DESCRIPTION

Title of Invention: PLANT EMBRYO FOR CELL-FREE PROTEIN SYNTHESIS

Technical Field

[0001]

This invention relates to plant germ for cell-free protein synthesis; an extract and a reaction liquid for cell-free protein synthesis, obtained using the plant germ; a method for obtaining an extract for cell-free protein synthesis; a method for cell-free protein synthesis; and a method for controlling the quality of plant germ.

Background Art

[0002]

Artificial synthesis of protein is not limited to academic applications. The need therefor has recently increased in various situations. An ideal method of artificial protein synthesis would be the one that can produce the target protein inexpensively, stably, and in large quantities and can be carried out conveniently without requiring special equipment or complicated processes.

[0003]

Methods for synthesizing proteins artificially include introducing a target gene into cells originating from various organisms, including mammals, insects, plants, and *E. coli*. Apart from this, cell-free protein synthesis systems that use materials derived from wheat or rabbit reticulocytes are known (see patent literature 1). Cell-free protein synthesis is a technique that performs the process of protein synthesis *in vitro*, for example, in a test tube. This process involves transcribing genetic information from DNA into mRNA, and then having ribosomes translate the mRNA information to synthesize the target protein.

Cell-free protein synthesis systems have advantages, such as accurate translation, and disadvantages, such as poor synthesis efficiency and yield. Various studies have been conducted to overcome these disadvantages. Examples of reported techniques for wheat germ-based cell-free protein synthesis systems include preparing a formulation by lyophilization of a cell extract-containing liquid (patent literature 2), adding a polyhydric

alcohol for stabilization during frozen storage (patent literature 3), removing impurities originating from animals and microorganisms (patent literature 4), maintaining the initial substrate concentration (patent literature 1), and concentrating an extracted liquid for use in protein synthesis and maintaining the resulting protein concentration at or above a given level (patent literature 5).

Citation List

Patent Literature:

[0004]

Patent literature 1: JP 1-503119A

Patent literature 2: JP 2000-316594A

Patent literature 3: US 2003/0199076A1

Patent literature 4: JP 2007-97438A

Patent literature 5: JP H6-225783A

Summary of Invention

[0005]

Using wheat, which is inexpensively available, as the material for cell-free protein synthesis systems is expected to ensure the large-volume synthesis of target proteins at low cost. However, the abovementioned conventional techniques relating to cell-free protein synthesis do not improve the yield as much as expected. Some of these techniques require special equipment. When an additive is used, the synthesized protein has limited applications. In addition, specific cell-free protein synthesis systems applicable to these techniques are limited, and these techniques thus lack versatility.

[0006]

The invention relates to a technique for plant germ-based cell-free protein synthesis systems that enables inexpensive, high-yield synthesis of desired proteins without requiring complicated equipment or processes.

[0007]

As a result of intensive investigations on wheat germ-based cell-free protein synthesis systems, the inventors have found that the moisture content of wheat germ

before extracting its components therefrom largely impacts the wheat germ-based cell-free protein synthesis activity. Further studies based on this finding have led to the completion of the invention.

[0008]

The invention provides plant germ for cell-free protein synthesis, the plant germ having a moisture content of 30% or less. Preferably, the plant germ is prepared from plant seeds and stored at or below normal temperature.

The invention also provides an extract for cell-free protein synthesis, the extract being obtained from the plant germ.

[0009]

The invention also provides a reaction liquid for cell-free protein synthesis, the reaction liquid containing the extract.

[0010]

The invention also provides a method for obtaining an extract for cell-free protein synthesis from plant germ. The method includes the following steps: (1) adding water to dry plant germ and leaving the plant germ to stand, (2) grinding and homogenizing the plant germ after standing, (3) adding a solution for extracting germ components to the plant germ to dissolve the germ, and (4) purifying the dissolved plant germ.

[0011]

The invention also provides a method for cell-free protein synthesis, the method including using the plant germ extract.

[0012]

The invention also provides a method for controlling the quality of plant germ. The method includes measuring the moisture content of the plant germ and determining whether the plant germ is usable for cell-free protein synthesis based on whether the measured moisture content is 30% or less.

Brief Description of Drawings

[0013]

[Fig. 1] Fig. 1 is a graph showing the evaluation results in Examples and Comparative Examples.

[Fig. 2] Fig. 2 is a graph showing the effect of storage period on protein synthesis activity.

