[DESCRIPTION]

[Invention Title]

EDIBLE MEDIUM COMPOSITION WITH EXCELLENT CELL PROLIFERATION ACTIVITY

[Technical Field]

The present invention relates to an edible medium composition having excellent cell proliferative activity.

[Background Art]

According to a report by the Food and Agriculture Organization of the United Nations (FAO), the global population is projected to increase from 7.6 billion in 2018 to 9.5 billion by 2050. However, due to abnormal global climates such as warming, crop yields are expected to decrease, whereas demand for feed grains will increase, thereby increasing production costs and decreasing production area, and livestock products as food resources are expected to become expensive foods.

Although animal-derived foods such as livestock products are expensive in terms of energy and production, they have directly contributed to securing human nutrition because they are the best sources of high-quality proteins and micronutrients essential for normal human growth and health. In particular, essential amino acids are nutrients that must be ingested through food because they are not synthesized in the body or are synthesized only in very small amounts. In view of the prediction that demand for animal-derived foods will reach 550 million tons by 2050, twice the current level, conventional livestock-production methods have limitations in supplying the proteins required to provide essential amino acids.

Cell-cultured food products are part of alternative foods that have become a focus of development by numerous companies worldwide as a means for addressing public-

health, environmental, and animal-welfare issues associated with animal husbandry and agriculture.

Culture media currently used for cell-cultured foods use highly purified or purified growth media as components required to aid the proliferation of plant or animal cells, and this approach is similar to biotechnology used in pharmaceutical manufacturing.

That is, because media for culturing cell-cultured foods use expensive and energy-intensive pharmaceutical-grade materials and processes, more resources are used to purify the components included in the media, which suggests that cell-cultured foods may inherently have a more adverse impact on the environment.

Accordingly, there is a need to develop an edible medium that, while capable of reducing future environmental impact, improves performance and at the same time lowers the cost of cell culture media.

[Disclosure]

[Technical Problem]

An object of the present invention is to provide an edible medium composition having excellent cell proliferative activity.

Another object of the present invention is to provide a food composition comprising cells cultured in the edible medium composition or a culture thereof.

Another object of the present invention is to provide a method for preparing a cell-cultured food using the edible medium composition.

Another object of the present invention is to provide a method for increasing cell proliferative activity.

Another object of the present invention is to provide a method for inhibiting cellular senescence.

[Technical Solution]

To achieve the objects described above, the present invention provides an edible medium composition having excellent cell proliferative activity, the edible medium composition comprising one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine); one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

Further, the present invention provides a food composition comprising cells cultured in the edible medium composition or a culture thereof.

Further, the present invention provides a method for preparing a cell-cultured food, the method comprising: culturing animal cells, plant cells, or microbial cells in the edible medium composition according to the present invention; and recovering the cultured cells or a culture thereof.

Further, the present invention provides a method for increasing cell proliferative activity, the method comprising culturing cells in a medium composition comprising one

or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine); one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

Furthermore, the present invention provides a method for inhibiting cellular senescence, the method comprising culturing cells in a medium composition comprising one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine); one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and

one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

[Advantageous Effects]

The edible medium composition according to the present invention has effects of enhancing cell proliferative activity and inhibiting cellular senescence. In addition, because the edible medium of the present invention, as the term indicates, is composed of materials that can be eaten by humans, the edible medium may be usefully used as a culture medium for a cell-cultured food. Further, cells cultured in the edible medium composition of the present invention have advantages of higher food stability and lower cost than cells cultured in a conventional culture medium.

[Description of Drawings]

FIG. 1 is a diagram schematically illustrating a process of rendering a culture medium edible according to an embodiment of the present invention.

FIG. 2 is a diagram illustrating results of comparing morphologies of bovine adipose-derived stem cells according to culture media, according to an embodiment of the present invention.

FIG. 3 is a diagram illustrating results of comparing cell proliferative capacities of P3 adipose-derived stem cells according to culture media, according to an embodiment of the present invention.

FIG. 4 is a diagram illustrating results of comparing cell proliferative capacities of P4 adipose-derived stem cells according to culture media, according to an embodiment of the present invention.

FIG. 5 is a diagram illustrating results of comparing levels of cellular senescence after long-term culture of adipose-derived stem cells according to culture media, according to an embodiment of the present invention.

FIG. 6 is a diagram illustrating results of comparing expression levels of mesenchymal stem-cell markers in adipose-derived stem cells according to culture media, according to an embodiment of the present invention.

