

Specification

EQUOL-CONTAINING FERMENTATION PRODUCT OF SOYBEAN EMBRYONIC AXIS,
AND METHOD FOR PRODUCTION THEREOF

5

Technical Field

The present invention relates to fermented soybean hypocotyl containing equol and a method of producing the same.

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Background Art

Isoflavones (soybean isoflavones: daidzein, genistein, glycitein) contained in soybeans have structures similar to estradiol, and have anti-estrogen actions associated with binding to estrogen receptors (hereinafter referred to as ER) and estrogen-like actions. The epidemiological studies and intervention studies of soybean isoflavones that have been done before suggest their preventive effects due to their anti-estrogen actions on breast cancer, prostate cancer and other hormone-dependent cancers and improving effects due to estrogen-like actions on menopausal disorders, postmenopausal osteoporosis and hyperlipidemia.

Recently, it has been pointed out that the active principle of the physiological effects of these soybean isoflavones may be a metabolite of daidzein, equol. More specifically, it has been reported that equol has an ability to bind to ER (especially to ER β) greater than soybean isoflavones and that it has remarkably high transition capability to target organs such as breast and prostate tissues. Moreover, a case-control study reports that there are significantly less patients who produce equol in the patients of breast cancer and prostate cancer. The effects of soybean isoflavones to improve the bone density and lipid metabolism were examined regarding postmenopausal women categorized into two groups: those who produce equol and those who do not. A significant improvement in those who produce equol was observed.

Equol is produced by metabolism of daidzein by enteric bacteria. The abilities to produce equol vary between individuals, and the percentage of Japanese who produce equol production is reportedly about 50%. That is, about 50% of Japanese are not able
5 to produce equol (non-equol-producing individuals). Such an individual cannot enjoy useful physiological benefits based on the action of equol even if they ingest soybeans and processed soybean foods. Therefore, in order to attain useful physiological benefits based on the action of equol in a non-equol-producing
10 individual, ingesting equol itself is thought to be effective.

A known method of producing equol is subjecting a raw material containing daidzein compounds to fermentation treatment by microorganisms (hereinafter referred to as equol-producing bacteria) which metabolize daidzein to produce equol. Known
15 starting materials containing daidzein for use in this production method include soybeans, Ge Gen Tang (Chinese traditional medicine, also known as Kakkonto), red globe grapes, alfalfa or others. Moreover, equol-producing bacteria are already known. For example, bacteroides E-23-15 (FERM BP-6435), streptococcus E-
20 23-17 (FERM BP-6436), streptococcus A6G225 (FERM BP-6437) and lactococcus 20-92 (FERM BP-10036) have been isolated from human excrement by the inventors of the present invention (refer to patent documents 1 and 2).

However, simply subjecting the above-mentioned starting
25 materials containing daidzein compounds to fermentation treatment by using equol-producing bacteria cannot yield sufficient amount of equol in the fermented product, and there has been the problem that desired useful benefits based on the action of equol cannot be sufficiently expected by ingesting the fermented product as it
30 is.

In contrast, the hypocotyl portions of soybeans have been known to contain isoflavones, saponins and other useful components in a proportion higher than in the cotyledon portions which are used as processed soybean foods, and various uses have
35 been developed for its extract (e.g., patent document 3). However,

soybean hypocotyl extract itself is disadvantageously expensive. Moreover, when the soybean hypocotyl extract is used as a starting material for producing equol, addition of other nutrients are necessary to allow fermentation by equol-producing
5 bacteria, which can be another problem. For these reasons, the soybean hypocotyl extract cannot be currently used as a starting material for industrially producing equol.

Meanwhile, since the soybean hypocotyl itself has a characteristic bitterness, there is a trend to avoid using the
10 substance itself as it is, and much of the soybean hypocotyl is currently disposed. Furthermore, likewise soybean cotyledon portions, soybean hypocotyls contain allergens, therefore the soybean hypocotyl could not be taken by or administer to people suffering from soybean allergy. Therefore, to effectively utilize
15 the soybean hypocotyl itself, it is important to impart added values to increase its usefulness.

[Patent document 1] International Publication
WO99/007392

[Patent document 2] International Publication
20 WO2005/000042

[Patent document 3] Japanese Unexamined Patent
Publication No. 2002-234844

Disclosure of the invention

25 Problems to be solved by the invention

An object of the invention is to provide an equol-containing fermented soybean hypocotyl material that is useful for foods, pharmaceutical preparations, cosmetic products, etc. Another object of the invention is to provide a method for
30 producing an equol-containing fermented soybean hypocotyl material.

Means for solving the problem

The present inventors conducted intensive research to
35 achieve the above objects and found that an equol-containing

fermented soybean hypocotyl material can be obtained very efficiently by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound. The present inventors also found
5 that the thus-obtained equol-containing fermented soybean hypocotyl material is useful as an allergen-reduced material, because the allergens contained in the soy bean hypocotyl are reduced. The present invention has been accomplished based on these finding.

10 In other words, the present invention provides equol-containing fermented soybean hypocotyl materials and uses thereof as below:

Item 1: An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least
15 one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein.

Item 2: A fermented soybean hypocotyl material according to Item 1, wherein said at least one microorganism is a
20 lactic acid bacterium of the genus *Lactococcus*.

Item 3: A fermented soybean hypocotyl material according to Item 1, wherein said at least one microorganism belongs to *Lactococcus garvieae*.

Item 4: A fermented soybean hypocotyl material
25 according to Item 1, which contains 0.1 to 20 wt% of equol per total weight of dry fermented soybean hypocotyl material.

Item 5: A fermented soybean hypocotyl material according to Item 1, which further contains daidzin compounds, genistin compounds, genistei compounds, glycitei compounds and
30 glycitei compounds.

Item 6: A fermented soybean hypocotyl material according to Item 1, which further contains ornithine.

Item 7: A food containing a fermented soybean hypocotyl material of Item 1.

35 Item 8: A food according to Item 7, which is a dietary

supplement.