[Fig. 3] Fig. 3 is a graph showing the effect of storage temperature on protein synthesis activity.

Description of Embodiments

[0014]

The invention (plant germ, an extract, and a reaction liquid for cell-free protein synthesis; a method for obtaining a germ extract; and methods for cell-free protein synthesis and for controlling plant germ quality) enables inexpensive, high-yield synthesis of desired proteins in cell-free protein synthesis systems without requiring complicated equipment or processes.

The invention provides an inexpensive, convenient way to supply desired proteins not only for research but also for a variety of applications, such as medical and diagnostic drugs, research reagents, and protein substitutes in animal feed and human food products, which will increase the industrial utilization of protein synthesis.

[0015]

The invention will be described on the basis of its preferred embodiments.

The invention relates to plant germ-based cell-free protein synthesis systems. As used herein, the term “cell-free protein synthesis systems” includes cell-free translation systems, where a protein or polypeptide is synthesized based on the information read from mRNA, and systems that include both a cell-free transcription system, where RNA is synthesized using DNA as a template, and a cell-free translation system.

Plant germ is germ of plant origin. Germs of various plants, such as wheat, barley, rice, corn, and spinach, can be used. From the standpoint of, for example, improvement in protein synthesis capability and/or ease of availability, germs from gramineous plant seeds are preferred, with wheat germ being more preferred. Examples of the proteins synthesized by the cell-free method in the invention include polypeptides

and proteins with molecular sizes generally synthesizable through cell-free methods, and specifically, polypeptides and proteins with molecular weights ranging from 10 to 200 kD may be targeted. The invention will be described sometimes specifically with reference to wheat germ, but the description equally applies to other plant germ types.

[0016]

Ribosome-containing plant germ, which is useful for cell-free protein synthesis, can be prepared from plant seeds using commonly known techniques. Methods for preparing plant germ include, for example, seed grinding, sieving, heavy liquid separation, removing seed coat and endosperm fractions through visual inspection or the like, washing out endosperm contaminants; and grinding, extraction, and purification, following the endosperm removal.

[0017]

The plant germ is preferably prepared by a method involving (A1) a plant seed grinding process and (A2) a germ separation process.

[0018]

(A1) Plant Seed Grinding Process:

To efficiently obtain the germ, it is preferable to remove as much of the parts other than germ as possible prior to grinding the plant seeds, because the germ is scarce in seeds.

The grinding process is generally performed by applying mechanical force to the plant seeds to obtain a ground mass of the plant seeds, the ground mass containing germ, crushed endosperm, and crushed seed coat. The grinding can be achieved using a known grinding apparatus. The grinding apparatus can be a device that applies an impact force to the material to be ground, such as a pin mill or a hammer mill. The degree of grinding may be appropriately selected according to the size of the germ of the plant seeds to be used. For example, wheat seeds are preferably ground to a maximum length of 4 mm or smaller, and more preferably to a maximum length of 2 mm or smaller. The grinding may be performed under dry or wet condition, and dry grinding is preferable in terms of, for example, allowing for or facilitating moisture content adjustment by controlling the amount of water added in the moisture content adjustment described later.

[0019]

(A2) Germ Separation Process:

The germ separation process preferably involves the separation of a crude germ fraction. The separation of the crude germ fraction is preferably followed by an enrichment treatment to increase the proportion of germ-derived components.

In the separation of the crude germ fraction, the crushed endosperm, crushed seed coat, and the like are removed from the ground seeds resulting from the grinding process to yield a crude germ fraction. The separated crude germ fraction contains germ as a main component, along with the ground endosperm and ground seed coat. The crude germ fraction is collected from the ground mass of the plant seeds using known classifiers, such as sieves. For example, a crude germ fraction with a mesh size of 0.5 to 2.0 mm, preferably 0.7 to 1.4 mm can be typically obtained in cases of wheat seeds. If necessary, the resulting crude germ fraction may be freed of the seed coat, endosperm, dust, and the like by wind power or static electricity.

Subsequently, or in place of the abovementioned classification, for example, with sieves, a crude germ fraction may also be obtained using a separation technique that takes advantage of the difference in specific gravity between the germ and the seed coat and endosperm, for example, a heavy liquid separation process. The heavy liquid separation process involves placing the ground plant germ in a liquid and removing the seed coat and endosperm fraction by utilizing the difference in specific gravity, yielding the crude germ fraction. A mixture of carbon tetrachloride and cyclohexane is generally used as the liquid. Chlorinated organic solvents, such as carbon tetrachloride, are preferable, since the solvent may be selected with due consideration of environmental and health aspects, depending on the intended use of the protein to be synthesized. To obtain a crude germ fraction containing as much germ as possible, two or more of the above-described techniques can be used in combination.

