Summary of Invention

Technical Problem

[0007]

An object of the present invention is to provide a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair. ~~Another object is to provide a polyphosphoric acid composition showing the effect of regulating intestinal function which protects intestines from invasion of toxic substances and pathogenic bacteria by narrowing the distance of intestinal epithelium cells.~~ ~~Yet~~ another object of the present invention is to provide a polyphosphoric acid composition which is highly safe and contains naturally synthesized polyphosphoric acid.

[0008]

An object of the present invention is to provide a method for producing a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair.

Solution to Problem

[0009]

As a result of repeated intensive research to solve the above problem, the present inventors could produce a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid, thereby completing the present invention.

[0010]

A first aspect of the present invention relates to a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the

polyphosphoric acid or solvates of the polyphosphoric acid. The polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid has a high cell permeation ability. Further, when the polyphosphoric acid composition is applied to the skin, ~~gums~~ and scalp, the polyphosphoric acid composition effectively acts on cell proliferation, prolongation of cell life, hair elongation and maintenance of the growth phase of hair.

[0011]

As a preferred embodiment of the first aspect of the present invention, yeast is budding yeast. Budding yeast is yeast which has been used for food since long ago. The polyphosphoric acid composition containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid can be efficiently obtained by using budding yeast. Further, a polyphosphoric acid composition in which a cell permeation ability is high can be obtained by using budding yeast. Further, when the polyphosphoric acid composition is applied to the skin, ~~gums~~ and scalp, the polyphosphoric acid composition effectively acts on cell proliferation, prolongation of cell life, hair elongation and maintenance of the growth phase of hair.

[0012]

As a preferred embodiment of the first aspect of the present invention, an extract further contains at least one of cell components of yeast and protein hydrolysates. When a polyphosphoric acid composition contains cell components and protein hydrolysates, the polyphosphoric acid composition can contain nutrients derived from yeast. Because of this, the polyphosphoric acid composition has an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life, and further more effectively acts to promote hair elongation and maintain the growth phase of hair.

[0013]

As a preferred embodiment of the first aspect of the present invention, polyphosphoric acid has a shape with a greatest diameter of 10 nm or more and 1000 nm or less. When polyphosphoric acid has a nanoparticle size, the polyphosphoric acid composition easily permeates into skin and scalp cells.

[0017]

As a preferred embodiment of the first aspect of the present invention, yeast is obtained by co-culturing with a lactic acid bacterium. As shown by way of examples, when yeast is co-cultured with a lactic acid bacterium, an ability to accumulate polyphosphoric acid is increased. Because of this, when yeast is obtained by co-culturing with a lactic acid bacterium, the concentration of polyphosphoric acid increases, and accordingly an excellent polyphosphoric acid composition can be obtained.

~~[0018]~~

~~A second aspect of the present invention relates to cosmetic material. The cosmetic material contains, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast. By mixing an effective amount of a polyphosphoric acid composition with a cosmetic product, the cosmetic product operates to promote cell proliferation and prolongation of cell life.~~

~~[0019]~~

A ~~third~~ aspect of the present invention relates to hair tonics. The hair tonics contain, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast. By mixing an effective amount of a polyphosphoric acid composition with a hair tonic, the hair tonic operates to promote hair elongation and maintain the growth phase of hair.

~~[0020]~~

~~A fourth aspect of the present invention relates to mouthwashes. The mouthwashes contain, as an effective ingredient, an effective amount of a~~

~~polyphosphoric acid composition extracted from yeast. By mixing an effective amount of a polyphosphoric acid composition with a mouthwash, the mouthwash effectively acts to prevent periodontal diseases by suppression of inflammation and regenerate alveolar bones by the effect of promoting regeneration.~~

~~[0021]~~

~~An aspect different from the above of the present invention relates to food products, food additives, supplements or intestinal drugs. Polyphosphoric acid is known to show the effect of regulating intestinal function which protects intestines from invasion of toxic substances and pathogenic bacteria by narrowing the distance of intestinal epithelium cells. Therefore, a product with the effect of regulating intestinal function can be provided by utilizing a polyphosphoric acid composition produced by yeast as a food product, a food additive or a supplement.~~

~~[0022]~~

A ~~fifth~~ aspect of the present invention relates to a method for producing a substance containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. This method comprises a step of obtaining an yeast mass, a step of obtaining a yeast broth, a step of obtaining yeast precipitates, a step of obtaining a yeast suspension, a step of obtaining a yeast extract and a step of obtaining a polyphosphoric acid composition. The step of obtaining an yeast mass is a step of obtaining an yeast mass by standing yeast cells under temperature conditions of 1°C or more and 10°C or less. The step of obtaining a yeast broth is a step of obtaining a broth containing yeast containing and accumulating polyphosphoric acid by culturing the yeast mass in a broth containing phosphate. The step of obtaining yeast precipitates is a step of obtaining precipitates by separating the yeast broth by centrifugation. The step of obtaining a yeast suspension is a step of, by suspending yeast cells obtained as precipitates in a liquid, obtaining a suspension in which yeast cells are suspended. The step of obtaining a yeast extract is a step of obtaining a yeast

extract by extracting polyphosphoric acid from yeast bodies by heat treatment of the yeast suspension. The step of obtaining a polyphosphoric acid composition is a step of, by separating the yeast extract by centrifugation into supernatant containing polyphosphoric acid and precipitates containing a fraction of yeast bodies, obtaining a polyphosphoric acid composition in the supernatant. An objective polyphosphoric acid composition is produced in the above step. The culture step in the production method involved in the present invention consists of one step and the culture time is drastically shortened. The obtained polyphosphoric acid composition has an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life, and further more effectively acts to promote hair elongation and maintain the growth phase of hair.

~~[0023]~~

As a preferred embodiment of the ~~fifth~~ aspect of the present invention, the step of obtaining a yeast extract is a step of obtaining the polyphosphoric acid and protein hydrolysates by heat treatment at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. Protein hydrolysates can be obtained by heat treatment at a temperature of 60°C or more and 80°C or less for not less than a certain time. In addition to polyphosphoric acid, a polyphosphoric acid composition containing protein hydrolysates has an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life, and further more effectively acts to promote hair elongation and maintain the growth phase of hair.

~~[0024]~~

~~An aspect different from the above of the present invention relates to polyphosphoric acid-containing yeast and supplements (food products, food additives and intestinal drugs) containing such polyphosphoric acid-containing yeast. The polyphosphoric acid-containing yeast can be produced as follows. As specific conditions, the method described above which is properly modified can be used. A~~

~~yeast broth containing and accumulating polyphosphoric acid is obtained by culturing an yeast mass in a broth containing a lactic acid bacterium and phosphate. Next, yeast precipitates are obtained by separating the yeast broth by centrifugation. Then, polyphosphoric acid-containing yeast containing any of polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid is obtained by using the yeast precipitates.~~

Advantageous Effects of Invention

~~[0025]~~

According to the present invention, there can be provided a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair. The polyphosphoric acid composition of the present invention contains polyphosphoric acid naturally synthesized in yeast bodies and there can be provided an excellent polyphosphoric acid composition in terms of safety.

~~[0026]~~

According to the present invention, a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair can be further produced. ~~In the present invention, there can be provided food products, food additives, supplements or intestinal drugs which contain an effective amount of yeast containing polyphosphoric acid, which repairs intestinal cells and provides the effect of regulating intestinal function, or extracts thereof as an effective ingredient.~~ The production method of the present invention is excellent in terms of being able to efficiently obtain a polyphosphoric acid composition.

Brief Description of Drawings

~~[0027]~~

Fig. 1 shows the molecular weight distribution of polyphosphoric acid obtained from yeast by heat extraction.

Fig. 2 shows an electron micrograph of polyphosphoric acid particles extracted from yeast.

Fig. 3 shows an ability to promote fibroblast proliferation by a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast.

Fig. 4 is photographs substitute for drawings which show the formulation of formazan on artificial skin treated with polyphosphoric acid extracted from yeast.

Fig. 5 shows the effect of promoting hair elongation by polyphosphoric acid extracted from yeast.

Fig. 6 shows examples of hair follicles in the elongation phase, regression phase and no changes of hair.

Fig. 7 shows the effect of maintaining the elongation phase of hair by polyphosphoric acid extracted from yeast.

Description of Embodiments

~~[0028]~~

A first aspect of the present invention relates to a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid. The polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid has a high cell permeation ability. Further, when the polyphosphoric acid composition is applied to the skin~~,~~ ~~gums~~ and scalp, the polyphosphoric acid composition effectively acts on cell

proliferation, prolongation of cell life, hair elongation and maintenance of the growth phase of hair.

~~[0029]~~

The term "the polyphosphoric acid composition" means a composition containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. The polyphosphoric acid composition can be in the various shapes such as liquid, condensed liquid, powder and solid. The polyphosphoric acid composition is preferably water-soluble since the composition is mixed with agents such as ~~cosmetic material,~~ hair tonics ~~and mouthwashes~~. In addition, the polyphosphoric acid composition can further contain at least one of cell components, protein hydrolysates, vitamins and minerals along with polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. Substances contained in the polyphosphoric acid composition are not limited to the above-described substances and other effective ingredients can be contained therein.

~~[0030]~~

Yeast is basically eukaryotic and unicellular microorganisms. Yeast has a cell wall on the outermost layer and the cell wall has glucan and mannan which are polysaccharides as main ingredients. A cell membrane is just under the cell wall and various receptors and transfer pumps exist therein. Budding yeast is eukaryotes and has a nuclear membrane and a nucleus. Besides, budding yeast has organelles such as cytoplasm, mitochondria and microtubule. In general, yeast can be roughly classified into budding yeast and fission yeast. The yeast used in the present invention are not particularly limited insofar as the yeast are generally called yeast, and budding yeast and fission yeast can be properly used.

~~[0031]~~

As a preferred embodiment of the present invention, yeast is budding yeast. Budding yeast has been used for the processing of food since long ago. Budding yeast

can decompose sugars in food into alcohol by fermenting the food. Yeast has been used for the production of food products such as beer, bread and Japanese fermented soybean paste since long ago and safety in use for food has been confirmed since long ago. Budding yeast is yeast which proliferate by budding. Preferred examples of budding yeast are Saccharomyces genus, Torulopsis genus, Mycotorula genus, Torulaspora genus, Candida genus, Rhodotorula genus and Pichia genus. In the present invention, a polyphosphoric acid composition containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid can be efficiently obtained by using budding yeast. Further, a polyphosphoric acid composition which more effectively acts on skin and scalp cells can be obtained by using budding yeast.

~~[0032]~~

As a further preferred embodiment, yeast is co-cultured with a lactic acid bacterium. By co-culturing, yeast is affected from the lactic acid bacterium or metabolites to be produced thereby and then the accumulated amount of polyphosphoric acid increases.

~~[0033]~~

Microorganisms have an ability to incorporate phosphoric acid into the bodies of microorganisms and synthesize polyphosphoric acid. Microorganisms have an enzyme to synthesize polyphosphoric acid utilizing adenosine triphosphate (ATP) and thus can synthesize polyphosphoric acid from the incorporated phosphoric acid. Yeast is microorganisms and thus, as described above, have an ability to incorporate phosphoric acid into the bodies of microorganisms and synthesize polyphosphoric acid.