[Best Mode]

The present invention will be described in detail below through embodiments of the present invention with reference to the accompanying drawings. However, the following embodiments are provided as examples of the present invention. When it is determined that a detailed description of a technique or configuration well known to those skilled in the art may unnecessarily obscure the gist of the present invention, the detailed description may be omitted, and the present invention is not limited thereby. Various modifications and applications of the present invention are possible within the scope of the following claims and equivalents interpreted therefrom.

In addition, the terminology used in the present specification is terminology used to appropriately describe preferred embodiments of the present invention and may vary depending on the intentions of a user or operator or customs in the field to which the present invention pertains. Accordingly, definitions of the terminology should be made based on the contents throughout the present specification. Throughout the specification, when a certain part is described as “comprising” a certain component, this means that the certain part may further comprise another component rather than excluding the other component, unless specifically stated otherwise.

Throughout the present specification, “%” used to indicate a concentration of a specific material is, unless otherwise stated, (w/w) % for solid/solid, (w/v) % for

solid/liquid, and (v/v) % for liquid/liquid.

The present invention will be described in greater detail below.

In one aspect, the present invention relates to an edible medium composition having excellent cell proliferative activity, the edible medium composition comprising one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine); one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

According to an embodiment of the present invention, the arginine (Arginine) may replace arginine monohydrochloride (Arginine monohydrochloride), which is toxic when orally ingested.

According to an embodiment of the present invention, the cystine (Cystine) may replace cystine dihydrochloride (Cystine Dihydrochloride), which may cause burns to the gastrointestinal tract and respiratory tract when orally ingested.

According to an embodiment of the present invention, the tyrosine (Tyrosine) may replace tyrosine disodium salt dihydrate (Tyrosine Disodium Salt Dihydrate), which is toxic when orally ingested.

According to an embodiment of the present invention, the ferrous sulfate (Ferrous Sulfate) may replace iron(III) nitrate nonahydrate (Iron(III) nitrate nonahydrate), which is toxic when orally ingested.

According to an embodiment of the present invention, the edible medium composition may further comprise a carbon source, but is not limited thereto.

In addition, glucose (Glucose), fructose (Fructose), mannose (Mannose), ribose (Ribose), xylose (Xylose), sucrose (Sucrose), melibiose (Melibiose), cellobiose (Cellobiose), lactose (Lactose), amylose (Amylose), carbohydrate (Carbohydrate), raffinose (Raffinose), sorbitol (Sorbitol), mannitol (Mannitol), and glycerol (Glycerol), or the like may be used as the carbon source. Any material known in the art to which the present invention pertains as a carbon source that is included in a medium and used as an energy source for cells may be used without limitation.

According to an embodiment of the present invention, the edible medium composition may comprise glycine at 0.0255 to 0.0345 g/L, L-arginine at 0.0589 to 0.0798 g/L, L-cystine at 0.041 to 0.0556 g/L, L-glutamine at 0.4964 to 0.6716 g/L, L-histidine at 0.0263 to 0.0357 g/L, L-isoleucine at 0.0892 to 0.1208 g/L, L-leucine at 0.0892 to 0.1208 g/L, L-lysine hydrochloride at 0.1241 to 0.1679 g/L, L-methionine at 0.0255 to 0.0345 g/L, L-phenylalanine at 0.0561 to 0.0759 g/L, L-serine at 0.0357 to 0.0483 g/L, L-threonine at 0.0807 to 0.1093 g/L, L-tryptophan at 0.0136 to 0.0184 g/L, L-tyrosine at 0.0612 to 0.0829 g/L, L-valine at 0.0799 to 0.1081 g/L, choline chloride at 0.0034 to 0.0046 g/L, calcium pantothenate at 0.0034 to 0.0046 g/L, folic acid at 0.0034 to 0.0046 g/L, inositol at 0.0061 to 0.0083 g/L, nicotinamide at 0.0034 to 0.0046 g/L,

vitamin B6 hydrochloride at 0.0034 to 0.0046 g/L, vitamin B2 at 0.0003 to 0.0005 g/L, vitamin B1 hydrochloride at 0.0034 to 0.0046 g/L, calcium chloride at 0.17 to 0.23 g/L, ferrous sulfate at 0.0003 to 0.0005 g/L, magnesium sulfate at 0.083 to 0.1124 g/L, potassium chloride at 0.34 to 0.46 g/L, sodium bicarbonate at 3.145 to 4.255 g/L, salt at 5.44 to 7.36 g/L, sodium dihydrogen phosphate at 0.1062 to 0.1438 g/L, and anhydrous crystalline glucose at 3.825 to 5.175 g/L.

