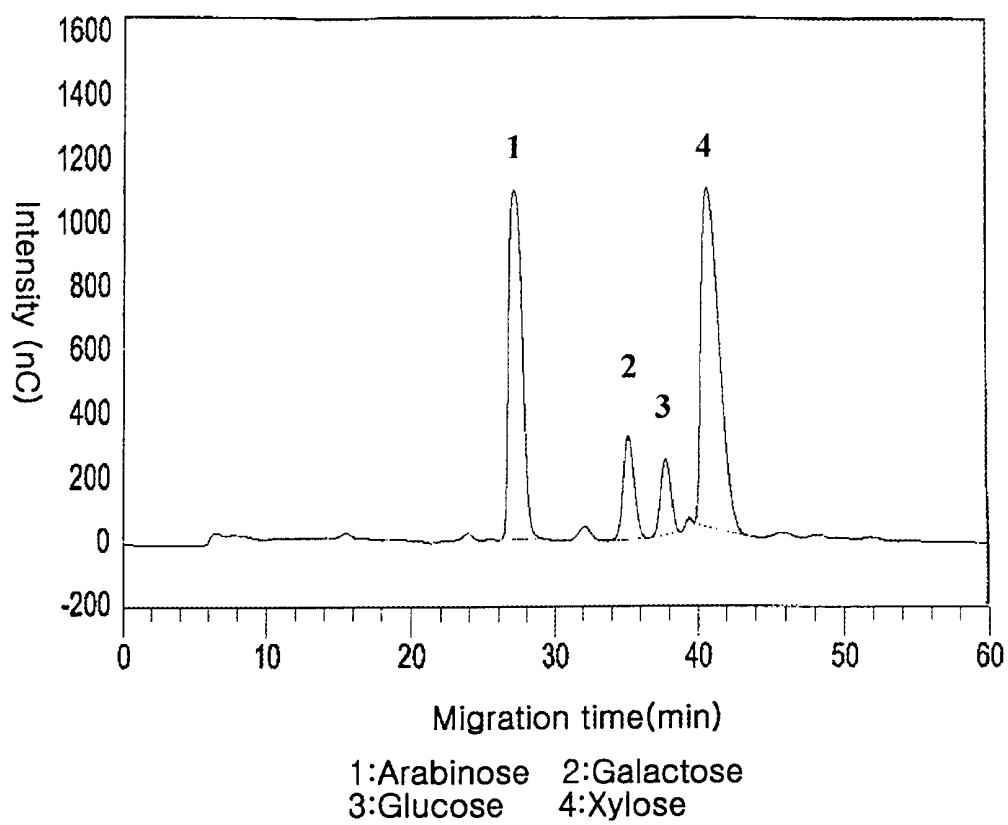
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FIG 6.



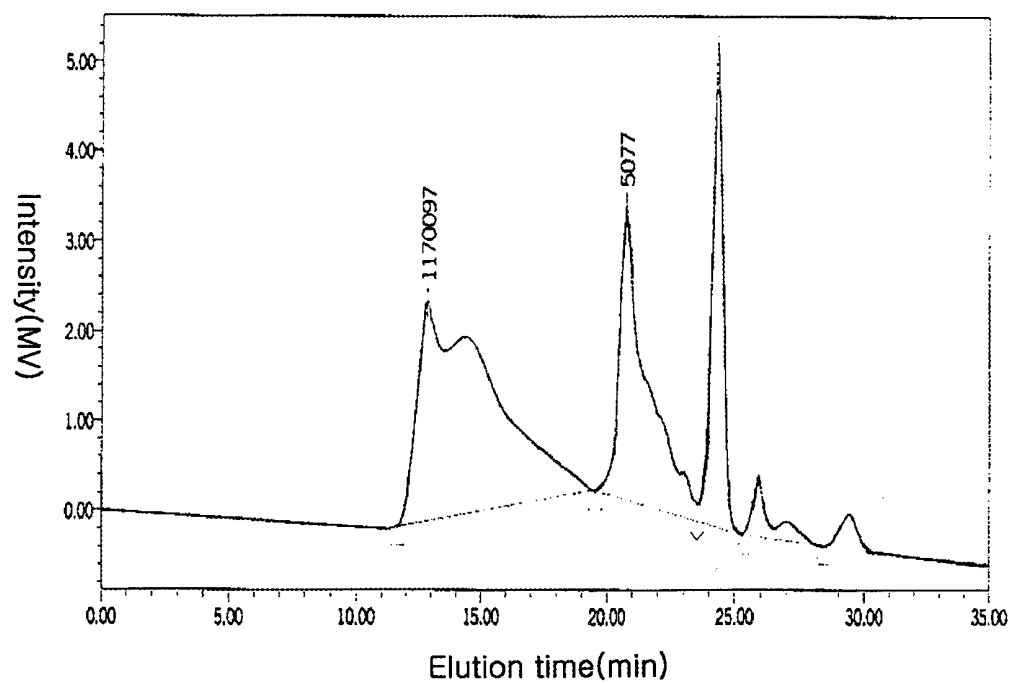
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FIG. 7