~~[0034]~~

Yeast cells incorporate phosphoric acid into yeast bodies by culturing yeast in a broth containing phosphoric acid. The broth is preferably pH 3 to 6 and may be also pH 3 to 8 depending on the characteristics of target proteins to be produced by yeast. The culture temperature is preferably 20°C or more and 35°C or less. The broth can

also contain minerals such as magnesium, calcium and zinc, and vitamins such as vitamin B2, vitamin B6, vitamin C, pantothenic acid and folic acid at a high concentration. Vitamins and minerals each contained in total mass in the broth can be 0.1 mass% to 3 mass%. Yeast can incorporate minerals and vitamins existing in the broth into yeast bodies.

~~[0035]~~

Polyphosphoric acid is synthesized by synthetic enzymes in yeast cells from phosphoric acid incorporated into yeast bodies. Polyphosphoric acid is contained and accumulated in yeast bodies in this manner. The degree of polymerization of synthesized polyphosphoric acid varies depending on types of yeast species and types of polyphosphoric acid-synthetic enzymes. In the same type of yeast species, the yeast have the same type of synthetic enzymes, and thus the degree of polymerization of polyphosphoric acid to be obtained is within a certain range. Further, the desired degree of polymerization of polyphosphoric acid can be obtained by controlling enzyme activity by adjusting the culture temperature and culture time of yeast.

~~[0036]~~

Polyphosphoric acid synthesized in yeast bodies can be extracted by applying heat shock to yeast to destroy the yeast bodies. The heating conditions are preferably a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. The cell wall and cell membrane of yeast are destroyed by heat treatment and a yeast extract containing polyphosphoric acid synthesized in yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid can be obtained. When the cell wall and cell membrane are destroyed, a part of cell component of yeast such as cell wall, cell membrane, mitochondria, nuclear membrane, intranuclear substances and microtubule is also extracted in an extract.

~~[0037]~~

The obtained yeast extract is separated by centrifugation into supernatant

containing polyphosphoric acid and precipitates containing a fraction of yeast bodies, and a polyphosphoric acid composition is obtained. When the size of cell fragments is not smaller than a certain size, the cell fragments are removed by precipitation in a step of separating a yeast extract by centrifugation. Fine cell components however remain in supernatant of the extract. Because of this, a part of cell component of yeast is contained in a polyphosphoric acid composition along with polyphosphoric acid. Further, when cell components of yeast and proteins produced by yeast are decomposed by enzymes, protein hydrolysates are contained in a yeast extract. In this case, protein hydrolysates are contained in a polyphosphoric acid composition along with polyphosphoric acid and a part of cell component. When a polyphosphoric acid composition contains cell components and protein hydrolysates, the polyphosphoric acid composition can contain nutrients derived from yeast.

~~[0038]~~

As described above, when a broth contains minerals such as magnesium, calcium or zinc and vitamins such as vitamin B2, vitamin B6, vitamin C, pantothenic acid and folic acid at a high concentration, the minerals and vitamins are contained and accumulated in yeast bodies along with polyphosphoric acid. Therefore, vitamins and minerals contained and accumulated in yeast bodies are also contained in an extract extracted from yeast by heat treatment along with polyphosphoric acid.

~~[0039]~~

The cell components of yeast, protein hydrolysates, vitamins and minerals are nutrient sources for skin and scalp cells and useful for the maintenance or growth of cells. Therefore, when these substances are added to ~~cosmetic material,~~ hair tonics ~~and mouthwashes~~, these substances act as effective ingredients. Further, an ability to promote cell proliferation and skin permeability of polyphosphoric acid can be increased by interacting cell components of yeast, protein hydrolysates, vitamins and minerals with polyphosphoric acid.

~~[0040]~~

Polyphosphoric acid may be linear chain polyphosphoric acid, branched polyphosphoric acid, cyclic polyphosphoric acid and polyphosphoric acid which is a highly branched (reticulated state) phosphoric acid polymer (ultraphosphoric acid) and may be also mixtures thereof and derivatives thereof. Linear chain polyphosphoric acid has a structure in which two or more PO_4 tetrahedrons share the oxygen atom on the top by dehydration and condensation of orthophosphoric acid to stretch in a row. Branched polyphosphoric acid has a structure in which organic groups are introduced into a straight branched chain.

~~[0041]~~

Polyphosphoric acid has H_2O and P_2O_5 as constituent molecules and is linear chain or cyclic polyphosphoric acid represented by $H_{n+2}(P_nO_{3n+1})$ or $(HPO_3)_n$. Herein, the number of polymerized phosphoric acid is represented by n. Polyphosphoric acid mainly has polyphosphoric acid with a degree of polymerization of 20 to 100 phosphate residues (medium-chain polyphosphoric acid) and polyphosphoric acid with a degree of polymerization of 100 to 1000 phosphate residues (long-chain polyphosphoric acid). In the present invention, the degree of polymerization of polyphosphoric acid is 20 to 300 phosphate residues, preferably 50 to 250 phosphate residues and more preferably 80 to 200 phosphate residues. Polyphosphoric acid with a degree of polymerization of 20 or more phosphate residues and 300 or less phosphate residues is water-soluble and has an ability to promote cell proliferation by stabilizing cell growth factors such as fibroblast growth factors.

~~[0042]~~

The polyphosphoric acid composition of the present invention contains polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid.

~~[0043]~~

The salts of polyphosphoric acid mean salts of polyphosphoric acid,

particularly pharmaceutically acceptable salts of polyphosphoric acid. The salts of polyphosphoric acid are preferably alkali metal salts and more preferably sodium salts. In the present description, salts of polyphosphoric acid may contain not only anhydrous salts but also hydrated salts. These salts, for example, are ionized in a solution to operate in the same manner as polyphosphoric acid.

~~[0044]~~

The solvates of polyphosphoric acid mean solvates of polyphosphoric acid. The solvates include hydrates. In addition, the polyphosphoric acid composition of the present invention can become a hydrate by absorption of water and attachment of adsorbed water in a yeast body or an extract. Such solvates formed are also included in the solvates of polyphosphoric acid. These solvates are ionized in a solution to operate in the same manner as polyphosphoric acid.

~~[0045]~~

As the polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid used in the present invention, a kind thereof may be used or a mixture of several kinds thereof may be used. Several kinds of polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid contain polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid with different degrees of polymerization or different molecular structures and polyphosphoric acid salts with different metal ions.

~~[0046]~~

In the present invention, it is preferred that polyphosphoric acid contained in the total mass of a polyphosphoric acid composition be 0.01 mass% or more and 70 mass% or less, preferably 0.02 mass% or more and 50 mass% or less and further preferably 0.1 mass% or more and 50 mass% or less. Polyphosphoric acid may be salts of polyphosphoric acid or solvates of polyphosphoric acid, and the percentage content of polyphosphoric acid to a polyphosphoric acid composition is a percentage

content of the total mass of these three types of polyphosphoric acid. By containing an effective amount of polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid in a polyphosphoric acid composition, the polyphosphoric acid composition effectively acts on the promotion of cell proliferation, prolongation of skin cell life, maintenance of hair elongation and the growth phase of hair and the effect of regulating intestinal function.

~~[0047]~~

The polyphosphoric acid obtained in the present invention is synthesized in yeast bodies and contained and accumulated in the yeast bodies. Polyphosphoric acid exists in a folded structure to stably exist in a yeast body. Polyphosphoric acid in the folded state has a shape with a greatest diameter of 10 nm or more and 1000 nm or less, preferably 30 nm or more and 800 nm or less, and further preferably 50 nm or more and 500 nm or less. The polyphosphoric acid obtained in the present invention has a particle size in the order of nanometers and thus permeability in skin surfaces is excellent. Polyphosphoric acid in the above shape easily fits to scalp and the layer of oil and fat on the skin and permeability is increased. The folded structure means a particle shape in which polyphosphoric acid coheres and the shape includes various shapes such as a globular shape, an oval shape, a cylindrical shape, a square shape and a block with unevenness.

~~[0048]~~

~~A second aspect of the present invention relates to cosmetic material. The cosmetic material contains, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast.~~

~~[0049]~~

~~The cosmetic material involved in the present invention contains a polyphosphoric acid composition as an effective ingredient. A polyphosphoric acid composition is added to a cosmetic product in a liquid, condensed liquid, powder or~~

~~solid state. It is preferred that polyphosphoric acid contained in the total mass of a cosmetic product be 0.01 mass% or more and 70 mass% or less, preferably 0.02 mass% or more and 50 mass% or less and further preferably 0.1 mass% or more and 50 mass% or less.~~

~~[0050]~~

~~To the cosmetic material involved in the present invention, other known effective ingredients can be further added. Examples thereof are, for lotions, medicines such as purified water to supply water to horny layers such as ion changed water, alcohols to dissolve oil-soluble components and provide a refreshed feeling such as ethanol and propanol, humectants to retain moisture in horny layers such as glycerin, PEG and hyaluronic acid, emollients (oil components to prevent water from evaporating) to improve moisture retention properties and usability such as ester oil and plant oil, solubilizing agents to solubilize raw material ingredients such as polyoxyethylene oleyl alcohol ether, buffers to adjust the pH of products such as citric acid, lactic acid and amino acids, perfumes to add flavors such as vanillin, orange flavor, lemon flavor, milk flavor, geraniol and linalool, antiseptics to inhibit microorganisms and prevent putrefaction such as methylparaben and phenoxyethanol, coloring agents to color, sequestering agents, and anti-fading agents to prevent fading and discoloration such as UV absorbers, astringents, bactericides, activators, anti-inflammatory agents and whitening agents. One or two or more medicines selected from these effective ingredients of lotions can be added.~~

~~[0051]~~

~~In general, cosmetic material is applied to skin surfaces to clean skin and retain healthy skin. Examples of cosmetic material are lotions, milky lotions, cream, gels, beauty essence (essence), pack cosmetics and the like. The cosmetic material containing a polyphosphoric acid composition as an effective ingredient can be produced according to a conventional method.~~

~~[0052]~~

~~The basic function of lotions is to supply water and moisturizing components to horny layers of skin and lotions also have a function to soften skin. The effective ingredient of lotions is desired to permeate into horny layers and those with a high skin permeation ability are preferred. As described above, polyphosphoric acid synthesized in yeast bodies has a particle size in the order of nanometers. Therefore, polyphosphoric acid synthesized in yeast bodies has a high skin permeation ability and an ability to promote cell proliferation and operates to increase the effect of prolonging skin cell life. Because of this, skin cells can be maintained in a healthy state.~~

~~[0053]~~

A ~~third~~ aspect of the present invention relates to hair tonics. The hair tonics contain, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast.

~~[0054]~~

The hair tonics involved in the present invention contain a polyphosphoric acid composition as an effective ingredient. A polyphosphoric acid composition is added to a hair tonic in a liquid, condensed liquid, powder or solid state. It is preferred that polyphosphoric acid contained in the total mass of a hair tonic be 0.01 mass% or more and 70 mass% or less, preferably 0.02 mass% or more and 50 mass% or less and further preferably 0.1 mass% or more and 50 mass% or less. The hair tonics may be administered in a range of 0.01 g or more and 10 g or less (preferably 0.1 g or more and 5 g or less) in terms of the weight of a polyphosphoric acid composition once to ten times a day.