[0020]

A germ fraction may be obtained by separating the germ from the resulting crude germ fraction, for example, through visual inspection or using a color sorter. To remove any endosperm components that may still be attached, it is preferable that the germ fraction be further enriched by washing. The washing is preferably carried out by, for example, dispersing and suspending the germ fraction in water or an aqueous solution typically cooled to 10°C or lower, preferably 4°C or lower, repeatedly until the washing

no longer becomes cloudy. This treatment is more preferably performed by dispersing and suspending the germ fraction in an aqueous solution of a surfactant typically cooled to 10°C or lower, preferably 4°C or lower, repeatedly until the washing no longer becomes cloudy. The surfactant is preferably nonionic. A wide range of nonionic surfactants can be utilized, and preferable examples thereof include Brij, Triton, Nonidet® P40, and Tween, which are polyoxyethylene derivatives, with Nonidet® P40 being the most suitable. These nonionic surfactants can be used in concentrations, e.g., of approximately 0.5%.

The washing using water or an aqueous solution and the washing using a surfactant may be used individually or in combination. The washing may be combined with ultrasonication.

[0021]

The plant germ prepared and separated through the aforementioned processes is preferably stored at temperatures below normal temperature. The temperatures equal to or lower than normal temperature are typically 25°C or lower, preferably 4°C or lower, more preferably -20°C or lower, and even more preferably -80°C or lower. Storing the plant germ at temperatures in the above range brings about improved efficiency in the cell-free protein synthesis that occurs subsequently. Since the processes for preparing wheat germ are performed at 4°C, it is important that the wheat germ be rapidly placed in an environment lower than normal temperature.

From the same viewpoint, the plant germ from the grinding process is preferably maintained at or below normal temperature during the storage until cell fluid extraction or during the storage and treatments.

[0022]

(Moisture Content Adjustment)

In order to enhance the protein synthesis activity and improve the protein yield when the plant germ, its extract, or a similar product of the invention is used for cell-free protein synthesis, it is preferable to adjust the moisture content of the plant germ prior to the process of extracting the components that participate in protein synthesis from the plant germ. The adjustment of the moisture content is conducted on, for example, the germ fraction resulting from the grinding process (A1) and the separation process (A2).

The germ's moisture content (% by mass) after the adjustment is preferably 30% or less, more preferably 28.6% or less, and even more preferably 24.8% or less. Although no particular lower limit is imposed, the moisture content may be, for example, 1.6% or more, and is preferably 18.8% or less.

The adjustment of the germ's moisture content can be accomplished in the following manner, for example. For instance, 1.6 to 8.0 mL of water is added to 20 g of wheat germ, and after storing the mixture under refrigeration at 4°C for one day, the moisture content of the germ is determined.

[0023]

The method for adjusting the moisture content of the plant germ is not particularly restricted as long as the final moisture content is as designed (e.g., 30% or less). The amount of water to be added and the way of adding water (conditions) are selected as appropriate. Regarding the way of adding water, for example, the plant germ may be immersed in water, or atomized water may be applied to the germ using a spray. Following the addition of water, the germ may be refrigerated for approximately one day to ensure that the moisture content is evenly distributed. The moisture content, whether excessive or insufficient, affects protein synthesis. In a preferred embodiment of the invention, therefore, the moisture content may be controlled appropriately by lyophilization, in addition to by adjusting the amount of water added. When lyophilization is carried out, the moisture content resulting from the lyophilization may be used as such, or may be adjusted to a desired level by adding a predetermined amount of water to the germ after lyophilization.

The germ's moisture content after the moisture content adjustment can be calculated from formula:

Germ's moisture content after adjustment (mass%) = [(mass after moisture content adjustment – dry mass before moisture content adjustment)/(mass after moisture content adjustment)] × 100

The dry mass is obtained by weighing 2 g of wheat germ into a heat-resistant container, heating the wheat germ at 135°C for one hour, reweighing the heated germ, and subtracting the mass loss from the initial mass of the wheat germ. The weight is measured in increments of 0.001 g.

[0024]

The water added to the germ for moisture content adjustment includes, but is not limited to, pure water and ion-exchanged water.