Item 9: A food according to Item 7, which contains 0.1 to 90 g of the fermented soybean hypocotyl material per 100 g of the food.

5 Item 10: A pharmaceutical preparation containing a fermented soybean hypocotyl material of Item 1.

Item 11: A pharmaceutical preparation according to Item 10, which is used for preventing or treating menopausal disorders, osteoporosis, prostatic hypertrophy, or metabolic syndrome.

10 Item 12: A pharmaceutical preparation according to Item 10, which is used for lowering the blood cholesterol level.

Item 13: Use of a fermented soybean hypocotyl material of Item 1 for producing preparations for preventing or treating menopausal disorders, osteoporosis, prostatic hypertrophy, or
15 metabolic syndrome.

Item 14: Use of a fermented soybean hypocotyl material of Item 1 for producing preparations for lowering the blood cholesterol level.

Item 15: A method for treating menopausal disorders
20 comprising the step of administering an effective amount of a fermented soybean hypocotyl material of Item 1 to a patient suffering from menopausal disorders.

Item 16: A method for lowering the blood cholesterol level comprising the step of administering an effective amount of
25 a fermented soybean hypocotyl material of Item 1 to a patient who is in need of lowering the blood cholesterol level.

Item 17: A cosmetic product containing the fermented soybean hypocotyl material of Item 1.

Item 18: A cosmetic product according to Item 17, which
30 contains 0.1 to 10 g of the fermented soybean hypocotyl material per 100 g of the cosmetic product.

The present invention also provides a method for producing an equol-containing fermented soybean hypocotyl material as below:

Item 19: A method for producing an equol-containing fermented soybean hypocotyl material comprising the step of fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one
5 daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein.

Effects of the Invention

The fermented soybean hypocotyl material of the
10 invention contains equol and other active ingredients, such as isoflavones and saponins, and can thereby find a variety of applications in the food, pharmaceutical, cosmetic and other fields. The fermented soybean hypocotyl material of the invention, in particular, has a significantly higher equol content than
15 those of the materials obtained by fermenting a raw material containing daidzein compounds such as soybean, Ge Gen Tang (Chinese traditional medicine, also known as Kakkonto), red globe grapes, alfalfa, and the like, and can thereby attain much superior equol-derived active physiological effects.

20 Furthermore, because the thus-obtained fermented soybean hypocotyl material is reduced in the amount of the allergens inherently contained in soy bean hypocotyls, it can be safely taken by or administered to people suffering from soybean allergy. Moreover, the fermented soybean hypocotyl material of
25 the invention is made from soybean hypocotyls that are discarded during soybean food processing, and therefore has high industrial potential in terms of effective use of resources.

Brief Description of the Drawings

30 Fig. 1 shows the equol concentrations of the fermented liquids obtained in Examples 1 to 3.

Fig. 2 shows the detection results of total proteins contained in the fermented soybean hypocotyl material of Example 1, soybean cotyledons, and soybean hypocotyls
35 (electrophoretogram).

Fig. 3 shows the detection results of major allergens (Gym4, Gm30K, and Gm28K) contained in the fermented soybean hypocotyl material of Example 1, soybean cotyledons, and soybean hypocotyls (electrophoretogram).

5 Fig. 4 shows the detection results of major allergens (7S globulin mix, oleocine, and trypsin inhibitor) contained in the fermented soybean hypocotyl material of Example 1, soybean cotyledons, and soybean hypocotyls (electrophoretogram).

10 BEST MODE FOR CARRYING OUT THE INVENTION

Embodiments of the present invention are described below in detail.

Microorganisms having an ability to produce equol (metabolic activity) by utilizing at least one daidzein compound
15 selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein are used as equol-producing bacteria in the present invention. Specific examples of diadzein glycosides include daidzin, malonyldaidzin, acetyldaidzin, etc.

Such microorganisms (equol-producing bacteria) are not
20 particularly limited as long as they have equol-producing ability and are acceptable for foods, pharmaceuticals, or cosmetics. It has been revealed that microorganisms having the above-mentioned include, for example, bacteria of the genus *Lactococcus*, such as *Lactococcus garvieae*, etc.; bacteria of the genus *Streptococcus*,
25 such as *Streptococcus intermedius*, *Streptococcus constellatus*, etc.; and bacteria of the genus *Bacteroides* such as *Bacteroides ovatus*. Amongst equol-producing bacteria, lactic acid bacteria belonging to the genera *Lactococcus*, *Streptococcus*, etc. are preferable; lactic acid bacteria of the genus *Lactococcus* are
30 particularly preferable; and *Lactococcus garvieae* is particularly preferable. Bacteria having equol-producing ability can be isolated from human feces using the presence or absence of equol production as an indicator. Equol-producing bacteria isolated from human feces and identified by the present inventors, as well
35 as others, i.e., *Lactococcus* 20-92 (FERM BP-10036), *Streptococcus*

E-23-17 (FERM BP-6436), *Streptococcus* A6G225 (FERM BP-6437), and *Bacteroides* E-23-15 (FERM BP-6435) have been deposited. Such deposited bacteria can be used in the present invention. Among these, *Lactococcus* 20-92 is preferable.

5 In the present invention, soybean hypocotyls are used as a fermentation material. Soybean hypocotyls are portions that develop into plumules and radicles when the soybeans germinate, and are known to contain large amounts of daidzein compounds such as daidzein glycosides, daidzein, etc. Soybean hypocotyls for use
10 in the present invention are not limited in origin, and may be processed or unprocessed, as long as their daidzein compound contents are not lost. Usable examples include raw soybean hypocotyls; hypocotyls separated from heated, dried, steamed or otherwise treated soybeans; materials obtained by heating, drying,
15 steaming or otherwise treating hypocotyls separated from unprocessed soybeans. Soybean hypocotyls that have been subjected to removal of fat and/or protein are also usable in the present invention. The form of the soybean hypocotyls for use in the present invention is also not limited, and may be powdered,
20 ground or crushed. From the viewpoint of efficient production of equol, it is preferable to use powdered soybean hypocotyls.