~~[0055]~~

To the hair tonics involved in the present invention, other known hair growth components can be further added. Examples thereof are activators of vital cells such as laurel extract and chlorophyll, blood flow-promoting agents to promote blood flow in

peripheral blood vessels such as Asian ginseng extract, Swertia herb extract, cepharanthin, vitamin E and derivatives thereof and γ-oryzanol, local irritants such as capsicum tincture, ginger tincture, cantharis tincture and nicotinic acid benzyl ester, nutrients for supplying nutrition to the surroundings of hair matrix cells such as vitamins such as vitamin A, B1, B2, B6, E and derivatives thereof, pantothenic acid and derivatives thereof and biotin, and amino acids such as cystine, cysteine, methionine, leucine, tryptophan and amino acid extracts, female hormones having antagonism against male hormones such as estradiol and ethinyl estradiol, hair root activators to improve hypofunction of hair matrix cells such as pantothenic acid and derivatives thereof, placenta extract, allantoin and quaternium-51, anti-inflammatory agents such as dipotassium glycyrrhizinate, β-glycyrrhetinic acid, allantoin and hinokitiol, and humectants to prevent scalp from drying such as glycerin and pyrrolidone carboxylic acid. One or two or more components selected from these hair growth components can be added.

~~[0056]~~

In general, hair tonics have effects on promotion of hair emergence, promotion of hair growth, hair restoration, hair nourish and prevention of hair loss. In general, hair tonics are directly applied to scalp. Examples of hair tonics are liquid medicines, emulsions, cream, lotions, gels, foam, sprays and the like. In addition, a polyphosphoric acid composition may be contained in shampoo, rinse and hair treatment as an effective ingredient. Fomentations in which an effective ingredient is soaked in nonwoven fabric or cotton may be also used. The hair tonics containing a polyphosphoric acid composition as an effective ingredient can be produced according to a conventional method.

~~[0057]~~

The basic function of hair tonics is to sustain the activation of hair follicle cells of scalp and elongate hair. Hair tonics are preferably those with a high permeation

ability into hair follicle cells to permeate an effective ingredient into hair follicle cells of scalp. As described above, polyphosphoric acid synthesized in yeast bodies has a particle size in the order of nanometers. Therefore, polyphosphoric acid synthesized in yeast bodies have a high permeation ability into hair follicle cells and can sustain the activation of hair follicle cells. Therefore, hair tonics can promote hair elongation and further can maintain hair follicle cells in the state of the growth phase.

~~[0058]~~

~~A fourth aspect of the present invention relates to mouthwashes. The mouthwashes contain, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast.~~

~~[0059]~~

~~The mouthwashes involved in the present invention contain a polyphosphoric acid composition as an effective ingredient. A polyphosphoric acid composition is added to a mouthwash in a liquid, gel, paste, condensed liquid, powder or solid state. It is preferred that polyphosphoric acid contained in the total mass of a mouthwash be 0.01 mass% or more and 70 mass% or less, preferably 0.02 mass% or more and 50 mass% or less and further preferably 0.1 mass% or more and 50 mass% or less. The mouthwashes may be administered in a range of 0.01 g or more and 10 g or less (preferably 0.1 g or more and 5 g or less) in terms of the weight of a polyphosphoric acid composition once to ten times a day.~~

~~[0060]~~

~~To the mouthwashes involved in the present invention, known mouthwash components can be further added. Examples thereof are washing agents such as polyethylene glycol, polyvinylpyrrolidone, phytic acid, dibasic sodium phosphate, sodium phosphate and zeolite, bleaching agents such as hydrogen peroxide, urea peroxide, calcium peroxide and sodium chlorite, anti-inflammatory agents such as allantoin, ε-aminocaproic acid, dipotassium glycyrrhizinate, β-glycyrrhetinic acid,~~

ascorbic acid, vitamin E and azulene sodium sulfonate, antibacterial agents such as isopropyl methylphenol, chlorhexidine hydrochloride, triclosan, hinokitiol, cetylpyridinium chloride, sodium copper chlorophyllin, benzalkonium chloride, dequalinium chloride, benzethonium chloride, alkyldiaminoethylglycine hydrochloride, pyridoxine hydrochloride, lysozyme chloride and catechin, surface active agents such as polyoxyethylene lauryl ether and sodium lauroyl sarcosinate, seasonings such as menthol, mastic extract, rose extract and mint flavor, fluorine compounds such as sodium fluoride, sodium monofluorophosphate and stannic fluoride, antiseptics such as paraben, phenoxy ethanol and alkyldiaminoethylglycine hydrochloride. One or two or more components selected from these mouthwash components can be added.

[0061]

In general, mouthwashes remain in the mouth for a certain time to clean up the mouth, prevent drying, provide a refreshed feeling and the like, or have the preventive or therapeutic effects of oral diseases. Examples of mouthwashes include tooth powder, polishers, mouthwashes, spray type mouthwashes, gargles, oral rinses and the like. The mouthwashes containing a polyphosphoric acid composition as an effective ingredient can be produced according to a conventional method.

[0062]

The basic function of mouthwashes is to clean the mouth and promote the health of periodontia containing alveolar bones and teeth. Since mouthwashes permeate an effective ingredient into periodontal cells to suppress the inflammation of gums, those with a high permeation ability into periodontal pockets are preferred. As described above, polyphosphoric acid synthesized in yeast bodies has a particle size in the order of nanometers. Therefore, a polyphosphoric acid composition synthesized in yeast bodies has a high permeation ability into gum cells and alveolar bones of periodontia and can promote gum cell proliferation, and simultaneously suppress inflammation and stimulate regeneration of alveolar bones.

~~[0063]~~

~~A preferred aspect of the present invention relates to food products containing, as an effective ingredient, an effective amount of any polyphosphoric acid composition described above. The types of food products are not particularly limited. Insofar as the food products show any effects previously described, a polyphosphoric acid composition may be contained therein in an amount which can show the effect. Examples of food products are drinks. When using as a drink, the polyphosphoric acid composition of the present invention can be easily orally taken. Examples of drinks are soft drinks, tea, coffee, black tea and lactic acid drinks. The food products may be administered to subjects in a range of 0.01 g or more and 10 g or less (preferably 0.1 g or more and 5 g or less) in terms of the weight of a polyphosphoric acid composition once to ten times a day.~~

~~[0064]~~

~~A preferred aspect of the present invention relates to food additives containing, as an effective ingredient, an effective amount of any polyphosphoric acid composition described above. The food additives of this embodiment are properly added to food products to show the above-described effects of polyphosphoric acid composition.~~

~~[0065]~~

~~A preferred aspect of the present invention relates to supplements containing, as an effective ingredient, an effective amount of any polyphosphoric acid composition described above. Supplements themselves are known. That is, supplements can be produced by mixing known carriers (starch etc.) with the polyphosphoric acid composition of the present invention and forming with a tableting machine. The food products, food additives or supplements involved in the present invention contain a polyphosphoric acid composition. Polyphosphoric acid contained in the total mass of a supplement is 0.01 mass% or more and 50 mass% or less, preferably 0.02 mass% or more and 30 mass% or less and further preferably 0.1 mass% or more and 15 mass% or~~

~~less. Supplements may properly contain various types of useful components such as saccharides such as lactose and oligosaccharides, diluents such as dextrin and cellulose, various types of inorganic bases, vitamins, collagen, hyaluronic acid, coenzyme Q10, α-lipoic acid and various types of plant extracts, perfumes, sweeteners and the like. The supplements may be administered to subjects in a range of 0.01 g or more and 10 g or less (preferably 0.1 g or more and 5 g or less) in terms of the weight of a polyphosphoric acid composition once to ten times a day.~~

~~[0066]~~

~~A preferred aspect of the present invention relates to intestinal drugs containing, as an effective ingredient, an effective amount of any polyphosphoric acid composition described above. The intestinal drugs can be produced in the same manner as for the above-described supplements. The intestinal drugs may be administered to subjects in a range of 0.01 g or more and 10 g or less (preferably 0.1 g or more and 5 g or less) in terms of the weight of a polyphosphoric acid composition once to ten times a day.~~

~~[0067]~~

Another aspect of the present invention relates to a method for producing a substance containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. This method comprises a step of obtaining an yeast mass, a step of obtaining a yeast broth, a step of obtaining yeast precipitates, a step of obtaining a yeast suspension, a step of obtaining a yeast extract and a step of obtaining a polyphosphoric acid composition. The step of obtaining an yeast mass is a step of obtaining an yeast mass by standing yeast cells under temperature conditions of 1°C or more and 10°C or less. The step of obtaining a yeast broth is a step of obtaining a broth containing yeast containing and accumulating polyphosphoric acid by culturing the yeast mass in a broth containing phosphate. The step of obtaining yeast precipitates is a step of obtaining precipitates by separating the yeast broth by centrifugation. The step of obtaining a yeast suspension is a step of, by suspending

yeast cells obtained as precipitates in a liquid, obtaining a suspension in which yeast cells are suspended. The step of obtaining a yeast extract is a step of obtaining a yeast extract by extracting polyphosphoric acid from yeast bodies by heat treatment of the yeast suspension. The step of obtaining a polyphosphoric acid composition is a step of, by separating the yeast extract by centrifugation into supernatant containing polyphosphoric acid and precipitates containing a fraction of yeast bodies, obtaining a polyphosphoric acid composition in the supernatant.

~~[0068]~~

Yeast cells can incorporate phosphoric acid into yeast bodies and synthesize polyphosphoric acid in the cell. Yeast incorporate phosphoric acid into fungal bodies by culturing yeast in a broth containing phosphate. As a method in which a yeast body incorporates phosphoric acid and synthesizes polyphosphoric acid, a method having two steps as a culture step is generally known. The first step is a step of obtaining the phosphoric acid starvation state of yeast by culturing yeast cells in a broth which does not contain phosphoric acid at all for the purpose that yeast easily incorporates phosphoric acid. The second step is a step of incorporating phosphoric acid into yeast bodies by culturing yeast cells in a broth rich in phosphoric acid. It has been believed that yeast can efficiently incorporate phosphoric acid into cell bodies by the culture step having two steps. In the above-described method, however, two types of broths are required, and thus yeast cells cultured in the first type of broth are required to be collected and cultured in a new broth again. Therefore, a conventional culture step has had problems in terms of handling and costs.

~~[0069]~~

In the production method involved in the present invention, there is a step of obtaining an yeast mass first. The step of obtaining an yeast mass is a step of obtaining an yeast mass by standing yeast at 1°C or more and 10°C or less. In the step of obtaining an yeast mass, yeast are left to stand under conditions where the water

content is 30 mass% or more and 50 mass% or less relative to an yeast mass. In the step of obtaining an yeast mass, yeast are preferably left to stand at 1°C or more and 10°C or less for preferably 72 hours or more. In the step of obtaining an yeast mass, a medium or a broth is not required and a broth is not required to be exchanged after obtaining an yeast mass. Therefore, the culture step procedure is simplified. Further, the culture time in the step of obtaining a yeast broth is drastically shortened by undergoing the step of obtaining an yeast mass.

~~[0070]~~

The step of obtaining a yeast broth is a step of culturing an yeast mass in a broth containing phosphate. The broth can contain minerals such as magnesium, calcium and zinc, and vitamins such as vitamin B2, vitamin B6, vitamin C, pantothenic acid and folic acid at a high concentration. In the production method involved in the present invention, the step of obtaining a yeast broth consists of only one step because of undergoing the step of obtaining an yeast mass. In the production method involved in the present invention, approximately 1.5 times amount of polyphosphoric acid can be obtained as compared to a conventional production step containing a culture step consisting of two steps. Further, the culture time is within an hour or more to 3 hours and is drastically shortened as compared to the culture time required in the conventional culture step consisting of two steps. Further, by culturing yeast in the presence of minerals and vitamins, not only a polyphosphoric acid composition favorable for ~~cosmetic material, hair~~ tonics ~~and mouthwashes~~ can be obtained but also the culture time can be shortened.