The amount of the plant germ to be used in a cell-free protein synthesis system can be controlled on the basis of the dry mass before water addition.

[0025]

(Permeation Treatment in Low-Temperature Environment)

The plant germ after water addition may be subjected to a permeation treatment where the plant germ is left in a low-temperature environment for a predetermined period of time. The germ is preferably left to stand during the treatment. This treatment allows the water to permeate the interior of the plant germ aggregate, which leads to the improvement of cell-free protein synthesis capability. The permeation treatment is carried out by leaving the germ after the water addition to stand at 4°C for 24 hours, for example.

[0026]

When the permeation treatment in a low-temperature environment is performed, the treatment may be incorporated as part of the moisture content adjustment.

The plant germ resulting from the moisture content adjustment is a preferred embodiment of the plant germ of the invention for cell-free protein synthesis. When used for protein synthesis according to cell-free methods, the plant germ of this embodiment improves protein synthesis capability as compared with plant germ with a moisture content exceeding 30%. Additionally, performing the moisture content adjustment stably and reliably improves the protein synthesis capability as compared with not performing the moisture content adjustment.

The plant germ with an adjusted moisture content of 30% or less is preferably used in the form of its extract in cell-free protein synthesis.

[0027]

A plant germ extract is obtained from the resulting plant germ. The method for obtaining the plant germ extract may be any known method. The plant germ may be pulverized and the resulting pulverized germ may be used. For example, the plant germ

may be frozen with liquid nitrogen and then pulverized, and an extraction solvent is added to the pulverized germ. After stirring, a liquid containing germ extract is collected from the mixture by centrifugation or other means. If necessary, the liquid can be purified by gel filtration, for example.

[0028]

The method for obtaining an extract or a reaction liquid of the invention for cell-free protein synthesis from plant germ with an adjusted moisture content will be described on the basis of its preferred embodiment.

The method for obtaining the extract from the plant germ preferably includes (B1) a grinding process and (B2) an extraction process and a purification process.

(B1) Grinding Process

The grinding process is preferably a grinding and homogenizing process, in which a raw feed is stirred during grinding thereby to accomplish homogenization of the ground product.

In the grinding and homogenizing process, the plant germ is ground in a grinding apparatus, such as a mill. Preferably, the plant germ after the moisture content adjustment is frozen with, for example, liquid nitrogen, and the frozen germ is ground in a grinding apparatus. Homogenization can be achieved by employing a grinding method that involves stirring.

Homogenization by stirring during grinding or grinding followed by a separate stirring process is preferred for reliably improving the protein synthesis capability in a cell-free protein synthesis method. Grinding the germ in a frozen state is preferred for suppressing the decomposition or denaturation of the components extracted from the germ that participate in protein synthesis.

When the plant germ after the moisture content adjustment is stored until extraction, the storage temperature is preferably 25°C or lower, more preferably 10°C or lower, for suppressing the decomposition or denaturation of the components participating in protein synthesis. It is preferable that the plant germ be stored in a frozen state in a centrifuge tube, as described later.

[0029]

(B2) Extraction Process and Purification Process

The extraction process is carried out, for example, as follows. An adequate amount of the ground germ is weighed out and placed in a centrifuge tube, a flask, or another suitable container. A solution for extracting germ components is added, and the mixture is shaken. After shaking, the mixture is subjected to centrifugation, either directly as it is in the centrifuge tube or after being transferred to a centrifuge tube, and the resulting supernatant is collected. The collected supernatant is preferably sterilized and/or subjected to gel filtration. A desired germ liquid extract is thus obtained.

An extraction buffer can be used as the solution for extracting germ components. Examples of the extraction buffer includes Extraction buffers 1 and 2 used in Examples given later. The solution for extracting germ components preferably contains at least potassium acetate, magnesium acetate, and a reducing agent, such as dithiothreitol (hereinafter DTT), and more preferably further contains calcium chloride, etc. The sterilization process is preferably a treatment that can be performed at low temperatures for preventing denaturation of the components contained in the resulting wheat germ extract. The gel filtration process is designed to purify the plant germ dissolved in the solution for extracting germ components.

[0030]

The germ liquid extract obtained from plant germ usually contains at least ribosomes and translation factors. The translation factors include initiation factors, elongation factors, and release factors. The liquid extract obtained from the plant germ with its moisture content adjusted to 30% or less or a liquid or solid obtained from the germ liquid extract by, for example, lyophilization, spray drying, or concentration is a preferred embodiment of the extract of the invention, which is preferably used in cell-free protein synthesis systems.