More preferably, the edible medium composition may comprise glycine at 0.026 to 0.032 g/L, L-arginine at 0.06 to 0.07 g/L, L-cystine at 0.045 to 0.05 g/L, L-glutamine at 0.5 to 0.6 g/L, L-histidine at 0.027 to 0.032 g/L, L-isoleucine at 0.09 to 0.110 g/L, L-leucine at 0.09 to 0.110 g/L, L-lysine hydrochloride at 0.14 to 0.15 g/L, L-methionine at 0.028 to 0.032 g/L, L-phenylalanine at 0.06 to 0.07 g/L, L-serine at 0.035 to 0.045 g/L, L-threonine at 0.09 to 0.1 g/L, L-tryptophan at 0.015 to 0.017 g/L, L-tyrosine at 0.07 to 0.08 g/L, L-valine at 0.085 to 0.1 g/L, choline chloride at 0.0038 to 0.0042 g/L, calcium pantothenate at 0.0038 to 0.0042 g/L, folic acid at 0.0038 to 0.0042 g/L, inositol at 0.0065 to 0.0075 g/L, nicotinamide at 0.0035 to 0.0042 g/L, vitamin B6 hydrochloride at 0.0038 to 0.0045 g/L, vitamin B2 at 0.00038 to 0.00042 g/L, vitamin B1 hydrochloride at 0.0038 to 0.0042 g/L, calcium chloride at 0.19 to 0.22 g/L, ferrous sulfate at 0.0004 to 0.00043 g/L, magnesium sulfate at 0.09 to 0.1 g/L, potassium chloride at 0.38 to 0.45 g/L, sodium bicarbonate at 3.5 to 3.8 g/L, salt at 6 to 6.6 g/L, sodium dihydrogen phosphate at 0.11 to 0.13 g/L, and anhydrous crystalline glucose at 4 to 4.6 g/L.

According to an embodiment of the present invention, the edible medium composition may enhance cell proliferative activity and inhibit cellular senescence, but is not limited thereto.

According to an embodiment of the present invention, the edible medium composition may be for culturing a cell-cultured food.

As used in the present invention, the term “cell-cultured food” means food produced or cultured using cells. Examples of the cell-cultured food include cell-cultured meat alternatives, proliferated lactic acid bacteria, cell-cultured protein alternatives, and the like.

According to an embodiment of the present invention, the cell-cultured food may be cultured meat, a fungi-based meat alternative, or an algae-based meat alternative, but is not limited thereto.

As used in the present invention, the term “cultured meat” means a meat alternative produced using cell culture technology and manufactured through a process of growing tissue or muscle using animal cells.

As used in the present invention, the term “fungi-based meat alternative” means a meat alternative produced by culturing fungi. The fungi-based meat alternative is produced using fungal cells or mycelia and is manufactured through cell culture technology or a fermentation process.

As used in the present invention, the term “algae-based meat alternative” means a meat alternative produced by culturing microalgae. Microalgae (for example, Chlorella and Spirulina) are known to have a much higher growth rate, a higher protein content, and better nutrient quality than terrestrial crops used as plant-based proteins.

In one aspect, the present invention relates to a food composition comprising cells cultured in the edible medium composition or a culture thereof.

According to an embodiment of the present invention, the cells may be animal cells, plant cells, or microbial cells, but are not limited thereto.

In the examples of the present invention, it was confirmed that, when adipose-derived stem cells were cultured using the medium composition according to the present

invention, cell proliferative activity was significantly superior to that in DMEM.

As used in the present invention, the term “culture” means a product obtained after culturing cells and includes both a form containing cultured cells and a form from which the cultured cells have been removed.

In one aspect, the present invention relates to a method for preparing a cell-cultured food, the method comprising: culturing animal cells, plant cells, or microbial cells in the edible medium composition having excellent cell proliferative activity of claim 1; and recovering the cultured cells or a culture thereof.

As used in the present specification, “culturing” may be performed according to a cell-culture method commonly used in the art to which the present invention pertains.

In addition, cell culture according to the present invention may be performed by various methods established in industrial biotechnology and, for example, by various fermentation methods established in industrial biotechnology. Continuous and discontinuous cell-culture methods may be used with the edible medium composition according to the present invention. Other known reactor technologies, for example, perfusion techniques, may also be used. A batch method is one preferred embodiment.