~~[0071]~~

It is preferred that the broth contain a lactic acid bacterium. The lactic acid bacterium is preferably added at 0.1 mass% or more and 50 mass% or less of yeast, and may be 0.5 mass% or more and 20 mass% or less, 1 mass% or more and 15 mass% or less, and 2 mass% or more and 10 mass% or less. Examples of lactic acid bacteria are

any or two or more of Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus delbrueckii, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus kefir, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius, Streptococcus thermophilus, Lactococcus lactis, Lactococcus plantarum, Lactococcus raffinolactis, Leuconostoc lactis and Leuconostoc mesenteroides. The lactic acid bacterium may be added to a medium in advance, may be gradually added during the course of culture, or may be added during the course of culture.

~~[0072]~~

The step of obtaining yeast precipitates is a step of obtaining yeast to be precipitated by separating a fluid, in which yeast cells are cultured, by centrifugation. Centrifugation is carried out in conditions of a centrifugal force of 3000 × g to 15000 × g for 3 minutes to 30 minutes. Centrifugation can be carried out by a conventional method.

~~[0073]~~

The step of obtaining a yeast suspension is a step of suspending yeast precipitates obtained by centrifugation in a cell suspension solvent. For the cell suspension solvent, solutions which are commonly used for suspending cells such as distilled water, a physiological saline solution, a phosphate buffer and a pH buffer can be used.

~~[0074]~~

The step of obtaining a yeast extract is a step of applying heat to a yeast extract. By applying heat shock to yeast, cell membranes are destroyed and polyphosphoric acid is eluted outside fungal bodies. The heat treatment can be carried out at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. It is preferred that the heat treatment be adjusted to the conditions of temperature and time at which synthesized polyphosphoric acid is not decomposed.

~~[0075]~~

The step of obtaining a polyphosphoric acid composition is a step of, by separating the yeast extract by centrifugation into supernatant containing polyphosphoric acid and a fraction of yeast bodies, obtaining a polyphosphoric acid composition. Polyphosphoric acid eluted from yeast cells is water-soluble and dissolved in a liquid. Therefore, the extract can be separated by centrifugation into precipitates of yeast bodies and a polyphosphoric acid composition containing polyphosphoric acid. After centrifugation, supernatant is collected and a polyphosphoric acid composition can be obtained. Centrifugation is carried out in conditions of a centrifugal force of 5000 × g to 10000 × g for 3 minutes to 30 minutes. Centrifugation can be carried out by a conventional method. The yeast extract contains cell components of yeast, protein hydrolysates of proteins, vitamins and minerals along with polyphosphoric acid. The cell components of yeast, protein hydrolysates, vitamins and minerals are useful for the maintenance or the growth of skin and scalp cells and operate as an effective ingredient of ~~cosmetic material, hair tonics and mouthwashes~~. Therefore, it is preferred that during centrifugation in the step of obtaining a polyphosphoric acid composition, these substances be left in supernatant along with polyphosphoric acid. As centrifugal conditions, therefore, separation is preferably carried out at a relatively low centrifugal force which can be set in a common centrifuge such as 5000 × g to 10000 × g.

~~[0076]~~

As a preferred embodiment of the present invention, the step of obtaining a yeast extract is a step of heat treatment at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. The above-described step of obtaining a yeast extract in the production method of a polyphosphoric acid composition is a step of heat treatment at a temperature of 60°C or more and 80°C or less and preferably 65°C or more and 75°C or less for 20 minutes or more and 40

minutes or less and preferably 25 minutes or more and 35 minutes or less. Synthesized polyphosphoric acid may be decomposed depending on conditions of heating temperature and heating time and thus the treatment time of heat treatment is preferably 40 minutes or less. Further, protein hydrolysates can be obtained in the step of obtaining a yeast extract. Protein hydrolysates can be obtained by hydrolyzing proteins in yeast bodies. Therefore, heat treatment is preferably carried out at a temperature of 80°C or less for not less than a certain time not to deactivate enzymes in protein hydrolysates in yeast bodies.

~~[0077]~~

Although the production step in the present invention contains a step of heat treatment at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less, the denaturation of proteins and vitamins which are weak against heat is suppressed. This is because, when proteins and vitamins are extracted together with polyphosphoric acid from yeast, nano particles of polyphosphoric acid protect proteins and vitamins by inclusion. Polyphosphoric acid extracted from yeast also has a function to stabilize components unstable in heat.

~~[0078]~~

~~An aspect different from the above of the present invention relates to polyphosphoric acid-containing yeast and supplements (food products, food additives and intestinal drugs) containing such polyphosphoric acid-containing yeast. The polyphosphoric acid-containing yeast can be produced as follows. As specific conditions, the method described above which is properly modified can be used. A yeast broth containing and accumulating polyphosphoric acid is obtained by culturing an yeast mass in a broth containing a lactic acid bacterium and phosphate. Next, yeast precipitates are obtained by separating the yeast broth by centrifugation. Then, polyphosphoric acid-containing yeast containing any of polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid is obtained by using the yeast~~

~~precipitates. At this time, yeast (and a lactic acid bacterium) may be died out by treatment such as heating. When polyphosphoric acid-containing yeast contains dead bodies of the yeast and lactic acid bacterium, since they become food of enterobacteria, more preferred effects are shown and further the production of supplements and the like becomes easy.~~

Example 1

~~[0079]~~

(Production of intra-cellular polyphosphoric acid using yeast)

As the yeast used for culture, live yeast for food (Oriental yeast, regular, Oriental Yeast Co., ltd.) was used. First, the above yeast cells were stored at 10°C or less for 3 days to obtain an yeast mass. Next, the yeast was cultured only in a broth B with a composition described in Table 1. The culture time and the amount of yeast bodies are as described in Table 2. The amount of yeast bodies is shown by a percentage of the amount of fungal bodies to the amount of broth. The broth B was prepared in an amount of 40 mL and the yeast was put therein to a percentage described in Table 2. Thereafter, the broth with the yeast was cultured with shaking at 25°C for 2 hours. After shaking culture, the broth was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. The collected yeast cells were washed with distilled water once and stored at -30°C.

~~[0080]~~

Comparative Example 1

In order that the yeast would be in a phosphate starvation state, yeast cells were cultured in a broth A not containing phosphoric acid at all. The culture time and the amount of yeast bodies are as described in Table 2. The amount of yeast bodies are shown by a percentage of the amount of fungal bodies to each amount of broth. The broth A was prepared in an amount of 300 mL and 6 g of the yeast were put therein.

Thereafter, the broth with the yeast was cultured with shaking at 25°C for each time described in Table 2. After shaking culture, the broth was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. Next, the broth B was prepared in an amount of 40 mL and the collected yeast bodies were put therein to a percentage described in Table 2. Thereafter, the broth with the yeast was cultured with shaking at 25°C for 2 hours. After shaking culture, the broth was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. The collected yeast cells were washed with distilled water once and stored at -30°C. Both the broths A and B were adjusted to pH 5.2 with a succinate buffer or a phosphate buffer.

~~[0081]~~

[Table 1]

Table 1

Broth A		Broth B	
$(NH_4)_2SO_4$	0.50%	$(NH_4)_2SO_4$	0.50%
KCl	0.07%	KCl	0.07%
$MgSO_4 \cdot 7H_2O$	0.04%	$MgSO_4 \cdot 7H_2O$	0.04%
D-Glucose	1%	D-Glucose	1%
Succinic acid	100mM	$NaH_2PO_4 \cdot 2H_2O$	100mM

~~[0082]~~

[Table 2]

Table 2

	Broth A		Broth B	
	Amount of yeast bodies	Culture time	Amount of yeast bodies	Culture time
Comparative Example 1-1	3.33%	15 hours	5.0%	2 hours
Comparative Example 1-2	6.67%	5 hours	2.0%	2 hours
Comparative Example 1-3	2.00%	15 hours	1.5%	2 hours
Comparative Example 1-4	2.00%	15 hours	2.0%	3 hours
Example 1-5	—	—	5.0%	2 hours
Example 1-6	—	—	7.5%	2 hours
Example 1-7	—	—	10.0%	2 hours

~~[0083]~~

(Comparative measurement of the contained and accumulated amount of polyphosphoric acid)

After culturing the yeast in the conditions in Table 2, the contained and accumulated amount of polyphosphoric acid in yeast was evaluated. The yeast cells were suspended in distilled water with twice the weight of the cells originally put and the obtained suspension was subjected to heat treatment at a temperature of 80°C for 30 minutes. Thereafter, centrifugation was carried out in conditions of 10000 × g for 10 minutes and supernatant was collected to obtain an extract. To the extract, nitric acid was added so that the final concentration would be 3.4%, and by heat treatment at a temperature of 100°C for 30 minutes, polyphosphoric acid was completely hydrolyzed. Next, a vanadate-molybdate reagent was added to determine a phosphoric acid amount. A polyphosphoric acid amount was calculated from the determined phosphoric acid amount (Japanese Standard of Food Additives, Hirokawa Shoten Co., Method for determining potassium polyphosphate). The calculated results are shown in Table 3. From the results in Table 3, in Comparative Examples 1-1, 1-2, 1-3 and 1-4, the accumulated amount of polyphosphoric acid per g of yeast was 0.03 to 0.13 mmol by the number of moles when a phosphoric acid residue was considered as a unit.

Contrarily, it was found that the accumulated amount was large in Examples 1-5, 1-6 and 1-7 not using the broth A, which was 0.10 to 0.22 mmol. In Example 1-5, the contained and accumulated amount of polyphosphoric acid was highest and was 1.5 times or more that of Comparative Example 1-3. In addition, in Examples 1-5, 1-6 and 1-7, the culture time was also short, which was for 2 hours. Further, the broth was not required to be exchanged. It was found that in the production method of a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, the production method of Example 1 was excellent as compared to the production method of Comparative Example 1 in terms of handling and costs.

~~[0084]~~

[Table 3]

Table 3

	Accumulated amount of polyphosphoric acid per g of yeast (mmol)
Comparative Example 1-1	0.03
Comparative Example 1-2	0.08
Comparative Example 1-3	0.14
Comparative Example 1-4	0.13
Example 1-5	0.22
Example 1-6	0.16
Example 1-7	0.10

Example 2

~~[0085]~~

(Evaluation of a method for extracting polyphosphoric acid)

The condition for extracting polyphosphoric acid from yeast was considered. Heat treatment was carried out so that the temperature (60 to 0°C) and time (0 to 60

min) of heat treatment would be conditions described in Table 4 and the extraction rate of polyphosphoric acid was compared. The broth of yeast was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. The collected yeast bodies were frozen at -20°C. Thereafter, the yeast cells were liquefied and suspended in distilled water again. The obtained suspension was dispensed to a microtube at each 50 μL. After dispensing and stirring, centrifugation was carried out in conditions of 8000 × g for 5 minutes and supernatant was collected as an extract. The obtained extract was used as a sample and electrophoresis was carried out using 15% polyacrylamide gel (150 V, 45 min). Thereafter, the gel was stained by 0.05% toluidine blue. The result of electrophoresis is shown in Fig. 1. When the molecular weight distribution of natural polyphosphoric acid extracted was confirmed by electrophoresis, it was found that molecular species with a large molecular weight decreased depending on heating time by heat treatment at 80°C for 40 minutes or more. This is because polyphosphoric acid has a possibility of being decomposed by heating for a long period of time. Because of this, it was found that the heat treatment at 80°C for approximately 30 minutes is preferred for the extraction of polyphosphoric acid.