 Such soybean hypocotyls are fermented by adding a suitable amount of water to the soybean hypocotyls to adjust the water content, and inoculating equol-producing bacteria as
25 mentioned above.

 The amount of water added to the soybean hypocotyls can be suitably selected according to the type of equol-producing bacteria, the type of fermentation tank, etc. It is usually appropriate that, at the start of fermentation, water be present
30 with soybean hypocotyls in a proportion of 400 to 4000 parts by weight, preferably 500 to 2000 parts by weight, and more preferably 600 to 1000 parts by weight, per 100 parts by weight of soybean hypocotyls (on a dry weight basis).

 In order to improve the fermentation efficiency, flavor
35 and taste of the fermented product, etc., nutritional ingredients

can be added, as necessary, to the soybean hypocotyls used as a fermentation material. Examples of usable nutritional ingredients include yeast extracts, polypeptones, meat extracts, and other nitrogen sources; glucose, sucrose, and other carbon sources; 5 phosphate, carbonate, sulfate, and other mineral salts; vitamins; amino acids; etc. In particular, when using a microorganism having an ability to convert arginine to ornithine (hereinafter referred to as "ornithine/equol-producing microorganism") as an equol-producing microorganism, it is possible to obtain a 10 fermented material containing ornithine by adding arginine to soybean hypocotyls and then performing fermentation. In such a case, the amount of added arginine may be, for example, about 0.5 to about 3 parts by weight per 100 parts by weight of soybean hypocotyls (on a dry weight basis). Equol-producing 15 microorganisms having an ability to convert arginine to ornithine can be selected from *Lactococcus garvieae* strains, and specific examples include *Lactococcus* 20-92 (FERM BP-10036).

The pH of the fermentation material (starting material which is subjected to fermentation) is not limited as long as the 20 equol-producing bacteria are viable. From the viewpoint of good proliferation of equol-producing microorganisms, it is desirable to adjust the pH of the fermentation material to about 6 to about 7, and preferably about 6.3 to about 6.8.

Isoflavones (including daidzein compounds as mentioned 25 above) can be added to the fermentation material. Addition of isoflavone(s) to the fermentation material makes it possible to increase the equol content of the resulting fermented soybean hypocotyl material, improving the utility of the fermented soybean hypocotyl material.

30 The fermentation of soybean hypocotyls is performed under environmental conditions that are suitable for the growth characteristics of the equol-producing bacteria used. For example, when using the equol-producing bacteria specifically listed above, the fermentation (cultivation) is performed under anaerobic 35 conditions.

The fermentation temperature is not limited as long as it is suitable for the growth of the equol-producing bacteria, and may be, for example, 20 to 40°C, preferably 35 to 40°C, and more preferably 36 to 38°C.

5 The fermentation time can be suitably selected according to the amount of equol produced, the residual amount of daidzein compounds, the type of equol-producing microorganism, etc., and it is usually 1 to 10 days, preferably 2 to 7 days, and more preferably 3 to 5 days.

10 Equol is produced and accumulated in the fermented soybean hypocotyl material produced by a fermentation treatment under the conditions mentioned above, and thus the fermented soybean hypocotyl material is capable of exhibiting the useful physiological activity of equol. The equol content of such a
15 fermented soybean hypocotyl material varies depending on the equol-producing bacteria used, fermentation conditions, etc.; and equol is usually contained in an amount of 0.1 to 1 g, preferably 0.2 to 1 g, and more preferably 0.5 to 0.8 g, per 100 g, on a dry weight basis, of fermented soybean hypocotyl material.

20 The fermented soybean hypocotyl material contains various isoflavones other than equol, such as daidzin, malonyldaidzin, acetylmalonyldaidzin, daidzein, dihydrodaidzein, and other daidzein compounds (these components may be referred to as "daidzein compounds"); genistin, malonylgenistin,
25 acetylgenistin, genistein, dihydrogenistein, and other genistein compounds (these components may be referred to as "genistein compounds"); glycitin, malonylglycitin, acetylglycitin, glycitein, dihydroglycitein, and other glycitein compounds (these components may be referred to as "glycitein compounds"); etc. Thus, the
30 fermented material also exhibits the useful physiological activities of such isoflavones. The isoflavone (including equol) content of the fermented soybean hypocotyl material may be, for example, on a dry weight basis, about 0.5 to about 2 g, preferably about 0.5 to about 1.5 g, and more preferably about

0.8 to about 1.5 g, per 100 g of the fermented soybean hypocotyl material.

The proportions of isoflavones other than equol in the fermented soybean hypocotyl material are different from those in
5 unfermented soybean hypocotyls. In particular, in the fermented soybean hypocotyl material, the total content of genistein compounds, whose activities as endocrine disrupters raise concerns, is as low as 14 wt.% or less, and preferably 12 wt.% or less. Therefore, the fermented soybean hypocotyl material is also
10 more advantageous than unfermented soybean hypocotyls from the viewpoint of isoflavone proportions.

Specific examples of proportions of isoflavones in the fermented soybean hypocotyl material include the following, in which "mg" indicates the total content of each isoflavone per 1g
15 of fermented soybean hypocotyl material on a dry weight basis.

Equol: 1 to 20 mg, and preferably 2 to 10 mg;

Daidzein compounds: 0.1 to 3 mg, and preferably 0.1 to 1.5 mg;

Genistein compounds: 0.05 to 2.5 mg, and preferably 0.05 to 2 mg;

Glycitein compounds: 0.1 to 4 mg, and preferably 2 to 3.5 mg.

20 The proportions of these isoflavones contained in the fermented soybean hypocotyl material are, for example, as below, in which "wt.%" indicates the percentage relative to the total amount of isoflavones contained in the fermented soybean hypocotyl material.