The synthesis of a target protein by a cell-free protein synthesis system using the extract of the invention for cell-free protein synthesis can be carried out with the resulting germ extract using any known method. The extract may be used in a cell-free protein synthesis reaction with a commercially available kit for the synthesis. The cell-free protein synthesis reaction can be performed using any known techniques. Commercially available kits for synthesis reactions may be used.

[0031]

In a cell-free translation synthesis system, where a protein or polypeptide is synthesized based on the information read from mRNA, mRNA carrying the information of a target protein is added to the plant germ extract containing ribosomes and factors necessary for protein synthesis, such as translation factors, to cause a reaction, whereby the target protein can be produced efficiently in an increased yield.

When a cell-free protein synthesis method includes a cell-free transcription system for RNA synthesis using DNA as a template and a cell-free translation system, the synthesis reaction kit preferably includes components for the cell-free translation system, such as RNA polymerase, deoxynucleotide triphosphate, an amino acid solution, and creatine kinase; and those for the cell-free transcription system, such as DNA polymerase, magnesium sulfate, and various proteins participating in transcription. The plant germ extract containing ribosomes and factors necessary for protein synthesis, such as translation factors, and DNA carrying the information about a target protein are added to such a synthesis reaction kit to cause a reaction, whereby the target protein can be produced efficiently in an increased yield.

[0032]

The protein synthesis efficiency in a cell-free protein synthesis method using the extract of the invention can be determined using, for example, the method described below. Determining the synthesis efficiency in advance enables estimating the yield of the protein to be synthesized. While “the extract of the invention” is preferably the liquid extract prepared from plant germ with a moisture content adjusted to 30% or less using the method described above, the method of extraction, the properties of the extract, and other conditions are not particularly limited, as long as the extract is obtained from plant germ with a moisture content of 30% or less.

[0033]

Using a cell-free protein synthesis kit, an mRNA sequence is synthesized from the DNA sequence of a protein whose properties resemble the properties of a target protein. Examples of the proteins used to determine the synthesis efficiency include, but are not limited to, fluorescent proteins, such as GFP, and any protein can be used to determine the synthesis efficiency. A fluorescent protein, such as GFP, is preferable

since a value reflecting the protein quantity can be readily obtained from the intensity of the fluorescence generated upon irradiation with specific excitation light. To determine the synthesis efficiency, protein synthesis is performed by, for example, using the synthesized mRNA as a template, and the yield is measured after a predetermined time has elapsed. For instance, after a predetermined period of time has elapsed since translation initiation, the translation reaction solution placed in a plate reader is irradiated with excitation light at 485 nm, and the fluorescence intensity is measured.

[0034]

The cell-free protein synthesis method of the invention encompasses a broad range of protein synthesis methods in cell-free systems using the above-described plant germ or its extract for cell-free protein synthesis or a reaction liquid for cell-free protein synthesis containing the extract, and is not particularly limited by the type of proteins to be synthesized, type of template DNA used, production method, etc.

A typical reaction liquid of the invention for cell-free protein synthesis is a mixture including the liquid extract obtained by the aforementioned method from plant germ with a moisture content adjusted to 30% or less, and other components necessary for cell-free protein synthesis. The liquid extract typically contains ribosomes and translation factors. Examples of the other components include an amino acid solution, creatine kinase, and an mRNA solution obtained by transcription from the template DNA.

[0035]

The method of the invention for obtaining a germ extract is a method for enhancing protein synthesis capability in cell-free protein synthesis. The method includes the following steps (1) to (4), preferably in the order described:

(1) adding water to dry plant germ and leaving the plant germ to stand,

(2) grinding and homogenizing the plant germ after standing,

(3) adding a solution for extracting germ components to the plant germ to dissolve the plant germ, and

(4) purifying the dissolved plant germ.

[0036]

In a preferred embodiment, Step (1) corresponds to the moisture content

adjustment process. Leaving the plant germ to stand is exemplified by the permeation treatment in a low-temperature environment. The term “dry plant germ” refers to wheat germ collected during wheat milling, and also encompasses lyophilized wheat germ. The permeation treating time or standing time following the water addition is preferably 1 hour or longer, and more preferably 6 hours or longer. There is no upper limit to the treating or standing time; for example, it may be as long as 3 months. Step (2) corresponds to the grinding and homogenizing process of the grinding process (B1). Step (3) and Step (4) correspond to the extraction and purification processes (B2).