~~[0086]~~

To the extract obtained by the above method, nitric acid was added so that the final concentration would be 3.4%, and by heat treatment at a temperature of 100°C for 30 minutes, polyphosphoric acid was completely hydrolyzed. Next, a vanadate-molybdate reagent was added to determine a phosphoric acid amount. A polyphosphoric acid amount was calculated from the determined phosphoric acid amount. The calculated results are shown in Table 4. From the results in Table 4, when heating at 80°C for 30 minutes, the greatest extraction amount of polyphosphoric acid was obtained.

~~[0087]~~

[Table 4]

Table 4

Extraction conditions	Heating temperature (°C)	Heating time (min)	Polyphosphoric acid concentration in yeast cell (mM)
Example 2-1	90	10	66.09
Example 2-2	80	0	7.24
Example 2-3	80	10	88.68
Example 2-4	80	20	86.13
Example 2-5	80	30	93.17
Example 2-6	80	40	91.92
Example 2-7	80	50	85.46
Example 2-8	80	60	85.20
Example 2-9	70	0	11.49
Example 2-10	70	10	72.14
Example 2-11	70	20	78.15
Example 2-12	70	30	83.13
Example 2-13	70	40	80.49
Example 2-14	70	50	68.89
Example 2-15	70	60	77.60
Example 2-16	60	10	19.65
Example 2-17	60	30	31.83
Example 2-18	60	60	48.18

Example 3

~~[0088]~~

(Shapes of polyphosphoric acid extracted from yeast)

A polyphosphoric acid composition containing polyphosphoric acid extracted from yeast was freeze-dried to obtain dry powder of a polyphosphoric acid extract. The fine structure of the dry powder was observed by a scanning electron microscope. As shown in Fig. 2, a large number of particles with a diameter of 1000 nm or less were

confirmed along with structures which are believed that constituents of yeast cells were dried and solidified. This could confirm that polyphosphoric acid extracted from yeast existed as particles with a diameter of the order of nanometers.

Example 4

~~[0089]~~

(Promotion of fibroblast proliferation by a polyphosphoric acid composition extracted from yeast)

The effect of a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast to fibroblast proliferation was evaluated by MTS method (CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit manufactured by Promega KK). Yeast was cultured by the procedure in Examples 1-5. For extraction, these suspensions of fungal bodies were dispensed at 0.2 mL each to an Eppendorf tube and heat treatment was carried out at 80°C for 30 minutes. Centrifugation was carried out in conditions of 10000 × g for 10 minutes and supernatant was collected to obtain an extract P. The obtained extracts P each were sterilized by filtration with a 0.45 μm sterilization filter and the protein concentration and the phosphoric acid amount were measured. As a result of the measurement of the phosphoric acid amount, the polyphosphoric acid concentration in the extract P was 91.1 mM. In addition, as a result of the measurement of the protein concentration, the protein concentration contained in the extract P was 15.89 mg/ml.

~~[0090]~~

Comparative Example 2

Yeast cultured only in a broth not containing phosphoric acid (broth A used in the procedure in Comparative Examples 1-3) for 15 hours was subjected to heat treatment in the same conditions as the extraction conditions of the extract P to prepare an extract Q which hardly contained polyphosphoric acid. The background level polyphosphoric acid concentration in the extract Q was 0.65 mM and the protein

concentration was 9.84 mg/ml.

~~[0091]~~

(Comparison of promotion of fibroblast proliferation)

Next, these extracts P and Q were added to a broth of dermal cells in normal human breast (HDF, manufactured by Cell Applications, Inc. USA) and the effect of promoting cell proliferation was evaluated by MTS method (CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit, manufactured by Promega KK). The cells were seeded in a 96-well plate at 3000 cells/well and cultured in D-MEM medium with 10% FBS (fetal bovine serum) for 24 hours. Thereafter, the cells were cultured in D-MEM with 1% FBS overnight. The cell broth was exchanged with D-MEM medium supplemented with each extract containing polyphosphoric acid. The MTS reaction was carried out at 24, 48 and 69 hours and OD_{492} nm was measured at 95 minutes from the onset of the reaction by a plate reader. Each extract was adjusted so that the protein concentration would be 0.174 mg/ml. In this case, the polyphosphoric acid concentration in the extract P was 1 mM in the medium. The polyphosphoric acid concentration in the extract Q was 0.0115 mM in the medium. The polyphosphoric acid concentration of the medium supplemented with the extract P is a concentration approximately 87 times the polyphosphoric acid concentration of the medium supplemented with the extract Q. In addition, a sample in which the concentration of polyphosphoric acid extracted from yeast was 0.2 mM in the extract was also evaluated.

~~[0092]~~

As comparative examples, a case in which polyphosphoric acid was not added to a medium and a case to which medium-chain sodium polyphosphate which was not derived from yeast and chemically synthesized (chemically synthesized polyphosphoric acid, the average polymerization number of phosphoric acid is approximately 60) was added at a concentration of 1 mM were evaluated. The promotion of fibroblast proliferation is compared and the results are shown in Table 5.

~~[0093]~~

[Table 5]

Table 5

Time/Group	Example 4-1	Example 4-2	Comparative Example 2-1	Comparative Example 2-2	Comparative Example 2-3
Details	1 mM Polyphosphoric acid extracted from yeast (extract P)	0.2 mM Polyphosphoric acid extracted from yeast (extract P)	Extract which hardly contains polyphosphoric acid (extract 0)	1 mM Chemically synthesized polyphosphoric acid	No treatment
24	0.3448 (1.17)	0.3117 (1.05)	0.3311 (1.12)	0.2913 (0.99)	0.2958 (1.00)
48	0.4334 (1.50)	0.3533 (1.22)	0.3279 (1.13)	0.3594 (1.24)	0.2892 (1.00)
69	0.532 (2.12)	0.4818 (1.92)	0.4107 (1.64)	0.4346 (1.73)	0.2507 (1.00)

Numbers in parentheses show proliferation rates at the time when a value of no treatment (Comparative Example 2-3) at each time is considered as 1.

~~[0094]~~

Table 5 shows the details of each example and comparative example and cell proliferation rates thereof (absorbance at 490 nm). Fig. 3 is a figure in which the values are shown by a graph. It was found that Example 4-1 had the highest proliferation rate and an extract containing polyphosphoric acid extracted from yeast at 1 mM had a very high activity of promoting cell proliferation. It was also found that the extract in Example 4-2 containing polyphosphoric acid extracted from yeast at 0.2 mM also had a relatively high activity of promoting cell proliferation. It was found that its ability to promote proliferation depended on a polyphosphoric acid content. In Comparative Example 2-1 which hardly contained polyphosphoric acid, an ability to promote proliferation was low as compared to samples containing polyphosphoric acid. It was also found that when polyphosphoric acid extracted from yeast was added, a proliferation promotion rate was approximately 1.5 times to twice higher than when the same concentration of chemically synthesized polyphosphoric acid was added. From the above, it was found that polyphosphoric acid extracted from yeast had a more excellent ability to promote cell proliferation than that of conventional polyphosphoric acid which was chemically synthesized.

Example 5

~~[0095]~~

(Permeability of polyphosphoric acid extracted from yeast into artificial skin)

The permeability of chemically synthesized polyphosphoric acid and polyphosphoric acid extracted from yeast was compared using artificial skin (TESTSKIN LSE-high, TOYOBO CO., LTD). To the horny layer surface of artificial skin, 0.1 ml of a 20 mM aqueous solution of polyphosphoric acid was added, and 1.2 ml of TBS (20mM Tris-HCl pH 7.5, 137 mM NaCl) was added to the lower part of artificial skin and temperature was retained under conditions at 37°C for 24 hours. Thereafter, the amount of polyphosphoric acid permeated into the lower part of artificial skin (under the dermal layer) and merged in TBS was determined. In a determination method, the phosphoric acid amount was determined by a vanadate-molybdate reagent in the same manner as in Example 1 and the polyphosphoric acid amount was calculated from the value. The permeability into artificial skin is compared and the results are shown in Table 6. The results in Table 6 confirmed that in artificial skin supplemented with polyphosphoric acid extracted from yeast, polyphosphoric acid extracted from yeast had permeation with approximately 2.1 times the amount of chemically synthesized polyphosphoric acid. Therefore, it was found that polyphosphoric acid extracted from yeast more easily permeated into skin than chemically synthesized polyphosphoric acid.

~~[0096]~~

[Table 6]

Table 6

	Permeation amount (μM)
Chemically synthesized polyphosphoric acid	0.053
Polyphosphoric acid extracted from yeast	0.109

Example 6

~~[0097]~~

(Effect of polyphosphoric acid extracted from yeast on cell proliferation)

To the horny layer surface of artificial skin (TESTSKIN LSE-high, TOYOBO CO., LTD), 0.1 ml of 20 mM or 100 mM each aqueous solution of polyphosphoric acid was added. Contrarily, 0.1 ml of TBS was added to the horny layer surface of artificial skin as a comparative example. To the lower part of the artificial skin, 1.2 ml of TBS was added, and temperature was retained at 37°C for 70 hours and the survival rate of artificial skin cells was then evaluated by MTT method. MTT[3-(4,5-dimethlthiazol-2-yl)-diphenyl tetrazolium bromide] was dissolved in TBS to 0.333 mg/ml and 1.2 ml of lower layer liquid of artificial skin was exchanged with TBS having MTT dissolved therein. Temperature was retained at 37°C for 4 hours and the formation of insoluble formazan was then confirmed. The results are shown in Fig. 4. Further, artificial skin was then collected and formazan precipitated in the artificial skin was dissolved in 0.3 ml of isopropanol and absorbance at 595 nm was measured. The results are shown in Table 7.

~~[0098]~~

[Table 7]

Table 7

sample	Absorbance
100 mM polyphosphoric acid extracted from yeast	0.8089
20 mM polyphosphoric acid extracted from yeast	0.1499
100 mM chemically synthesized polyphosphoric acid	0.1911
20 mM chemically synthesized polyphosphoric acid	0.0963
TBS	0.0208

~~[0099]~~

Fig. 4 is photographs substitute for drawings which show the precipitation amount of formazan formed by the reduction ability of mitochondria of living cells by MTT method. In artificial skin to which 20 mM or 100 mM polyphosphoric acid extracted from yeast was added and which was cultured, the formulation of formazan was confirmed with the naked eye. In a sample treated with 100 mM polyphosphoric acid extracted from yeast, the formation of formazan was confirmed more remarkably than the others. From the results in Table 7, the formulation amount of formazan was 0.1499 in a sample treated with 20 mM polyphosphoric acid extracted from yeast. The formulation amount of formazan was 0.8089 in a sample treated with 100 mM polyphosphoric acid extracted from yeast. This confirmed that a sample treated with 100 mM polyphosphoric acid extracted from yeast had a high cell survival rate. Contrarily, in a sample treated with chemically synthesized polyphosphoric acid for 70 hours, the formation of formazan was hardly observed with the naked eye (Fig. 4). In addition, the formation amount of formazan in a sample treated with 20 mM chemically synthesized polyphosphoric acid was 0.0963. The formation amount of formazan in a sample treated with 100 mM chemically synthesized polyphosphoric acid was 0.1911. It was found that since the formation amount of formazan on artificial skin treated with TBS was 0.0208, the cell survival rate slightly increased even by chemically synthesized polyphosphoric acid, but the survival rate was lower than those of samples treated with polyphosphoric acid in yeast cells.