25 Equol: 30 to 75 wt.%, preferably 40 to 70 wt.%, and more preferably 45 to 65 wt.%;

Daidzein compounds: 1 to 20 wt.%, preferably 2 to 15 wt.%, and more preferably 4 to 8 wt.%;

Genistein compounds: 0.1 to 20 wt.%, preferably 1 to 15 wt.%, and
30 more preferably 1 to 10 wt.%;

Glycitein compounds: 10 to 50 wt.%, preferably 15 to 35 wt.%, and more preferably 25 to 30 wt.%;

The fermented soybean hypocotyl material of the present invention contains isoflavones in such proportions that cannot be
35 achieved by known methods. Therefore, the fermented soybean

hypocotyl material of the present invention may be referred to as an isoflavone-containing material comprising the isoflavones in the above proportions.

For production of a fermented soybean hypocotyl material having an isoflavone proportions as above, *Lactococcus* 20-92 (FERM BP-10036) can be used particularly advantageously.

Further, since the fermented soybean hypocotyl material also contains saponins derived from soybean hypocotyls, it is also possible to achieve the useful physiological activity based on such saponins (e.g., antiviral activity). The saponin content of the fermented soybean hypocotyl material is usually 1 to 8 g, preferably 2 to 5 g, and more preferably 3 to 4 g, per 100 g, on a dry weight basis, of the fermented soybean hypocotyl material.

Further, as mentioned above, ornithine is contained in a fermented soybean hypocotyl material obtained by adding arginine to soybean hypocotyls and performing fermentation using an ornithine/equol-producing microorganism. Specifically, ornithine may be contained in such a fermented soybean hypocotyl material in an amount of, for example, about 0.5 to about 2.0 g, preferably about 0.8 to about 1.5 g, and more preferably about 0.9 to about 1.2 g, per 100 g, on a dry weight basis, of the fermented soybean hypocotyl material.

The fermented soybean hypocotyl material obtained by fermentation under the conditions described above may be in its post-fermentation state without additional treatment, or may be dried as necessary to form a dry solid product, for use as an ingredient for foods, pharmaceuticals, cosmetics, etc. In order to improve its storage stability, the fermented soybean hypocotyl material is preferably dried to solid form. The heated and dried fermented soybean hypocotyl material may be powdered, as necessary.

As previously described, because the fermented soybean hypocotyl material of the invention contains a variety of effective physiologically active substances such as equol, it expresses a variety of physiological and pharmacological

activities. For example, the fermented soybean hypocotyl material of the invention is useful for the prevention or alleviation of symptoms or diseases such as, e.g., menopausal disorders, osteoporosis, prostatic hypertrophy, metabolic syndrome, and for
5 lowering of the blood cholesterol level, skin whitening, acne treatment, treatment of intestinal disorders, obesity, urinary disorders, etc. Among such uses, the fermented soybean hypocotyl material of the invention is especially useful for the prevention or alleviation of general malaise-complaints and menopausal
10 symptoms (for example, osteoporosis, menopausal disorders, etc.) in middle-aged women. When a fermented soybean hypocotyl material is produced by fermenting an arginine-containing fermentation material using an ornithine/equol-producing bacteria, ornithine is also formed and stored therein. Such a fermented soybean
15 hypocotyl material can also exhibit active physiological effects derived from ornithine, such as improved liver function, improved growth hormone secretion, increased immunostimulation, increased amount of muscles, increased basal metabolism, etc.

When the fermented soybean hypocotyl material of the
20 invention is used as a food ingredient, it may be prepared in the form of, e.g., a drink, granules, fine grains, capsules, tablets, a powder, dairy product, gum, gum drop, pudding, bar, or other solid food. A food containing the fermented soybean hypocotyl material exhibits not only the equol-derived effective
25 physiological activities, but also physiological activities derived from isoflavones, saponins, and other like materials. Such foods, therefore, provide excellent health-keeping effects, and are highly useful. When a fermented soybean hypocotyl material produced by fermenting an arginine-containing fermentation
30 material using ornithine/equol-producing bacteria is used in a food, such a food also contains ornithine, and therefore has further enhanced usefulness.

Food containing the fermented soybean hypocotyl material of the invention are usable, not only as general foods,
35 but also as foods for specified health uses, dietary supplements,

functional foods, foods for invalids, etc. Foods containing the fermented soybean hypocotyl material of the invention are particularly usable as dietary supplements.

5 The proportion of the fermented soybean hypocotyl material of the invention in a food can be suitably determined according to the type of food, equol content, age and sex of the subject, expected effects, and other factors. For example, the total amount of the fermented soybean hypocotyl material per 100 g of a food may be generally 0.1 to 90 g, preferably 0.1 to 10 g, 10 and more preferably 0.5 to 2 g on a dry weight basis.

The daily dosage of a food containing the fermented soybean hypocotyl material depends upon the equol content of the fermented soybean hypocotyl material, age and body weight of the subject, daily number of doses, and other factors; but, for 15 example, an adult may take a daily dosage of 0.1 to 10 g of the fermented soybean hypocotyl material.

When the fermented soybean hypocotyl material of the invention is used as a pharmaceutical ingredient, the fermented soybean hypocotyl material is prepared in the form of, e.g., 20 tablets, pills, a powder, a liquid medicine, a suspension, an emulsion, granules, capsules, a suppository, or the like. A pharmaceutical preparation containing the fermented soybean hypocotyl material of the invention is useful for the prevention or alleviation of symptoms or diseases, e.g., menopausal 25 disorders (including menopausal complaints, osteoporosis, and hyperlipidemia), osteoporosis, prostatic hypertrophy, metabolic syndrome, and for the reduction of the blood cholesterol level, treatment of intestinal disorders, obesity, urinary disorders, etc. Such a pharmaceutical preparation is especially suitable for 30 use in the prevention or treatment of general malaise-complaints and menopausal symptoms (e.g., osteoporosis, menopausal disorders, etc.) in middle-aged women.

The dosage of a pharmaceutical preparation containing the fermented soybean hypocotyl material of the invention depends 35 upon the equol content of the fermented soybean hypocotyl

material, age and body weight of the subject, symptoms, number of doses per day, and other factors; but, for example, an adult may take a daily dosage of 0.5 to 6 g of the fermented soybean hypocotyl material on a dry weight basis.