Batch cell culture includes fed-batch culture or simple batch culture. The term “fed-batch cell culture” refers to cell culture in which mammalian cells and a cell-culture medium are initially supplied to a culture vessel, and additional culture nutrients are supplied to the culture continuously or in discontinuous increments during a culture process, with or without periodically harvesting cells and/or products before termination of the culture. The term “simple batch culture” relates to a procedure in which all components for cell culture, including mammalian cells and a cell-culture medium, are supplied to a culture vessel at the start of a culture process.

According to one preferred embodiment of the present invention, feeding of the culture is carried out by a fed-batch method. Such feeding is beneficial for replacing medium components and nutrients that are depleted from the medium by the cells during the culture process. Typically, a feed solution comprises amino acids, one or more carbohydrates as an energy source, trace elements, vitamins, or specific ions. The feed solution is added according to the requirements of the cells, and these requirements are based on a predetermined schedule determined for a specific cell line or cell clone and product, or are measured during the culture process. To prevent a large volume increase and dilution of the medium, use of a concentrated feed solution is particularly advantageous. In some embodiments, it may also be useful to have two or more different feed solutions. This allows two or more different groups of nutrients and components to be administered to the cells independently and thus allows better adjustment of feeding conditions relating to optimal supply of specific nutrients.

In one aspect, the present invention relates to a method for increasing cell proliferative activity, the method comprising culturing cells in a medium composition comprising one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine); one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine

Hydrochloride); and one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

In one aspect, the present invention relates to a method for inhibiting cellular senescence, the method comprising culturing cells in a medium composition comprising one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine); one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

According to an embodiment of the present invention, the medium composition may comprise glycine at 0.0255 to 0.0345 g/L, L-arginine at 0.0589 to 0.0798 g/L, L-cystine at 0.041 to 0.0556 g/L, L-glutamine at 0.4964 to 0.6716 g/L, L-histidine at 0.0263

to 0.0357 g/L, L-isoleucine at 0.0892 to 0.1208 g/L, L-leucine at 0.0892 to 0.1208 g/L, L-lysine hydrochloride at 0.1241 to 0.1679 g/L, L-methionine at 0.0255 to 0.0345 g/L, L-phenylalanine at 0.0561 to 0.0759 g/L, L-serine at 0.0357 to 0.0483 g/L, L-threonine at 0.0807 to 0.1093 g/L, L-tryptophan at 0.0136 to 0.0184 g/L, L-tyrosine at 0.0612 to 0.0829 g/L, L-valine at 0.0799 to 0.1081 g/L, choline chloride at 0.0034 to 0.0046 g/L, calcium pantothenate at 0.0034 to 0.0046 g/L, folic acid at 0.0034 to 0.0046 g/L, inositol at 0.0061 to 0.0083 g/L, nicotinamide at 0.0034 to 0.0046 g/L, vitamin B6 hydrochloride at 0.0034 to 0.0046 g/L, vitamin B2 at 0.0003 to 0.0005 g/L, vitamin B1 hydrochloride at 0.0034 to 0.0046 g/L, calcium chloride at 0.17 to 0.23 g/L, ferrous sulfate at 0.0003 to 0.0005 g/L, magnesium sulfate at 0.083 to 0.1124 g/L, potassium chloride at 0.34 to 0.46 g/L, sodium bicarbonate at 3.145 to 4.255 g/L, salt at 5.44 to 7.36 g/L, sodium dihydrogen phosphate at 0.1062 to 0.1438 g/L, and anhydrous crystalline glucose at 3.825 to 5.175 g/L.

More preferably, the medium composition may comprise glycine at 0.026 to 0.032 g/L, L-arginine at 0.06 to 0.07 g/L, L-cystine at 0.045 to 0.05 g/L, L-glutamine at 0.5 to 0.6 g/L, L-histidine at 0.027 to 0.032 g/L, L-isoleucine at 0.09 to 0.110 g/L, L-leucine at 0.09 to 0.110 g/L, L-lysine hydrochloride at 0.14 to 0.15 g/L, L-methionine at 0.028 to 0.032 g/L, L-phenylalanine at 0.06 to 0.07 g/L, L-serine at 0.035 to 0.045 g/L, L-threonine at 0.09 to 0.1 g/L, L-tryptophan at 0.015 to 0.017 g/L, L-tyrosine at 0.07 to 0.08 g/L, L-valine at 0.085 to 0.1 g/L, choline chloride at 0.0038 to 0.0042 g/L, calcium pantothenate at 0.0038 to 0.0042 g/L, folic acid at 0.0038 to 0.0042 g/L, inositol at 0.0065 to 0.0075 g/L, nicotinamide at 0.0035 to 0.0042 g/L, vitamin B6 hydrochloride at 0.0038 to 0.0045 g/L, vitamin B2 at 0.00038 to 0.00042 g/L, vitamin B1 hydrochloride at 0.0038 to 0.0042 g/L, calcium chloride at 0.19 to 0.22 g/L, ferrous sulfate at 0.0004 to 0.00043 g/L, magnesium sulfate at 0.09 to 0.1 g/L, potassium chloride at 0.38 to 0.45 g/L, sodium