Example 7

~~[0100]~~

(Effect of promoting hair elongation by organ culture of hair follicles)

The effects of chemically synthesized polyphosphoric acid and polyphosphoric acid extracted from yeast on promoting hair elongation were compared. A single hair follicle tissue was isolated from human scalp tissues collected at the time of the operation of hair implantation and the isolated hair follicle was cultured in a broth

containing medium to long-chain polyphosphoric acid (5 mM) with an average chain length of 60 or more phosphate residues and polyphosphoric acid extracted from yeast (5 mM) for 12 days. During the culture, the length of hair was measured with time and the whole length of the hair was shown in Fig. 5. From the results in Fig. 5, it was found that hair follicles cultured in a broth containing polyphosphoric acid extracted from yeast had a greater degree of elongation of hair than that of hair follicles cultured in a broth containing medium to long-chain polyphosphoric acid.

Example 8

~~[0101]~~

(Evaluation of the percentage of hair follicles in the growth phase by organ culture of hair follicles)

Using hair follicles, the percentage of hair follicles in the growth phase of hair was evaluated. A single hair follicle tissue was isolated by the same method as in Example 7 and the isolated hair follicle was cultured in a broth containing chemically synthesized polyphosphoric acid (5 mM) and polyphosphoric acid extracted from yeast (5 mM) for 12 days. Thereafter, as shown in Fig. 6, a sample in which hair growth could be confirmed by organ culture as hair follicles in the growth phase, a sample in which hair growth could not be observed and hair was falling out from hair follicles as hair follicles in the regression phase and a sample in which changes were not observed at all as unchanged hair follicles were distinguished. The changes in the percentage of each hair follicle treated with various types of polyphosphoric acid were examined. The evaluation of the elongation phase, regression phase and unchanged phase of hair follicles is shown in Fig. 6. The resulting percentages were shown in Fig. 7. In the hair follicles treated with polyphosphoric acid extracted from yeast, the percentage of the elongation phase was high, in which the elongation phase was 87% until the 4th day of culture and the elongation phase was 62% even on the 6th day, while in the hair follicles treated with chemically synthesized polyphosphoric acid, the percentage of hair

follicles in the elongation phase decreased with time, in which the elongation phase was 62% on the 2nd day, 62% on the 4th day and the elongation phase was 50% on the 6th day. Therefore, it was found that the elongation phase was retained long in the hair follicles treated with polyphosphoric acid extracted from yeast and hair follicles were activated.

Example 9

~~[0102]~~

(Increase in the production amount of polyphosphoric acid by mixed culture with a lactic acid bacterium)

A lactic acid bacterium (Lactobacillus Casei) was mixed with yeast (Oriental yeast, regular, Oriental Yeast Co., Ltd.) in a percentage of 2.5% of the weight of yeast (Example 9-1) or 5% (Example 9-2) and the accumulation treatment of polyphosphoric acid was carried out by the method in Example 1-5. A sample in which polyphosphoric acid was accumulated with only yeast (Comparative Example 9-1) and a sample in which polyphosphoric acid was accumulated with only a lactic acid bacterium were prepared as comparative examples and the amounts of polyphosphoric acid produced by extraction of polyphosphoric acid from the samples were compared. The results were shown in Table 8. Compared to Comparative Example 9-1, the accumulated amount of polyphosphoric acid increased, which was 106.3% in Example 9-1 which was mixed with the lactic acid bacterium and cultured and 121.7% in Example 9-2. Since the accumulation of polyphosphoric acid in the sample of only the lactic acid bacterium (Comparative Example 9-2) was not more than the limit value of measurement, it is believed that an ability to accumulate polyphosphoric acid of yeast themselves was increased by adding the lactic acid bacterium. Consequently, an ability to produce polyphosphoric acid can be increased by mixing yeast with a lactic acid bacterium and culturing the obtained mixture.

~~[0103]~~

Summary of Invention

Technical Problem

[0007]

An object of the present invention is to provide a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair. Another object of the present invention is to provide a polyphosphoric acid composition which is highly safe and contains naturally synthesized polyphosphoric acid.

[0008]

An object of the present invention is to provide a method for producing a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair.

Solution to Problem

[0009]

As a result of repeated intensive research to solve the above problem, the present inventors could produce a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid, thereby completing the present invention.

[0010]

A first aspect of the present invention relates to a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid. The polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the

As a preferred embodiment of the first aspect of the present invention, polyphosphoric acid has a shape with a greatest diameter of 10 nm or more and 1000 nm or less. When polyphosphoric acid has a nanoparticle size, the polyphosphoric acid composition easily permeates into skin and scalp cells.

[0017]

As a preferred embodiment of the first aspect of the present invention, yeast is obtained by co-culturing with a lactic acid bacterium. As shown by way of examples, when yeast is co-cultured with a lactic acid bacterium, an ability to accumulate polyphosphoric acid is increased. Because of this, when yeast is obtained by co-culturing with a lactic acid bacterium, the concentration of polyphosphoric acid increases, and accordingly an excellent polyphosphoric acid composition can be obtained.

[0018]

A second aspect of the present invention relates to hair tonics. The hair tonics contain, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast. By mixing an effective amount of a polyphosphoric acid composition with a hair tonic, the hair tonic operates to promote hair elongation and maintain the growth phase of hair.

[0019]

A third aspect of the present invention relates to a method for producing a substance containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. This method comprises a step of obtaining an yeast mass, a step of obtaining a yeast broth, a step of obtaining yeast precipitates, a step of obtaining a yeast suspension, a step of obtaining a yeast extract and a step of obtaining a polyphosphoric acid composition. The step of obtaining an yeast mass is a step of obtaining an yeast mass by standing yeast cells under temperature conditions of 1°C or more and 10°C or less. The step of obtaining a yeast broth is a step of obtaining a

broth containing yeast containing and accumulating polyphosphoric acid by culturing the yeast mass in a broth containing phosphate. The step of obtaining yeast precipitates is a step of obtaining precipitates by separating the yeast broth by centrifugation. The step of obtaining a yeast suspension is a step of, by suspending yeast cells obtained as precipitates in a liquid, obtaining a suspension in which yeast cells are suspended. The step of obtaining a yeast extract is a step of obtaining a yeast extract by extracting polyphosphoric acid from yeast bodies by heat treatment of the yeast suspension. The step of obtaining a polyphosphoric acid composition is a step of, by separating the yeast extract by centrifugation into supernatant containing polyphosphoric acid and precipitates containing a fraction of yeast bodies, obtaining a polyphosphoric acid composition in the supernatant. An objective polyphosphoric acid composition is produced in the above step. The culture step in the production method involved in the present invention consists of one step and the culture time is drastically shortened. The obtained polyphosphoric acid composition has an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life, and further more effectively acts to promote hair elongation and maintain the growth phase of hair.

[0020]

As a preferred embodiment of the third aspect of the present invention, the step of obtaining a yeast extract is a step of obtaining the polyphosphoric acid and protein hydrolysates by heat treatment at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. Protein hydrolysates can be obtained by heat treatment at a temperature of 60°C or more and 80°C or less for not less than a certain time. In addition to polyphosphoric acid, a polyphosphoric acid composition containing protein hydrolysates has an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life, and further more effectively acts to promote hair elongation and maintain the growth phase of hair.

Advantageous Effects of Invention

[0021]

According to the present invention, there can be provided a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair. The polyphosphoric acid composition of the present invention contains polyphosphoric acid naturally synthesized in yeast bodies and there can be provided an excellent polyphosphoric acid composition in terms of safety.

[0022]

According to the present invention, a polyphosphoric acid composition in which an ability to promote cell proliferation, cell permeability and an ability to promote prolongation of cell life are high and which further acts to promote hair elongation and maintain the growth phase of hair can be further produced. The production method of the present invention is excellent in terms of being able to efficiently obtain a polyphosphoric acid composition.

Brief Description of Drawings

[0023]

Fig. 1 shows the molecular weight distribution of polyphosphoric acid obtained from yeast by heat extraction.

Fig. 2 shows an electron micrograph of polyphosphoric acid particles extracted from yeast.

Fig. 3 shows an ability to promote fibroblast proliferation by a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast.

Fig. 4 is photographs substitute for drawings which show the formulation of formazan on artificial skin treated with polyphosphoric acid extracted from yeast.

Fig. 5 shows the effect of promoting hair elongation by polyphosphoric acid extracted from yeast.

Fig. 6 shows examples of hair follicles in the elongation phase, regression phase and no changes of hair.

Fig. 7 shows the effect of maintaining the elongation phase of hair by polyphosphoric acid extracted from yeast.

Description of Embodiments

[0024]

A first aspect of the present invention relates to a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid. The polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid has a high cell permeation ability. Further, when the polyphosphoric acid composition is applied to the skin and scalp, the polyphosphoric acid composition effectively acts on cell proliferation, prolongation of cell life, hair elongation and maintenance of the growth phase of hair.

[0025]

The term "the polyphosphoric acid composition" means a composition containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. The polyphosphoric acid composition can be in the various shapes such as liquid, condensed liquid, powder and solid. The polyphosphoric acid composition is preferably water-soluble since the composition is mixed with agents such as hair tonics. In addition, the polyphosphoric acid composition can further contain at least one of cell components, protein hydrolysates, vitamins and minerals along with polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. Substances contained in the polyphosphoric acid composition

are not limited to the above-described substances and other effective ingredients can be contained therein.

[0026]

Yeast is basically eukaryotic and unicellular microorganisms. Yeast has a cell wall on the outermost layer and the cell wall has glucan and mannan which are polysaccharides as main ingredients. A cell membrane is just under the cell wall and various receptors and transfer pumps exist therein. Budding yeast is eukaryotes and has a nuclear membrane and a nucleus. Besides, budding yeast has organelles such as cytoplasm, mitochondria and microtubule. In general, yeast can be roughly classified into budding yeast and fission yeast. The yeast used in the present invention are not particularly limited insofar as the yeast are generally called yeast, and budding yeast and fission yeast can be properly used.

[0027]

As a preferred embodiment of the present invention, yeast is budding yeast. Budding yeast has been used for the processing of food since long ago. Budding yeast can decompose sugars in food into alcohol by fermenting the food. Yeast has been used for the production of food products such as beer, bread and Japanese fermented soybean paste since long ago and safety in use for food has been confirmed since long ago. Budding yeast is yeast which proliferate by budding. Preferred examples of budding yeast are Saccharomyces genus, Torulopsis genus, Mycotorula genus, Torulaspora genus, Candida genus, Rhodotorula genus and Pichia genus. In the present invention, a polyphosphoric acid composition containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid can be efficiently obtained by using budding yeast. Further, a polyphosphoric acid composition which more effectively acts on skin and scalp cells can be obtained by using budding yeast.