5 When the fermented soybean hypocotyl material of the invention is used as a cosmetic ingredient, the fermented soybean hypocotyl material may be prepared in any desired form, such as, e.g., paste-like, mousse-like, gel-like, liquid, emulsion, suspension, cream, ointment, sheet-like, or like form. Such
10 cosmetic products can be used in a wide various usages: e.g., basic skin care products such as emulsions, creams, lotions, oils, and packs; cleansing products such as facial washes, cleansers, and body cleansers; cleansing wipes; purifying agents; etc. Cosmetic products containing the fermented soybean hypocotyl
15 material of the invention are used for skin whitening and clearing acne.

 The proportion of the fermented soybean hypocotyl material of the invention in a cosmetic product can be suitably determined according to the type of the cosmetic product, equal
20 content, and the like. For example, the total amount of the fermented soybean hypocotyl material per 100 g of a food may be 0.1 to 10 g, and preferably 0.5 to 5 g on a dry weight basis.

EXAMPLES

25 The present invention is described in detail with reference to Test Examples, Examples, etc. below, but is not limited to these examples.

Examples 1 to 3

 Powdered soybean hypocotyls, arginine, and water were
30 mixed in such a manner that the compositions of the mixture was as shown in Table 1 to prepare soybean hypocotyl solutions. Into 5 ml samples of the soybean hypocotyl solution was inoculated Lactococcus 20-92 (FERM BP-10036; *Lactococcus garvieae*), and subjected to static cultivation at 37 °C for 96 hours under
35 anaerobic conditions. After cultivation, the resulting culture

(fermented liquids) were sterilized by heating at 100 °C for one minute, subsequently dried at 80 °C, and further powdered using a homogenizer, thereby obtaining powdered fermented soybean hypocotyl materials.

5 Table 1 shows equol concentrations in culture 96 hours after cultivation. Table 1 also shows viable bacterial counts and pH of culture media 96 hours after cultivation, yields of the powdered fermented soybean hypocotyl materials, and equol concentrations in the powdered fermented soybean hypocotyl materials. The results established that fermentation of the powdered soybean hypocotyls using an equol-producing bacterium can produce equol very efficiently.

Table 1

		Ex. 1	Ex. 2	Ex. 3
Composition of soybean hypocotyl solution	Powdered soybean hypocotyls (dried wt.)	0.25 g	0.5 g	0.75 g
	Arginine	0.005 g	0.005 g	0.005 g
	Water	Appropriate quantity	Appropriate quantity	Appropriate quantity
	Total amount	5 ml	5 ml	5 ml
	pH	6.75 ± 0.03	6.54 ± 0.02	6.39 ± 0.03
Analytical result of fermented liquid	Viable bacterial counts of fermented liquid (log cfu/ml)	7.9 ± 0.1	8.2 ± 0.1	8.3 ± 0.2
	pH of fermented liquid	7.00 ± 0.03	6.88 ± 0.01	6.76 ± 0.02
Analytical result of powdered fermented soybean hypocotyls	Equol concentration in powdered soybean hypocotyl material (mg/100g)	385.6 ± 101.5	344.6 ± 62.1	417.5 ± 68.0

Note: Each example was carried out using powdered soybean hypocotyls of three different lots (N=3). The analytical results in the table are average ± SD values.

Example 4

Lactococcus 20-92 (FERM BP-10036; *Lactococcus garvieae*)

was inoculated into 5 ml of a soybean hypocotyl solution containing 10 wt.% of powdered soybean hypocotyls and 0.1 wt.% of L-arginine, and subjected to static cultivation at 37 °C for 96 hours under anaerobic conditions. After cultivation, the
5 resulting culture (fermented liquid) was sterilized by heating at 100 °C for one minute, then dried at 80 °C, and further powdered using a homogenizer, thereby obtaining a powdered fermented soybean hypocotyl material.

The powdered soybean hypocotyls used as starting
10 materials (referred to as "pre-fermentation" in Tables 2 and 3) and the obtained powdered fermented soybean hypocotyl material (referred to as "post-fermentation" in Tables 2 and 3) were analyzed for compositional components. Table 2 shows the analytical results for soybean isoflavones, and Table 3 shows the
15 analytical results for nutritional components. These results also established that fermented soybean hypocotyl materials containing high levels of equol can be produced by fermenting soybean hypocotyls with equal-producing bacterium. The results further revealed that the contents of oligosaccharides such as raffinose,
20 stachyose and the like after the fermentation remain almost the same as before, indicating that they are hardly influenced by fermentation. However, it was found that arginine is converted to ornithine by fermentation. Consequently, it was established that when arginine-added soybean hypocotyls are fermented with
25 *Lactococcus* 20-92, not only equol but also ornithine can be produced.

Table 2

Soybean isoflavones		Per 100 g
Component	Pre-fermentation	Post-fermentation
Equol	N.D.	632.0 mg
Daidzin	566.4 mg	29.7 mg
Malonyldaidzin	124.9 mg	N.D.
Acetyldaidzin	364.8 mg	25.4 mg
Daidzein	7.1 mg	24.4 mg
Dihydrodaidzein	N.D.	49.4 mg
Genistin	111.7 mg	3.2 mg
Malonylgenistin	35.1 mg	N.D.
Acetylgenistin	146.1 mg	3.7 mg
Genistein	0.9 mg	22.5 mg
Dihydrogenistein	N.D.	112.0 mg
Glycitin	331.7 mg	53.6 mg
Malonylglycitin	65.0 mg	N.D.
Acetylglycitin	169.2 mg	34.8 mg
Glycitein	19.1 mg	292.3 mg
Dihydroglycitein	N.D.	8.2 mg
Total isoflavones	1942.0 mg	1291.2 mg

N.D. refers to "Not Detected"