In Step (3), the phrase “to dissolve the plant germ” means to dissolve, into the solution for extracting the germ components, the ribosomes, translation factors, and other components which are necessary to translate protein from mRNA. In Step (4), the phrase “purifying the dissolved plant germ” means to purify and recover the components dissolved in Step (3).

The method of the invention for obtaining a germ extract enables efficient preparation of a germ extract that easily enhances protein synthesis capability of cell-free protein synthesis systems without requiring complicated apparatus and processes.

[0037]

It is widely known that reducing the water content of a reaction liquid used in cell-free protein synthesis is effective in increasing the reaction efficiency, specifically the yield of the target protein. This intends to achieve stable continuation of the reaction to thereby increase the yield. However, it is an unexpected effect to those skilled in the art that the moisture content of the germ before extraction affects the efficiency in the protein synthesis reaction, such that adjustment of the moisture content of plant germ improves the synthesis efficiency, despite the fact that a large quantity of an aqueous medium (i.e., water) is added to the germ extract in cell-free protein synthesis.

[0038]

In another aspect, the invention can be utilized as a method for controlling the quality of plant germ. The method for controlling the quality of plant germ includes measuring the moisture content of plant germ to determine its usefulness for cell-free protein synthesis.

The quality control method is typically carried out as follows: a given amount of

plant germ is weighed out, heated, and weighed again, and the mass ratio of the mass loss to the initial mass of the plant germ is calculated. Whether the plant germ is useful for cell-free protein synthesis is determined based on whether the found moisture content is above or below a previously set threshold. The threshold may be a range.

Plant germ with a moisture content outside the threshold can be made useful for cell-free protein synthesis by re-adjusting the moisture content so as to satisfy the threshold. Using such a threshold for the moisture content of plant germ is preferable when performing cell-free protein synthesis, since it achieves efficient synthesis with reduced variation in yield among reaction batches of the cell-free protein synthesis.

Examples

[0039]

The invention will now be illustrated in greater detail by way of Examples, but it should be understood that the invention is not limited thereto.

[0040]

Comparative Example 1

Pure water was added to 20 g of wheat germ from North American hard wheat (available in a refrigerated state from Nisshin Flour Milling, Inc., hereinafter designated as “Germ A”), and the mixture was allowed to stand at 4°C for 24 hours to prepare Germ 1 and 2 with different moisture contents. The amount of pure water added was as follows:

Germ 1: 6.4 mL of pure water to 20 g of the germ,

Germ 2: 8.0 mL of pure water to 20 g of the germ.

[0041]

[Example 1]

Germ A was lyophilized using a freeze dryer (FDU-1200, from Tokyo Rikakikai Co., Ltd.) to prepare Germs 3 and 4 with different moisture contents. The lyophilization times were as follows:

Germ 3: 16 hours for 20 g of the germ,

Germ 4: 6 hours for 20 g of the germ.

[Example 2]

Germ A as untreated was used as Germ 5. Separately, pure water was added to 20 g of Germ A, and the mixture was left to stand at 4°C for 24 hours to prepare Germs 6 to 9 different in moisture content. The amounts of pure water added were as follows:

Germ 5: untreated

Germ 6: 0.8 mL of pure water to 20 g of the germ,

Germ 7: 1.6 mL of pure water to 20 g of the germ,

Germ 8: 3.2 mL of pure water to 20 g of the germ, and

Germ 9: 4.8 mL of pure water to 20 g of the germ.

[0042]

[Example 3] (Measurement of Moisture Content)

The moisture content of each wheat germ prepared in Comparative Example 1 and Examples 1 and 2 was determined. 2 g of the wheat germ was weighed out and placed in a heat-resistant container, heated at 135°C for 1 hour, and reweighed. The ratio of the mass loss to the initial mass (2 g) before heating was calculated to give the moisture content (%). The measurement results of the moisture content are shown in Table 1. The weight was measured in increments of 0.001 g.

[0043]

[Table 1]

Moisture Content of Wheat Germ

	Comp. Ex. 1		Example 1		Example 2				
Germ No.	1	2	3	4	5	6	7	8	9
Moisture Content (%)	31.2	33	1.6	5.9	14.7	18.8	20.9	24.8	28.6

[0044]

In the invention, plant germ with a moisture content of 30% or less is preferably prepared by adjusting the water content of the starting germ to 30% or less.