bicarbonate at 3.5 to 3.8 g/L, salt at 6 to 6.6 g/L, sodium dihydrogen phosphate at 0.11 to 0.13 g/L, and anhydrous crystalline glucose at 4 to 4.6 g/L.

According to an embodiment of the present invention, the medium composition may further comprise a carbon source, but is not limited thereto.

In addition, glucose (Glucose), fructose (Fructose), mannose (Mannose), ribose (Ribose), xylose (Xylose), sucrose (Sucrose), melibiose (Melibiose), cellobiose (Cellobiose), lactose (Lactose), amylose (Amylose), carbohydrate (Carbohydrate), raffinose (Raffinose), sorbitol (Sorbitol), mannitol (Mannitol), and glycerol (Glycerol), or the like may be used as the carbon source. Any material known in the art to which the present invention pertains as a carbon source that is included in a medium and used as an energy source for cells may be used without limitation.

According to an embodiment of the present invention, the cells may be animal cells, plant cells, or microbial cells, but are not limited thereto.

The examples of the present invention will be described in greater detail below with reference to the accompanying drawings. However, the following examples are provided only to embody the contents of the present invention, and the present invention is not limited thereby.

<Preparation Example> Isolation of Adipose-Derived Mesenchymal Stem Cells

A 2.2 g sample of bovine subcutaneous fat was isolated and sufficiently washed with DPBS (Gibco) to remove blood. The tissue was finely chopped using forceps/scissors, collagenase (Sigma) was added thereto, and the tissue was shaken at 37°C. When the tissue had been completely digested and single cells were observed, the same volume of FBS was added to stop the enzymatic reaction. To separate some

undigested tissue, the tissue digest was filtered through a 100 μm nylon mesh and then filtered once more through a 70 μm mesh, thereby obtaining only pure single cells. After centrifugation (1500 rpm, 5 minutes), washing was performed with 1× DPBS to obtain a cell pellet of autologous adipose tissue-derived cells (Stromal Vascular Fraction, SVF), and the cells were cultured in a culture flask (FALCON).

<Example 1> Development of Edible Medium

Referring to FIG. 1, an edible medium was developed based on basal media (Gibco) commercially sold as cell culture media by replacing components that cannot be used for food with edible components.

The components of conventional basal media for cell culture are all publicly disclosed. However, because the respective components are made as a combination of reagents developed for research use, cells cultured in the media cannot be directly applied to food. Accordingly, to manufacture an edible medium, components that were not edible were to be replaced with edible components based on the medium composition of the basal media. Some components could not be used because the components themselves had not been developed for edible use. For such components, similar edible components were identified, a medium was manufactured, and cell proliferative activity was tested.

The edible medium ultimately developed in the present invention was named SPEM (Simple planet Edible Medium), and the composition of the edible medium is shown in Table 1.

[Table 1]

Type	Component	Content (g/L)
Amino acid	Glycine	0.03
Amino acid	L-arginine	0.06938
Amino acid	L-cystine	0.048329
Amino acid	L-glutamine	0.584
Amino acid	L-histidine	0.031
Amino acid	L-isoleucine	0.105
Amino acid	L-leucine	0.105
Amino acid	L-lysine hydrochloride	0.146
Amino acid	L-methionine	0.03
Amino acid	L-phenylalanine	0.066
Amino acid	L-serine	0.042
Amino acid	L-threonine	0.095
Amino acid	L-tryptophan	0.016
Amino acid	L-tyrosine	0.07207
Amino acid	L-valine	0.094
Vitamin	Choline chloride	0.004
Vitamin	Calcium pantothenate	0.004
Vitamin	Folic acid	0.004