Table 3

Nutritional component		Per 100 g
Component	Pre-fermentation	Post-fermentation
Moisture	3.2 g	6.2 g
Protein	38.1 g	38.3 g
Fat	13.0 g	14.5 g
Ash	4.3 g	4.0 g
Saccharide	30.9 g	26.8 g
Dietary fiber	10. g	10.2 g
Energy	414 kcal	411 kcal
Sucrose	7.95 g	7.42 g
Raffinose	1.37 g	1.34 g
Stachyose	9.04 g	8.38 g
Trans fatty acids	N.D.	N.D.
Phospholipids(as stear-, ole-, and lecitin)	3.33 g	2.92 g
Free arginine	881 mg	12 mg
Free ornithine	N.D.	1.06 g
Syasapogenol A	N.D.	N.D.
Soyasapogenol B	N.D.	N.D.
Soybean saponine	3.6 g	3.8 g

N.D. refers to "Not Detected"

Examples 5-11

Powdered fermented soybean hypocotyls (Examples 5-11) were produced under the same conditions as in Example 3, except that powdered soybean hypocotyls of seven different lots from that in Example 3 were used. Proportions of isoflavones contained in the thus-obtained fermented soybean hypocotyl materials were evaluated. As is clear from the results shown in Table 4, the fermented soybean hypocotyl materials of Examples 5-11 have a high equol content and contain isoflavones in such proportions that cannot be achieved by known methods.

Table 4

	Isoflavone Proportions			
	Equol	Daidzein Compounds	Genistein Compounds	Glycitein Compounds
Example 5	6.51 mg (62.2 wt.%)	0.71 mg (6.8 wt.%)	0.53 mg (5.1 wt.%)	2.71 mg (25.9 wt.%)
Example 6	6.25 mg (61.3 wt.%)	0.48 mg (4.7 wt.%)	0.35 mg (3.4 wt.%)	3.12 mg (30.6 wt.%)
Example 7	5.38 mg (48.9 wt.%)	1.18 mg (10.7 wt.%)	1.45 mg (13.2 wt.%)	3.00 mg (27.2 wt.%)
Example 8	6.43 mg (63.4 wt.%)	0.61 mg (6.0 wt.%)	0.48 mg (4.7 wt.%)	2.62 mg (25.8 wt.%)
Example 9	6.05 mg (64.2 wt.%)	0.51 mg (5.4 wt.%)	0.30mg (3.2 wt.%)	2.57 mg (27.3 wt.%)
Example 10	6.11 mg (65.6 wt.%)	0.37 mg (4.0 wt.%)	0.10 mg (1.1 wt.%)	2.74 mg (29.4 wt.%)
Example 11	6.3 mg (60.9 wt.%)	0.49 mg (4.73 wt.%)	0.37 mg (3.6 wt.%)	3.19 mg (30.8 wt.%)

In Table 4, the upper figures indicate the amount (mg) of each isoflavone per 1 g of fermented soybean hypocotyl material, and the lower figures indicate the percentage (wt%) of each isoflavone per total weight (100 %wt) of isoflavones contained in each fermented soybean hypocotyl material.

Example 12

Powdered fermented soybean hypocotyls were produced under the same conditions as in Example 3 above, except that powdered soybean hypocotyls of a different lot from that in Example 3 above were used. The obtained fermented soybean hypocotyl material contained 6.5 mg of equol, 0.6 mg of daidzein

compounds, 0.6 mg of genistein compounds, and 3.2 mg of glycitein compounds, per g. Aglycone accounted for 90 wt.% or more in the total isoflavone content in the fermented soybean hypocotyl material.

5 Tablets having the following formula (weight 2.51 g and 10.9 mg equol content per tablet) were prepared using the thus obtained fermented soybean hypocotyls.

	Fermented soybean hypocotyl material	66.7 wt.%
10	<u>Erythritol</u>	<u>33.2 wt.%</u>
	Total	100.0 wt.%

Example 13

15 Granules having the following formula were prepared using the fermented soybean hypocotyl material used in Example 5 above.

	Fermented soybean hypocotyl material	66.7 wt.%
	<u>Erythritol</u>	<u>33.2 wt.%</u>
	Total	100.0 wt.%

20

Example 14

Cosmetic product having the following formula were prepared using the fermented soybean hypocotyl material of Example 1 above.

	Fermented soybean hypocotyl material	10 g
25	Paraffin oil	60 ml
	Olive oil	40 ml
	Glycerol monostearic acid ester	50 ml
	Lanolin	10 ml
	propylene glycol	30 ml
30	<u>Water</u>	<u>balance</u>
	Total	1000 g

Example 15

35 Cosmetic product having the following formula were prepared using the fermented soybean hypocotyl material of Example 1 above.

	Fermented soybean hypocotyl material	10 g
	Paraffin oil	30 ml
	Olive oil	30 ml
	Glycerol monostearic acid ester	60 ml
5	Lanolin	20 ml
	propylene glycol	40 ml
	Water	balance
	Total	1000 g

10 Test Example 1

It is known that soybean hypocotyls inherently contain Gym4, Gm30K, Gm28K, 7S globulin mix (β -conglycine), oleocine, trypsin inhibitor and like allergens. The presence or absence of allergens in the fermented soybean hypocotyl material prepared in
15 Example 1 was detected by the following method.

First, an adequate amount of the fermented soybean hypocotyl material prepared in Example 1 was added to an extraction buffer (Tris HCl pH 7.5, containing 1 M EDTA and an adequate amount of protease inhibitor), followed by sufficient
20 agitation to extract water-soluble components from the fermented soybean hypocotyl material. Subsequently, solid matter was removed therefrom by filtration, giving an extract. Total protein in the thus-obtained extract was assayed using a Bio-Rad Protein Assay. Major allergens (Gym4, Gm30K, Gm28K, 7S globulin mix,
25 oleocine, and trypsin inhibitor) contained in the thus-obtained extract were then detected by western blotting. For comparison, detections of total protein and major allergens were conducted in the same manner as described above using soybean cotyledon and soybean hypocotyl powders instead of the fermented soybean
30 hypocotyl material.

Figs. 2 to 4 show the results. Fig. 2 shows the results of total protein detection. Fig. 3 shows the results of Gym4, Gm30K, and Gm28K detections. Fig. 4 shows the results of 7S globulin mix, oleocine, and trypsin inhibitor detections.