Germ 5 in Example 2, which was obtained from plant seeds, was germ that had been stored at or below normal temperature throughout the storage period. In this respect, Germ 5 is considered a previously unknown plant germ.

It is determined that Germ 5 in Example 2 is useful for cell-free protein synthesis, according to the plant germ quality control method in which the usefulness is determined based on whether or not the measured moisture content is 30% or less.

[0045]

[Example 4] (Preparation of Wheat Germ Extract)

A wheat germ extract was prepared from each of the germs in Comparative Example 1 and Examples 1 and 2 according to the following procedure, which is a partial modification of the method described in Methods in Enzymology (1983), Vol. 96, pp. 38-50.

(1) The wheat germ was ground and homogenized using a blender (Silent Millser from Iwatani Corp.).

(2) The wheat germ was weighed out to have a dry weight of 3 g based on the moisture content measured in Example 3, and placed in a 50-mL centrifuge tube. Ten milliliters of Extraction buffer 1, which had been prepared according to the composition of Table 1 below, was added thereto.

(3) The centrifuge tube was shaken for 5 minutes using a shaker (Utility Shaker US-S100 from AS ONE Corp.; preset speed 200 rpm).

(4) The centrifuge tube was subjected to centrifugation (23,000G, 10 mins.) using a centrifuge (Avanti JXN-30 from Beckman Coulter, Inc.).

(5) The aqueous phase of the supernatant was collected and sterilized using a 0.22-μm filter.

(6) After removing the storage solution from a PD-10 column (from Cytiva), Extraction buffer 1 shown in Table 1 was poured into the column to equilibrate the gel packed therein.

(7) A 2.5 mL portion of the impurity-free solution collected in (5) above was loaded onto the PD-10 column, and the flow-through was discarded.

(8) Through the column, 3.5 mL of Extraction buffer 2 with the composition of Table 2 was passed, and the collected flow-through was used as a wheat germ extract for the test.

[0046]

Table 2

Extraction Buffer 1	
800 mM HEPES-KOH, pH 7.8	5 mL
100 mM Magnesium Acetate	1 mL
1 M Potassium Acetate	10 mL
40 mM Calcium Chloride	5 mL
2 M DTT (dithiothreitol)	0.2 mL
Pure Water	78.8 mL
Total	100 mL

[0047]

Table 3

Extraction Buffer 2	
800 mM HEPES-KOH, pH 7.8	5 mL
100 mM Magnesium Acetate	5 mL
1 M Potassium Acetate	10 mL
2 M DTT (Dithiothreitol)	0.2 mL
Pure Water	79.8 mL
Total	100 mL

[0048]

[Example 5] (Cell-Free Protein Synthesis Method)

mRNA was synthesized from plasmid DNA having the base sequence derived from GFP, described in Table 4 below, using a cell-free protein synthesis kit (from NUProtein Co., Ltd.) in accordance with the protocol of the kit. GFP was translated using the synthesized mRNA and Amino Acid Mix from the kit in accordance with the kit protocol. Sixteen hours after the initiation of the translation, the translation reaction solution was irradiated with excitation light at 485 nm, and the fluorescence intensity of

GFP was measured at a measuring wavelength of 535 nm, using a plate reader (ARVO-X4, from Perkin-Elmer). The results obtained are shown in Fig. 1.

[0049]

[Table 4]

Base Sequence of GFP

A T G A G T A A A G G A G A A G A A C T T T T C A C T G G A G T T G T C C C A
A T T C T T G T T G A A T T A G A T G G T G A T G T T A A T G G G C A C A A A
T T T T C T G T C A G T G G A G A G G G T G A A G G T G A T G C A A C A T A C
G G A A A A C T T A C C C T T A A A T T T A T T T G C A C T A C T G G A A A A
C T A C C T G T T C C A T G G C C A A C A C T T G T C A C T A C T T T C T C T
T A T G G T G T T C A A T G C T T T T C A A G A T A C C C A G A T C A T A T G
A A A C G G C A T G A C T T T T T C A A G A G T G C C A T G C C C G A A G G T
T A T G T A C A G G A A A G A A C T A T A T T T T T C A A A G A T G A C G G G
A A C T A C A A G A C A C G T G C T G A A G T C A A G T T T G A A G G T G A T
A C C C T T G T T A A T A G A A T C G A G T T A A A A G G T A T T G A T T T T
A A A G A A G A T G G A A A C A T T C T T G G A C A C A A A T T G G A A T A C
A A C T A T A A C T C A C A C A A T G T A T A C A T C A T G G C A G A C A A A
C A A A A G A A T G G A A T C A A A G T T A A C T T C A A A A T T A G A C A C
A A C A T T G A A G A T G G A A G C G T T C A A C T A G C A G A C C A T T A T
C A A C A A A A T A C T C C A A T T G G C G A T G G C C C T G T C C T T T T A
C C A G A C A A C C A T T A C C T G T C C A C A C A A T C T G C C C T T T C G
A A A G A T C C C A A C G A A A A G A G A G A C C A C A T G G T C C T T C T T
G A G T T T G T A A C A G C T G C T G G G A T T A C A C A T G G C A T G G A T
G A A C T A T A C A A A