[0028]

As a further preferred embodiment, yeast is co-cultured with a lactic acid

bacterium. By co-culturing, yeast is affected from the lactic acid bacterium or metabolites to be produced thereby and then the accumulated amount of polyphosphoric acid increases.

[0029]

Microorganisms have an ability to incorporate phosphoric acid into the bodies of microorganisms and synthesize polyphosphoric acid. Microorganisms have an enzyme to synthesize polyphosphoric acid utilizing adenosine triphosphate (ATP) and thus can synthesize polyphosphoric acid from the incorporated phosphoric acid. Yeast is microorganisms and thus, as described above, have an ability to incorporate phosphoric acid into the bodies of microorganisms and synthesize polyphosphoric acid.

[0030]

Yeast cells incorporate phosphoric acid into yeast bodies by culturing yeast in a broth containing phosphoric acid. The broth is preferably pH 3 to 6 and may be also pH 3 to 8 depending on the characteristics of target proteins to be produced by yeast. The culture temperature is preferably 20°C or more and 35°C or less. The broth can also contain minerals such as magnesium, calcium and zinc, and vitamins such as vitamin B2, vitamin B6, vitamin C, pantothenic acid and folic acid at a high concentration. Vitamins and minerals each contained in total mass in the broth can be 0.1 mass% to 3 mass%. Yeast can incorporate minerals and vitamins existing in the broth into yeast bodies.

[0031]

Polyphosphoric acid is synthesized by synthetic enzymes in yeast cells from phosphoric acid incorporated into yeast bodies. Polyphosphoric acid is contained and accumulated in yeast bodies in this manner. The degree of polymerization of synthesized polyphosphoric acid varies depending on types of yeast species and types of polyphosphoric acid-synthetic enzymes. In the same type of yeast species, the yeast have the same type of synthetic enzymes, and thus the degree of polymerization of

polyphosphoric acid to be obtained is within a certain range. Further, the desired degree of polymerization of polyphosphoric acid can be obtained by controlling enzyme activity by adjusting the culture temperature and culture time of yeast.

[0032]

Polyphosphoric acid synthesized in yeast bodies can be extracted by applying heat shock to yeast to destroy the yeast bodies. The heating conditions are preferably a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. The cell wall and cell membrane of yeast are destroyed by heat treatment and a yeast extract containing polyphosphoric acid synthesized in yeast, salts of the polyphosphoric acid or solvates of the polyphosphoric acid can be obtained. When the cell wall and cell membrane are destroyed, a part of cell component of yeast such as cell wall, cell membrane, mitochondria, nuclear membrane, intranuclear substances and microtubule is also extracted in an extract.

[0033]

The obtained yeast extract is separated by centrifugation into supernatant containing polyphosphoric acid and precipitates containing a fraction of yeast bodies, and a polyphosphoric acid composition is obtained. When the size of cell fragments is not smaller than a certain size, the cell fragments are removed by precipitation in a step of separating a yeast extract by centrifugation. Fine cell components however remain in supernatant of the extract. Because of this, a part of cell component of yeast is contained in a polyphosphoric acid composition along with polyphosphoric acid. Further, when cell components of yeast and proteins produced by yeast are decomposed by enzymes, protein hydrolysates are contained in a yeast extract. In this case, protein hydrolysates are contained in a polyphosphoric acid composition along with polyphosphoric acid and a part of cell component. When a polyphosphoric acid composition contains cell components and protein hydrolysates, the polyphosphoric acid composition can contain nutrients derived from yeast.

[0034]

As described above, when a broth contains minerals such as magnesium, calcium or zinc and vitamins such as vitamin B2, vitamin B6, vitamin C, pantothenic acid and folic acid at a high concentration, the minerals and vitamins are contained and accumulated in yeast bodies along with polyphosphoric acid. Therefore, vitamins and minerals contained and accumulated in yeast bodies are also contained in an extract extracted from yeast by heat treatment along with polyphosphoric acid.

[0035]

The cell components of yeast, protein hydrolysates, vitamins and minerals are nutrient sources for skin and scalp cells and useful for the maintenance or growth of cells. Therefore, when these substances are added to hair tonics, these substances act as effective ingredients. Further, an ability to promote cell proliferation and skin permeability of polyphosphoric acid can be increased by interacting cell components of yeast, protein hydrolysates, vitamins and minerals with polyphosphoric acid.

[0036]

Polyphosphoric acid may be linear chain polyphosphoric acid, branched polyphosphoric acid, cyclic polyphosphoric acid and polyphosphoric acid which is a highly branched (reticulated state) phosphoric acid polymer (ultraphosphoric acid) and may be also mixtures thereof and derivatives thereof. Linear chain polyphosphoric acid has a structure in which two or more PO_4 tetrahedrons share the oxygen atom on the top by dehydration and condensation of orthophosphoric acid to stretch in a row. Branched polyphosphoric acid has a structure in which organic groups are introduced into a straight branched chain.

[0037]

Polyphosphoric acid has H_2O and P_2O_5 as constituent molecules and is linear chain or cyclic polyphosphoric acid represented by $H_{n+2}(P_nO_{3n+1})$ or $(HPO_3)_n$. Herein, the number of polymerized phosphoric acid is represented by n. Polyphosphoric acid

mainly has polyphosphoric acid with a degree of polymerization of 20 to 100 phosphate residues (medium-chain polyphosphoric acid) and polyphosphoric acid with a degree of polymerization of 100 to 1000 phosphate residues (long-chain polyphosphoric acid). In the present invention, the degree of polymerization of polyphosphoric acid is 20 to 300 phosphate residues, preferably 50 to 250 phosphate residues and more preferably 80 to 200 phosphate residues. Polyphosphoric acid with a degree of polymerization of 20 or more phosphate residues and 300 or less phosphate residues is water-soluble and has an ability to promote cell proliferation by stabilizing cell growth factors such as fibroblast growth factors.

[0038]

The polyphosphoric acid composition of the present invention contains polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid.

[0039]

The salts of polyphosphoric acid mean salts of polyphosphoric acid, particularly pharmaceutically acceptable salts of polyphosphoric acid. The salts of polyphosphoric acid are preferably alkali metal salts and more preferably sodium salts. In the present description, salts of polyphosphoric acid may contain not only anhydrous salts but also hydrated salts. These salts, for example, are ionized in a solution to operate in the same manner as polyphosphoric acid.

[0040]

The solvates of polyphosphoric acid mean solvates of polyphosphoric acid. The solvates include hydrates. In addition, the polyphosphoric acid composition of the present invention can become a hydrate by absorption of water and attachment of adsorbed water in a yeast body or an extract. Such solvates formed are also included in the solvates of polyphosphoric acid. These solvates are ionized in a solution to operate in the same manner as polyphosphoric acid.

[0041]

As the polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid used in the present invention, a kind thereof may be used or a mixture of several kinds thereof may be used. Several kinds of polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid contain polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid with different degrees of polymerization or different molecular structures and polyphosphoric acid salts with different metal ions.

[0042]

In the present invention, it is preferred that polyphosphoric acid contained in the total mass of a polyphosphoric acid composition be 0.01 mass% or more and 70 mass% or less, preferably 0.02 mass% or more and 50 mass% or less and further preferably 0.1 mass% or more and 50 mass% or less. Polyphosphoric acid may be salts of polyphosphoric acid or solvates of polyphosphoric acid, and the percentage content of polyphosphoric acid to a polyphosphoric acid composition is a percentage content of the total mass of these three types of polyphosphoric acid. By containing an effective amount of polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid in a polyphosphoric acid composition, the polyphosphoric acid composition effectively acts on the promotion of cell proliferation, prolongation of skin cell life, maintenance of hair elongation and the growth phase of hair and the effect of regulating intestinal function.

[0043]

The polyphosphoric acid obtained in the present invention is synthesized in yeast bodies and contained and accumulated in the yeast bodies. Polyphosphoric acid exists in a folded structure to stably exist in a yeast body. Polyphosphoric acid in the folded state has a shape with a greatest diameter of 10 nm or more and 1000 nm or less, preferably 30 nm or more and 800 nm or less, and further preferably 50 nm or more and 500 nm or less. The polyphosphoric acid obtained in the present invention has a

particle size in the order of nanometers and thus permeability in skin surfaces is excellent. Polyphosphoric acid in the above shape easily fits to scalp and the layer of oil and fat on the skin and permeability is increased. The folded structure means a particle shape in which polyphosphoric acid coheres and the shape includes various shapes such as a globular shape, an oval shape, a cylindrical shape, a square shape and a block with unevenness.

[0044]

A second aspect of the present invention relates to hair tonics. The hair tonics contain, as an effective ingredient, an effective amount of a polyphosphoric acid composition extracted from yeast.

[0045]

The hair tonics involved in the present invention contain a polyphosphoric acid composition as an effective ingredient. A polyphosphoric acid composition is added to a hair tonic in a liquid, condensed liquid, powder or solid state. It is preferred that polyphosphoric acid contained in the total mass of a hair tonic be 0.01 mass% or more and 70 mass% or less, preferably 0.02 mass% or more and 50 mass% or less and further preferably 0.1 mass% or more and 50 mass% or less. The hair tonics may be administered in a range of 0.01 g or more and 10 g or less (preferably 0.1 g or more and 5 g or less) in terms of the weight of a polyphosphoric acid composition once to ten times a day.

[0046]

To the hair tonics involved in the present invention, other known hair growth components can be further added. Examples thereof are activators of vital cells such as laurel extract and chlorophyll, blood flow-promoting agents to promote blood flow in peripheral blood vessels such as Asian ginseng extract, Swertia herb extract, cepharanthin, vitamin E and derivatives thereof and γ-oryzanol, local irritants such as capsicum tincture, ginger tincture, cantharis tincture and nicotinic acid benzyl ester,

nutrients for supplying nutrition to the surroundings of hair matrix cells such as vitamins such as vitamin A, B1, B2, B6, E and derivatives thereof, pantothenic acid and derivatives thereof and biotin, and amino acids such as cystine, cysteine, methionine, leucine, tryptophan and amino acid extracts, female hormones having antagonism against male hormones such as estradiol and ethinyl estradiol, hair root activators to improve hypofunction of hair matrix cells such as pantothenic acid and derivatives thereof, placenta extract, allantoin and quaternium-51, anti-inflammatory agents such as dipotassium glycyrrhizinate, β-glycyrrhetinic acid, allantoin and hinokitiol, and humectants to prevent scalp from drying such as glycerin and pyrrolidone carboxylic acid. One or two or more components selected from these hair growth components can be added.

[0047]

In general, hair tonics have effects on promotion of hair emergence, promotion of hair growth, hair restoration, hair nourish and prevention of hair loss. In general, hair tonics are directly applied to scalp. Examples of hair tonics are liquid medicines, emulsions, cream, lotions, gels, foam, sprays and the like. In addition, a polyphosphoric acid composition may be contained in shampoo, rinse and hair treatment as an effective ingredient. Fomentations in which an effective ingredient is soaked in nonwoven fabric or cotton may be also used. The hair tonics containing a polyphosphoric acid composition as an effective ingredient can be produced according to a conventional method.

[0048]

The basic function of hair tonics is to sustain the activation of hair follicle cells of scalp and elongate hair. Hair tonics are preferably those with a high permeation ability into hair follicle cells to permeate an effective ingredient into hair follicle cells of scalp. As described above, polyphosphoric acid synthesized in yeast bodies has a particle size in the order of nanometers. Therefore, polyphosphoric acid synthesized in

yeast bodies have a high permeation ability into hair follicle cells and can sustain the activation of hair follicle cells. Therefore, hair tonics can promote hair elongation and further can maintain hair follicle cells in the state of the growth phase.