35 From these results, it was confirmed that major

allergens inherently contained in soybeans or soybean hypocotyls are eliminated in the fermented soybean hypocotyl material.

CLAIMS

1. An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein, which contains 0.1 to 20 wt % of equol per total weight of dry fermented soybean hypocotyl material.

10

2. An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein, which further contains daidzin compounds, genistin compounds, genistei compounds, glycitei compounds and glycitei compounds.

15

3. An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein, which further contains ornithine.

20
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4. An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein, which is a dietary supplement.

30

5. An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting

35

of diadzein glycosides, daidzein, and dihydrodaidzein, which is a food containing 0.1 to 90 g of the fermented soybean hypocotyl material per 100 g of the food.

5 6. An equol-containing fermented soybean hypocotyl material obtained by fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein, which is
10 used for lowering the blood cholesterol level.

 7. A fermented soybean hypocotyl material according to any of claims 1 to 6, wherein said at least one microorganism is a lactic acid bacterium of the genus *Lactococcus*.

15

 8. A fermented soybean hypocotyl material according to any of claims 1 to 7, wherein said at least one microorganism belongs to *Lactococcus garvieae*.

20 9. A food containing a fermented soybean hypocotyl material of any of claims 1 to 3.

 10. The food according to claim 9, which is a dietary supplement.

25

 11. The food according to claim 9, which contains 0.1 to 90g of the fermented soybean hypocotyl material per 100 g of the food.

30 12. A pharmaceutical preparation containing a fermented soybean hypocotyl material of any of claims 1 to 3.

 13. The pharmaceutical preparation according to claim 12, which is used for preventing or treating menopausal disorders,
35 osteoporosis, prostatic hypertrophy, or metabolic syndrome.

14. A pharmaceutical preparation according to claim 12, which is used for lowering the blood cholesterol level.

5 15. Use of a fermented soybean hypocotyl material of any of claims 1 to 3 for producing preparations for preventing or treating menopausal disorders, osteoporosis, prostatic hypertrophy, or metabolic syndrome.

10 16. Use of a fermented soybean hypocotyl material of any of claims 1 to 3 for producing preparations for lowering the blood cholesterol level.

15 17. A cosmetic product containing the fermented soybean hypocotyl material of any of claims 1 to 3.

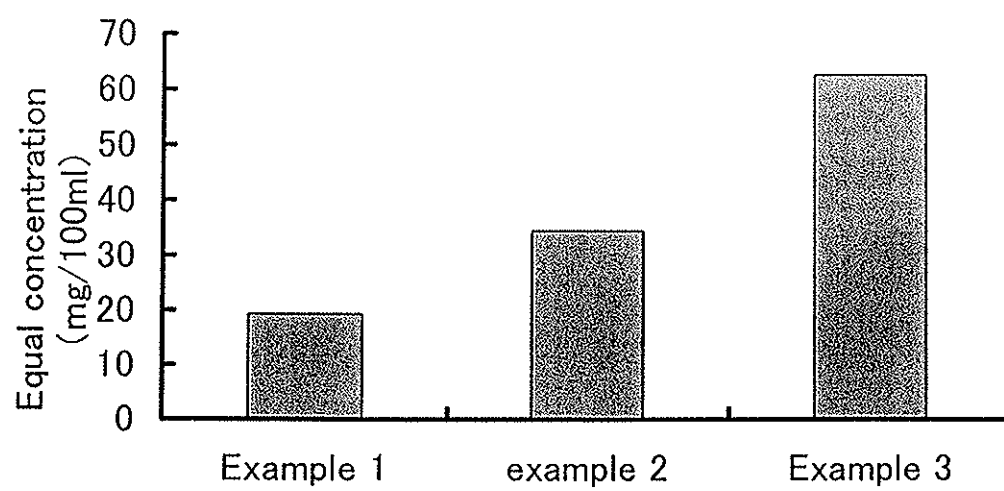
18. A cosmetic product according to claim 17, which contains 0.1 to 10 g of the fermented soybean hypocotyl material per 100 g of cosmetic product.

20

19. A method for producing an equol-containing fermented soybean hypocotyl material of any of claims 1 to 3, comprising the step of fermenting soybean hypocotyls using at least one microorganism having an equol-producing ability by utilizing at least one daidzein compound selected from the group consisting of diadzein glycosides, daidzein, and dihydrodaidzein.