Type	Component	Content (g/L)
Vitamin	Inositol	0.0072
Vitamin	Nicotinamide	0.004
Vitamin	Vitamin B6 hydrochloride	0.004
Vitamin	Vitamin B2	0.0004
Vitamin	Vitamin B1 hydrochloride	0.004
Inorganic salt	Calcium chloride	0.2
Inorganic salt	Ferrous sulfate	0.000417
Inorganic salt	Magnesium sulfate	0.09767
Inorganic salt	Potassium chloride	0.4
Inorganic salt	Sodium bicarbonate	3.7
Inorganic salt	Purified salt	6.4
Inorganic salt	Sodium dihydrogen phosphate	0.125
Other	Anhydrous crystalline glucose	4.5
Other	Purified water	up to 1 L

<Experimental Example 1> Comparison of Morphologies of P2 Bovine Adipose-Derived Stem Cells

To confirm a cell-growth pattern in the edible medium prepared in Example 1, passage 2 (P2) bovine adipose-derived stem cells were seeded in the edible medium (SPEM) of Example 1 or DMEM (Dulbecco's Modified Eagle Medium, Gibco), and cell

attachment, proliferation rate, and morphology were visually observed. Each medium was prepared by adding FBS (10%, Gibco) and an antibiotic-antimycotic solution (Antibiotic Antimycotic solution (1%, Sigma)) to DMEM or Edible media. For attachment, the two conditions were compared on day 1 (the first day) after seeding, and proliferation rates were compared for 3 to 4 days. On day 1 after seeding, attachment at approximately an RUO level was observed, and the proliferation rates were similar. When the cells reached 90% confluence, the cells were treated with Trypsin-EDTA (Welgene) and incubated at 37°C for only 2 minutes, after which FBS was added to inhibit the enzymatic reaction. Detached cells were collected in a 15 ml tube and centrifuged (1500 rpm, 5 minutes) to obtain the cells, and the cells were washed once with DPBS. After obtaining a cell pellet, only viable cells were counted by Trypan blue staining to determine the total number of cells.

As a result, as shown in FIG. 2, when cell attachment, proliferation, and morphology in conventional DMEM and the edible medium of Example 1 were compared during culture of adipose-derived mesenchymal stem cells, initial cell attachment and proliferation showed patterns similar to those in the conventional medium, but the size of the cells cultured in the edible medium of Example 1 was maintained smaller than the size of the cells cultured in DMEM.

<Experimental Example 2> Comparison of Proliferative Capacity of P3 Bovine Adipose-Derived Stem Cells

To confirm the cell proliferative capacity of the edible medium prepared in Example 1, passage 3 (P3) bovine adipose-derived stem cells were subcultured, seeded at 3×10^5 cells in a 100 mm dish (Falcon), and cultured in the edible medium (SPEM) of Example 1 or DMEM (Dulbecco's Modified Eagle Medium, Gibco). The morphology and

size of the cells were visually observed, the number of cells was counted by the same method as in Experimental Example 1, and PDT (Population doubling time) was measured.

As a result of culturing the P3 adipose-derived stem cells, as shown in FIG. 3, cells in DMEM were elongated and some senescent cells were observed during culture, whereas cells in the edible medium (SPEM) of Example 1 were small and exhibited rapid proliferation. Further, as a result of counting the cells, DMEM had 9.9×10^5 cells and the edible medium (SPEM) of Example 1 had 1.26×10^6 cells. Thus, approximately 27% more cells were observed in SPEM than in DMEM, and PDT was also measured to be lower in SPEM.

In view of stem-cell characteristics, a small size and rapid proliferation are evaluated as indicating high activity. Accordingly, it was confirmed that the stem cells cultured in the edible medium developed in the present invention maintained high activity.

<Experimental Example 3> Comparison of Proliferative Capacity of P4 Bovine Adipose-Derived Stem Cells

To confirm the cell proliferative capacity of the edible medium prepared in Example 1, passage 4 (P4) bovine adipose-derived stem cells were seeded at 2×10^5 cells per 100 mm plate and cultured in the edible medium (SPEM) of Example 1 or DMEM (Dulbecco's Modified Eagle Medium, Gibco). Thereafter, the morphology and size of the cells were visually observed, the number of cells was counted by the same method as in Experimental Example 1, and PDT (Population doubling time) was measured.

As a result of culturing the P4 adipose-derived stem cells, as shown in FIG. 4, cells in DMEM were elongated and some senescent cells were observed during culture, whereas cells in the edible medium (SPEM) of Example 1 were small and exhibited rapid

proliferation. In particular, in terms of cell yield during the same culture period, the edible medium (SPEM) of Example 1 made it possible to obtain 44% more cells, which is expected to be because there were fewer senescent cells and the cells had a lower PDT.