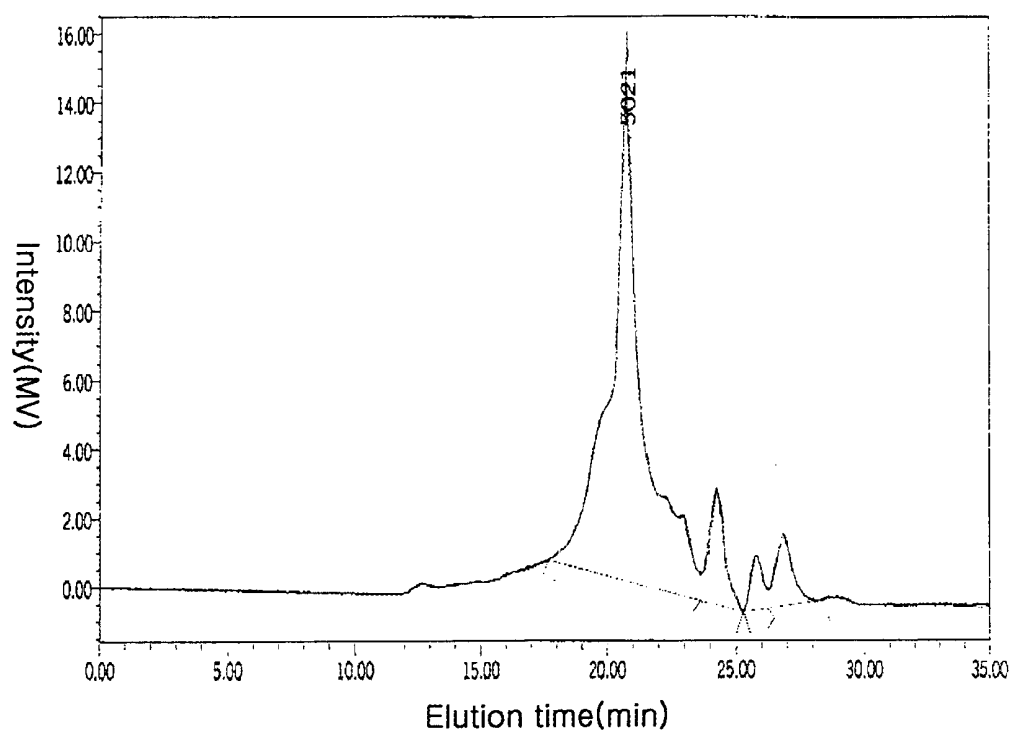
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FIG. 8



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FIG. 9



# INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR01/00395

## A. CLASSIFICATION OF SUBJECT MATTER

**IPC7 A23L 1/10**

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)

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 practicable, search terms used)  
NPS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                        | Relevant to claim No. |
|-----------|-----------------------------------------------------------------------------------------------------------|-----------------------|
| A         | KR 1998-0083335 A (Korea Food Research Institute) 05 December 1998 (05.12.98)<br>see abstract; claims 1-6 | 1-3                   |
| A         | KR 1991-0019540 A (KUREHA KAKAKU INDUSTRIAL INC.) 19 December 1991 (19.12.91)<br>see claims 1-8           | 1                     |
| A         | JP 4-023958 A (NIPPON FLOUR MILLS CO., LTD.) 28 January 1992 (28.01.92)<br>see abstract                   | 1                     |
| A         | US 4500558 A (GENERAL FOODS CORP.) 19 February 1985 (19.02.92)<br>see abstract                            | 1                     |

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

|                                                                                                                                                                 |                                                                                                                                                                                                                                                  |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| * Special categories of cited documents:                                                                                                                        | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention                                              |
| "A" document defining the general state of the art which is not considered to be of particular relevance                                                        | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone                                                                     |
| "E" earlier application or patent but published on or after the international filing date                                                                       | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified) | "&" document member of the same patent family                                                                                                                                                                                                    |
| "O" document referring to an oral disclosure, use, exhibition or other means                                                                                    |                                                                                                                                                                                                                                                  |
| "P" document published prior to the international filing date but later than the priority date claimed                                                          |                                                                                                                                                                                                                                                  |

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|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| Date of the actual completion of the international search<br>23 JULY 2001 (23.07.2001)                                                                                                                                    | Date of mailing of the international search report<br>25 JULY 2001 (25.07.2001) |
| Name and mailing address of the ISA/KR<br>Korean Intellectual Property Office<br>Government Complex-Daejeon, Dunsan-dong, Seo-gu, Daejeon<br>Metropolitan City 302-701, Republic of Korea<br>Facsimile No. 82-42-472-7140 | Authorized officer<br><br>LEE, Ho Jo<br><br>Telephone No. 82-42-481-5631        |



**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International application No.

PCT/KR01/00395

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|-------------------------------------------|---------------------|----------------------------|---------------------|
| KR 1998-0083335 A                         | 05.12.98            | None                       |                     |
| KR 1991-0019540 A                         | 19.12.91            | EP 457539 A1               | 21.11.91            |
|                                           |                     | AU 7702291 A1              | 28.11.91            |
|                                           |                     | CA 2042426 AA              | 16.11.91            |
|                                           |                     | DE 69101080 T2             | 05.05.94            |
|                                           |                     | ES 2048560 T3              | 16.03.94            |
|                                           |                     | US 5308618 A               | 03.05.94            |
|                                           |                     | JP 4218501 A2              | 10.08.92            |
| JP 4-023958 A                             | 28.01.92            | None                       |                     |
| US 4500558 A                              | 19.02.85            | None                       |                     |