25

Fig. 1



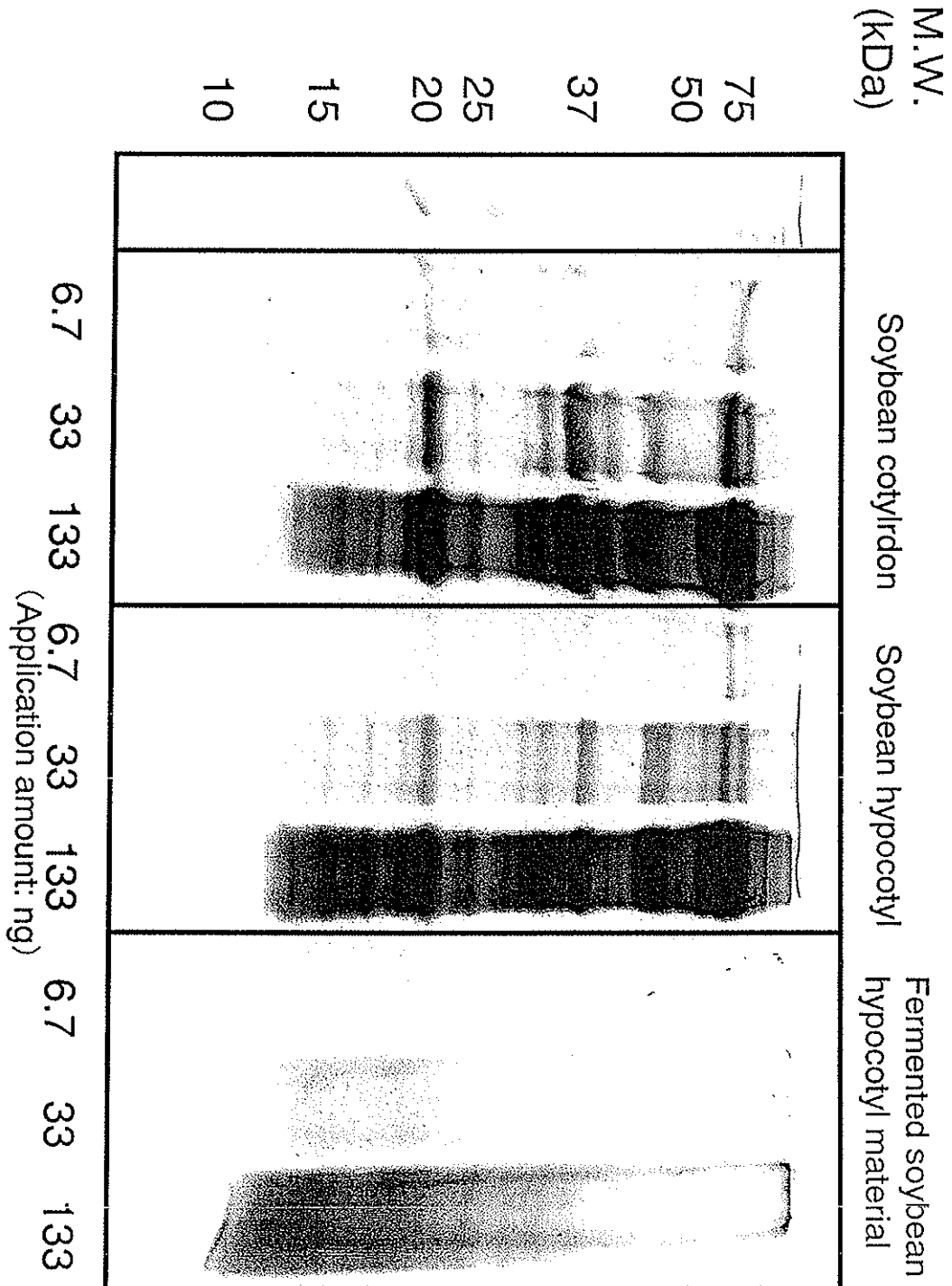


Fig. 3

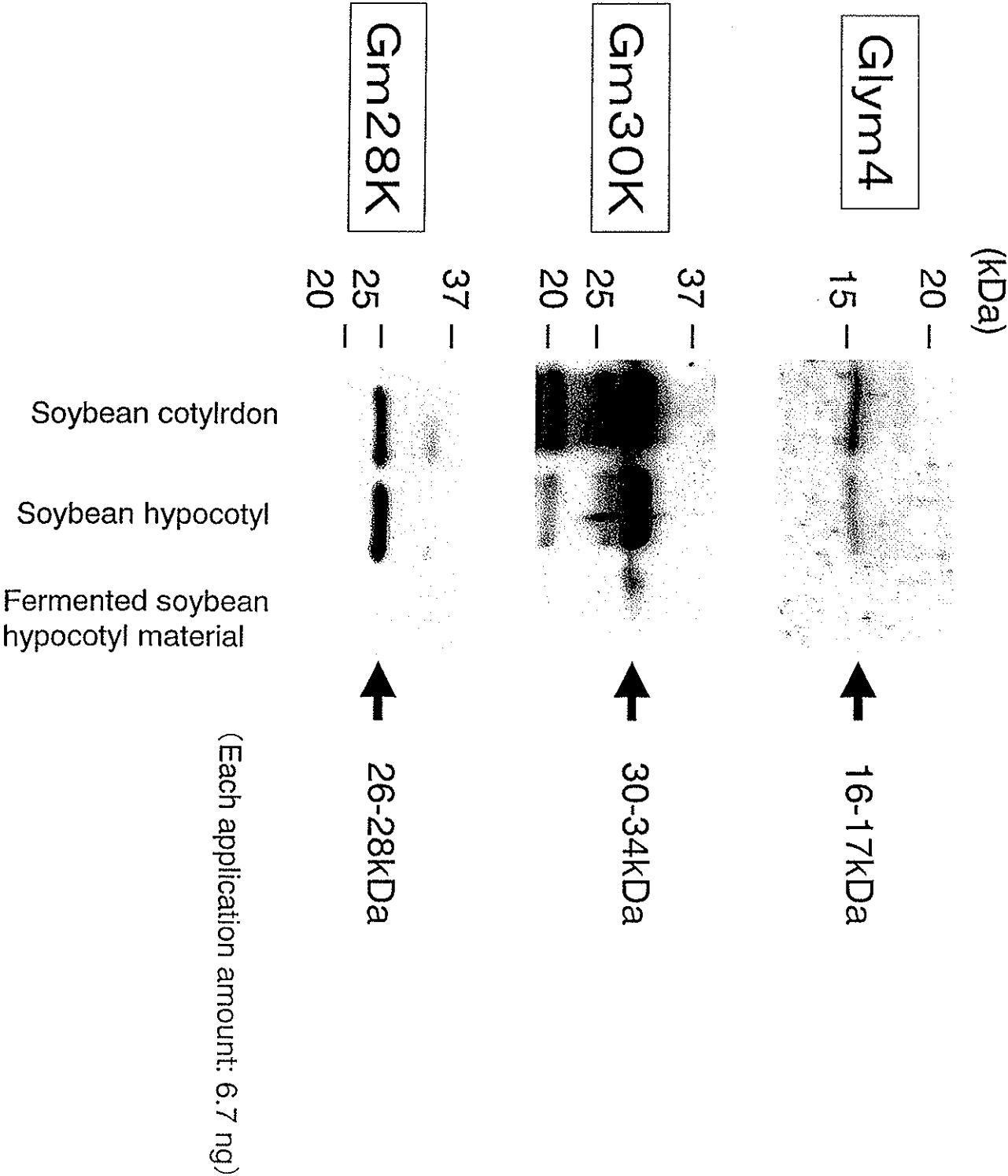


Fig. 4