<Experimental Example 4> Comparison of Senescence of Bovine Adipose-Derived Stem Cells

For cells cultured in DMEM, an increase in cell size was observed as the passage number increased; however, in Edible media, this phenomenon was hardly observed. To determine whether this difference was caused by a difference in the rate of cellular senescence, staining for a cellular-senescence marker was performed.

To confirm the degree of senescence of the cells cultured in the edible medium prepared in Example 1, bovine adipose-derived stem cells were cultured long-term in the edible medium (SPEM) of Example 1 or DMEM (Dulbecco's Modified Eagle Medium, Gibco), and senescent cells were identified using a Beta-galactosidase assay.

For staining, the cells were seeded at 1×10^5 cells in a 6-well plate and cultured in each medium. When the cells reached at least 90% confluence, the culture medium was discarded, and the cells were washed by adding 2 ml of DPBS. Then, 2 ml of 1× Fixing Buffer (the 10× Fixing buffer diluted with deionized H_2O at 1:10) was added, the cells were fixed at room temperature for 10 minutes, and the cells were washed twice with 2 ml of DPBS. Then, 2 ml of an X-gal staining mix was added, the cells were incubated at 37°C for 12 hours, and the number of stained cells was measured using an optical microscope.

As a result, as shown in FIG. 5, it was confirmed that the cells cultured in the edible medium of Example 1 exhibited a level of senescence approximately 50% lower than the level of senescence of the cells cultured in GM.

<Experimental Example 5> Comparison of Mesenchymal Stem-Cell Marker Expression

Bovine adipose-derived stem cells are a type of mesenchymal stem cells, and the ISCT designates three markers (CD73, CD90, and CD105) as essential expression markers.

To determine whether the adipose-derived stem cells cultured in the edible medium prepared in Example 1 maintained stem-cell characteristics, P4 bovine adipose-derived stem cells were cultured in the edible medium (SPEM) of Example 1 or DMEM (Dulbecco's Modified Eagle Medium, Gibco), and RT-PCR was performed using mRNA from each group of cells (GM and SPEM) as a template and primer sets targeting CD73, CD90, and CD105, which are known as mesenchymal stem-cell markers, thereby measuring mRNA expression levels of the markers.

Specifically, after cell seeding, cells in a confluent state were harvested, and 1 ml of Trizol (15596026, TRIzol™ Reagent, invitrogen) was added to 5-10 × 10^6 cells, followed by incubation at room temperature for 5 minutes. Then, 0.2 ml of chloroform was added, the mixture was mixed by inversion, incubated at room temperature for 5 minutes, and then centrifuged at 14,000 × g and 4°C for 20 minutes. A colorless, transparent supernatant was transferred to a new tube, 0.5 ml of isopropanol was added, the mixture was incubated at room temperature for 10 minutes, and then the mixture was centrifuged at 14,000 × g and 4°C for 10 minutes. After the supernatant was discarded, 1 ml of 75% ethanol was added, and the mixture was centrifuged at 14,000 × g and 4°C for 5 minutes. The supernatant was discarded, and the sample was dried for 5 to 10 minutes with the tube cap open. After the RNA pellet was dissolved by adding 20 μl of RNase-free water, absorbance at 260 nm was measured using a microvolume spectrophotometer

(ND-ONEC, NanoDrop™ One/OneC Microvolume UV/VIS Spectrophotometer, Thermo Fisher Scientific) to calculate the RNA concentration. cDNA was synthesized from 1 μg of RNA using a cDNA synthesis kit (18080051, SuperScript™ III First-Strand Synthesis System, Invitrogen). 1 μl of the synthesized cDNA was mixed with primers for CD90 (Forward: TTGTAGCAGGTGTGGTTGCT (SEQ ID NO: 1), Reverse: TTTCACCCGTGTCCCATTTG (SEQ ID NO: 2)), CD73 (Forward: AAGGTTCCTGTGGTCCAGGCCT (SEQ ID NO: 3), Reverse: TGCATTCTCGAAAGCGGCAGGA (SEQ ID NO: 4)), and CD105 (Forward: GAGCCTTCGTCTAGACTGCC (SEQ ID NO: 5), Reverse: CTGGCCTAGTGACCCCATTG (SEQ ID NO: 6)) and SYBR Green Master mix (A25918, PowerUp™ SYBR™ Green MasterMix, Applied Biosystems™), and mRNA expression levels were determined using a PCR instrument (A28572, QuantStudio™ 3 Real-Time PCR System, Applied Biosystems™).