[0050]

As demonstrated in Fig. 1, when the wheat germ extracts obtained from wheat germ having a moisture content adjusted to 30% or less are used, the fluorescence intensity of GFP significantly increases as compared with wheat germ extracts from germ with moisture contents exceeding 30%. It is seen from the result that using plant germ with a moisture content adjusted to 30% or less improves the protein synthesis capability and the protein yield in cell-free protein synthesis systems without requiring complicated equipment or processes.

[0051]

[Reference Example 1]

A wheat germ liquid extract was obtained from the germ of North American hard

wheat having been stored at 25°C for a storage period of from 0 days (0d) to 4 weeks (4w) after the preparation of the germ. The protein synthesis activity of the germ liquid extract was determined in the same manner as in Example 5. The results obtained are shown in Fig. 2.

It can be seen in Fig. 2 that when the germ is stored at or below normal temperature, even for an extended period, the reduction of protein synthesis activity due to the storage is suppressed to some extent. These results indicate that the plant germ is preferably stored at temperatures below normal temperature.

[0052]

[Reference Example 2]

Germ obtained from North American hard wheat was stored at -80°C, -20°C, 4°C, 25°C, or 40°C for one week after the preparation of the germ. Then, from each germ, a germ liquid extract was prepared in the same manner as in Example 4. The protein synthesis activity of each germ liquid extract was determined using the same method as in Example 5. The results obtained are shown in Fig. 3. The germ had a moisture content of not higher than 30% both before and after the storage at different temperatures. Specifically, the moisture content before the storage was 15.3%, and the plant germ was stored in an airtight container to ensure that the initial moisture content before storage was substantially maintained.

As shown in Fig. 3, the germ stored at or above normal temperature, specifically at 40°C exhibits significantly superior protein synthesis activity compared with the germ stored at or below normal temperature. It is thus proved that it is preferable to store plant germ at temperatures lower than normal temperature.

Industrial Applicability

[0053]

According to the invention, a material necessary for plant germ-based cell-free protein synthesis is efficiently extracted from plant germ so that a germ liquid extract with reduced impurities can be provided. Thus, the invention stably and efficiently provides a germ liquid extract and a solution for cell-free protein synthesis. Using the germ liquid extract or solution of the invention for protein synthesis enables stable and efficient cell-free protein synthesis. Therefore, the invention contributes to large-volume

production of proteins for use in not only research but also various fields, including medical drugs, food products, and industrial products.

CLAIMS

[Claim 1] Plant germ for cell-free protein synthesis, having a moisture content of 30% or less.

[Claim 2] The plant germ according to claim 1, prepared from plant seeds and stored at or below normal temperature.

[Claim 3] An extract for cell-free protein synthesis, from the plant germ according to claim 1 or 2.

[Claim 4] A reaction liquid for cell-free protein synthesis, comprising the extract according to claim 3.

[Claim 5] A method for obtaining an extract for cell-free protein synthesis from plant germ, comprising the steps of:

(1) adding water to dry plant germ and leaving the plant germ to stand,

(2) grinding and homogenizing the plant germ after standing,

(3) adding a solution for extracting germ components to the plant germ to dissolve the plant germ, and

(4) purifying the dissolved germ.

[Claim 6] The method according to claim 5, wherein steps (1) and (2) are performed in such a manner that the plant germ to which the solution is to be added in step (3) has a moisture content of 30% or less.

[Claim 7] A method for cell-free protein synthesis, comprising using the extract obtained by the method according to claim 5 or 6.

[Claim 8] A method for controlling plant germ quality, comprising measuring a moisture content of plant germ and determining whether the plant germ is usable for cell-free protein synthesis based on whether the measured moisture content is 30% or less.