As a result, as shown in FIG. 6, it was confirmed that the cells cultured in the edible medium of Example 1 had significantly higher mRNA expression levels of CD73, CD90, and CD105 than the cells cultured in GM.

Through these results, it was confirmed that culturing cells in the edible medium developed in the present invention enhances cell proliferative activity and inhibits cellular senescence as compared with a generally used medium. In addition, because the edible medium of the present invention is composed only of edible components, the edible medium can be usefully used as a culture medium for a cell-cultured food.

Although specific examples of the present invention have been described in detail above, those skilled in the art who understand the spirit of the present invention may

readily propose other regressive inventions or other embodiments included within the scope of the spirit of the present invention through the addition, modification, deletion, or the like of other components within the scope of the same spirit. Therefore, the embodiments described above should be understood in all respects as illustrative and not restrictive. The scope of the present invention is defined by the following claims rather than the foregoing detailed description, and all changes or modified forms derived from the meaning and scope of the claims and equivalents thereof should be construed as being included in the scope of the present invention.

[CLAIMS]

[Claim 1]

An edible medium composition having excellent cell proliferative activity, comprising:

one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine);

one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and

one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

[Claim 2]

The edible medium composition having excellent cell proliferative activity of claim 1, further comprising a carbon source.

[Claim 3]

The edible medium composition having excellent cell proliferative activity of claim 2, wherein the carbon source is one or more selected from the group consisting of

glucose (Glucose), fructose (Fructose), mannose (Mannose), ribose (Ribose), xylose (Xylose), sucrose (Sucrose), melibiose (Melibiose), cellobiose (Cellobiose), lactose (Lactose), amylose (Amylose), carbohydrate (Carbohydrate), raffinose (Raffinose), sorbitol (Sorbitol), mannitol (Mannitol), and glycerol (Glycerol).

[Claim 4]

The edible medium composition having excellent cell proliferative activity of claim 1, wherein the edible medium composition enhances cell proliferative activity and inhibits cellular senescence.

[Claim 5]

The edible medium composition having excellent cell proliferative activity of claim 1, wherein the edible medium composition is for culturing a cell-cultured food.

[Claim 6]

The edible medium composition having excellent cell proliferative activity of claim 5, wherein the cell-cultured food is cultured meat, a fungi-based meat alternative, or an algae-based meat alternative.

[Claim 7]

A food composition comprising cells cultured in the edible medium composition having excellent cell proliferative activity of claim 1 or a culture thereof.

[Claim 8]

The food composition of claim 7, wherein the cells are animal cells, plant cells, or microbial cells.

[Claim 9]

A method for preparing a cell-cultured food, comprising:

culturing animal cells, plant cells, or microbial cells in the edible medium composition having excellent cell proliferative activity of claim 1; and

recovering the cultured cells or a culture thereof.

[Claim 10]

A method for increasing cell proliferative activity, comprising:

culturing cells in a medium composition, the composition comprising:

one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride), methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine);

one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and

one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).

[Claim 11]

A method for inhibiting cellular senescence, comprising:

culturing cells in a medium composition, the composition comprising:

one or more amino acids selected from the group consisting of glycine (Glycine), arginine (Arginine), cystine (Cystine), glutamine (Glutamine), histidine (Histidine), isoleucine (Isoleucine), leucine (Leucine), lysine hydrochloride (Lysine Hydrochloride),

methionine (Methionine), phenylalanine (Phenylalanine), serine (Serine), threonine (Threonine), tryptophan (Tryptophan), tyrosine (Tyrosine), and valine (Valine);

one or more vitamins selected from the group consisting of choline chloride (Choline Chloride), calcium pantothenate (Calcium Pantothenate), folic acid (Folic Acid), inositol (Inositol), nicotinamide (Nicotinamide), vitamin B6 hydrochloride (Pyridoxine Hydrochloride), vitamin B2 (Riboflavin), and vitamin B1 hydrochloride (Thiamine Hydrochloride); and

one or more inorganic salts selected from the group consisting of calcium chloride (Calcium Chloride), ferrous sulfate (Ferrous Sulfate), magnesium sulfate (Magnesium Sulfate), potassium chloride (Potassium Chloride), sodium bicarbonate (Sodium Bicarbonate), salt (Salt), and sodium dihydrogen phosphate (Sodium Dihydrogen Phosphate).