[0049]

Another aspect of the present invention relates to a method for producing a substance containing polyphosphoric acid, salts of polyphosphoric acid or solvates of polyphosphoric acid. This method comprises a step of obtaining an yeast mass, a step of obtaining a yeast broth, a step of obtaining yeast precipitates, a step of obtaining a yeast suspension, a step of obtaining a yeast extract and a step of obtaining a polyphosphoric acid composition. The step of obtaining an yeast mass is a step of obtaining an yeast mass by standing yeast cells under temperature conditions of 1°C or more and 10°C or less. The step of obtaining a yeast broth is a step of obtaining a broth containing yeast containing and accumulating polyphosphoric acid by culturing the yeast mass in a broth containing phosphate. The step of obtaining yeast precipitates is a step of obtaining precipitates by separating the yeast broth by centrifugation. The step of obtaining a yeast suspension is a step of, by suspending yeast cells obtained as precipitates in a liquid, obtaining a suspension in which yeast cells are suspended. The step of obtaining a yeast extract is a step of obtaining a yeast extract by extracting polyphosphoric acid from yeast bodies by heat treatment of the yeast suspension. The step of obtaining a polyphosphoric acid composition is a step of, by separating the yeast extract by centrifugation into supernatant containing polyphosphoric acid and precipitates containing a fraction of yeast bodies, obtaining a polyphosphoric acid composition in the supernatant.

[0050]

Yeast cells can incorporate phosphoric acid into yeast bodies and synthesize polyphosphoric acid in the cell. Yeast incorporate phosphoric acid into fungal bodies by culturing yeast in a broth containing phosphate. As a method in which a yeast body

incorporates phosphoric acid and synthesizes polyphosphoric acid, a method having two steps as a culture step is generally known. The first step is a step of obtaining the phosphoric acid starvation state of yeast by culturing yeast cells in a broth which does not contain phosphoric acid at all for the purpose that yeast easily incorporates phosphoric acid. The second step is a step of incorporating phosphoric acid into yeast bodies by culturing yeast cells in a broth rich in phosphoric acid. It has been believed that yeast can efficiently incorporate phosphoric acid into cell bodies by the culture step having two steps. In the above-described method, however, two types of broths are required, and thus yeast cells cultured in the first type of broth are required to be collected and cultured in a new broth again. Therefore, a conventional culture step has had problems in terms of handling and costs.

[0051]

In the production method involved in the present invention, there is a step of obtaining an yeast mass first. The step of obtaining an yeast mass is a step of obtaining an yeast mass by standing yeast at 1°C or more and 10°C or less. In the step of obtaining an yeast mass, yeast are left to stand under conditions where the water content is 30 mass% or more and 50 mass% or less relative to an yeast mass. In the step of obtaining an yeast mass, yeast are preferably left to stand at 1°C or more and 10°C or less for preferably 72 hours or more. In the step of obtaining an yeast mass, a medium or a broth is not required and a broth is not required to be exchanged after obtaining an yeast mass. Therefore, the culture step procedure is simplified. Further, the culture time in the step of obtaining a yeast broth is drastically shortened by undergoing the step of obtaining an yeast mass.

[0052]

The step of obtaining a yeast broth is a step of culturing an yeast mass in a broth containing phosphate. The broth can contain minerals such as magnesium, calcium and zinc, and vitamins such as vitamin B2, vitamin B6, vitamin C, pantothenic

acid and folic acid at a high concentration. In the production method involved in the present invention, the step of obtaining a yeast broth consists of only one step because of undergoing the step of obtaining an yeast mass. In the production method involved in the present invention, approximately 1.5 times amount of polyphosphoric acid can be obtained as compared to a conventional production step containing a culture step consisting of two steps. Further, the culture time is within an hour or more to 3 hours and is drastically shortened as compared to the culture time required in the conventional culture step consisting of two steps. Further, by culturing yeast in the presence of minerals and vitamins, not only a polyphosphoric acid composition favorable for hair tonics can be obtained but also the culture time can be shortened.

[0053]

It is preferred that the broth contain a lactic acid bacterium. The lactic acid bacterium is preferably added at 0.1 mass% or more and 50 mass% or less of yeast, and may be 0.5 mass% or more and 20 mass% or less, 1 mass% or more and 15 mass% or less, and 2 mass% or more and 10 mass% or less. Examples of lactic acid bacteria are any or two or more of Lactobacillus casei, Lactobacillus acidophilus, Lactobacillus brevis, Lactobacillus delbrueckii, Lactobacillus fermentum, Lactobacillus helveticus, Lactobacillus kefir, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius, Streptococcus thermophilus, Lactococcus lactis, Lactococcus plantarum, Lactococcus raffinolactis, Leuconostoc lactis and Leuconostoc mesenteroides. The lactic acid bacterium may be added to a medium in advance, may be gradually added during the course of culture, or may be added during the course of culture.

[0054]

The step of obtaining yeast precipitates is a step of obtaining yeast to be precipitated by separating a fluid, in which yeast cells are cultured, by centrifugation. Centrifugation is carried out in conditions of a centrifugal force of 3000 × g to 15000 ×

g for 3 minutes to 30 minutes. Centrifugation can be carried out by a conventional method.

[0055]

The step of obtaining a yeast suspension is a step of suspending yeast precipitates obtained by centrifugation in a cell suspension solvent. For the cell suspension solvent, solutions which are commonly used for suspending cells such as distilled water, a physiological saline solution, a phosphate buffer and a pH buffer can be used.

[0056]

The step of obtaining a yeast extract is a step of applying heat to a yeast extract. By applying heat shock to yeast, cell membranes are destroyed and polyphosphoric acid is eluted outside fungal bodies. The heat treatment can be carried out at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. It is preferred that the heat treatment be adjusted to the conditions of temperature and time at which synthesized polyphosphoric acid is not decomposed.

[0057]

The step of obtaining a polyphosphoric acid composition is a step of, by separating the yeast extract by centrifugation into supernatant containing polyphosphoric acid and a fraction of yeast bodies, obtaining a polyphosphoric acid composition. Polyphosphoric acid eluted from yeast cells is water-soluble and dissolved in a liquid. Therefore, the extract can be separated by centrifugation into precipitates of yeast bodies and a polyphosphoric acid composition containing polyphosphoric acid. After centrifugation, supernatant is collected and a polyphosphoric acid composition can be obtained. Centrifugation is carried out in conditions of a centrifugal force of 5000 × g to 10000 × g for 3 minutes to 30 minutes. Centrifugation can be carried out by a conventional method. The yeast extract contains cell components of yeast, protein hydrolysates of proteins, vitamins and

minerals along with polyphosphoric acid. The cell components of yeast, protein hydrolysates, vitamins and minerals are useful for the maintenance or the growth of skin and scalp cells and operate as an effective ingredient of hair tonics. Therefore, it is preferred that during centrifugation in the step of obtaining a polyphosphoric acid composition, these substances be left in supernatant along with polyphosphoric acid. As centrifugal conditions, therefore, separation is preferably carried out at a relatively low centrifugal force which can be set in a common centrifuge such as 5000 × g to 10000 × g.

[0058]

As a preferred embodiment of the present invention, the step of obtaining a yeast extract is a step of heat treatment at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less. The above-described step of obtaining a yeast extract in the production method of a polyphosphoric acid composition is a step of heat treatment at a temperature of 60°C or more and 80°C or less and preferably 65°C or more and 75°C or less for 20 minutes or more and 40 minutes or less and preferably 25 minutes or more and 35 minutes or less. Synthesized polyphosphoric acid may be decomposed depending on conditions of heating temperature and heating time and thus the treatment time of heat treatment is preferably 40 minutes or less. Further, protein hydrolysates can be obtained in the step of obtaining a yeast extract. Protein hydrolysates can be obtained by hydrolyzing proteins in yeast bodies. Therefore, heat treatment is preferably carried out at a temperature of 80°C or less for not less than a certain time not to deactivate enzymes in protein hydrolysates in yeast bodies.

[0059]

Although the production step in the present invention contains a step of heat treatment at a temperature of 60°C or more and 80°C or less for 20 minutes or more and 40 minutes or less, the denaturation of proteins and vitamins which are weak against

heat is suppressed. This is because, when proteins and vitamins are extracted together with polyphosphoric acid from yeast, nano particles of polyphosphoric acid protect proteins and vitamins by inclusion. Polyphosphoric acid extracted from yeast also has a function to stabilize components unstable in heat.

Example 1

[0060]

(Production of intra-cellular polyphosphoric acid using yeast)

As the yeast used for culture, live yeast for food (Oriental yeast, regular, Oriental Yeast Co., ltd.) was used. First, the above yeast cells were stored at 10°C or less for 3 days to obtain an yeast mass. Next, the yeast was cultured only in a broth B with a composition described in Table 1. The culture time and the amount of yeast bodies are as described in Table 2. The amount of yeast bodies is shown by a percentage of the amount of fungal bodies to the amount of broth. The broth B was prepared in an amount of 40 mL and the yeast was put therein to a percentage described in Table 2. Thereafter, the broth with the yeast was cultured with shaking at 25°C for 2 hours. After shaking culture, the broth was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. The collected yeast cells were washed with distilled water once and stored at -30°C.

[0061]

Comparative Example 1

In order that the yeast would be in a phosphate starvation state, yeast cells were cultured in a broth A not containing phosphoric acid at all. The culture time and the amount of yeast bodies are as described in Table 2. The amount of yeast bodies are shown by a percentage of the amount of fungal bodies to each amount of broth. The broth A was prepared in an amount of 300 mL and 6 g of the yeast were put therein. Thereafter, the broth with the yeast was cultured with shaking at 25°C for each time

described in Table 2. After shaking culture, the broth was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. Next, the broth B was prepared in an amount of 40 mL and the collected yeast bodies were put therein to a percentage described in Table 2. Thereafter, the broth with the yeast was cultured with shaking at 25°C for 2 hours. After shaking culture, the broth was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. The collected yeast cells were washed with distilled water once and stored at -30°C. Both the broths A and B were adjusted to pH 5.2 with a succinate buffer or a phosphate buffer.

[0062]

[Table 1]

Table 1

Broth A		Broth B	
$(NH_4)_2SO_4$	0.50%	$(NH_4)_2SO_4$	0.50%
KCl	0.07%	KCl	0.07%
$MgSO_4 \cdot 7H_2O$	0.04%	$MgSO_4 \cdot 7H_2O$	0.04%
D-Glucose	1%	D-Glucose	1%
Succinic acid	100mM	$NaH_2PO_4 \cdot 2H_2O$	100mM

[0063]

[Table 2]

Table 2

	Broth A		Broth B	
	Amount of yeast bodies	Culture time	Amount of yeast bodies	Culture time
Comparative Example 1-1	3.33%	15 hours	5.0%	2 hours
Comparative Example 1-2	6.67%	5 hours	2.0%	2 hours
Comparative Example 1-3	2.00%	15 hours	1.5%	2 hours
Comparative Example 1-4	2.00%	15 hours	2.0%	3 hours
Example 1-5	—	—	5.0%	2 hours
Example 1-6	—	—	7.5%	2 hours
Example 1-7	—	—	10.0%	2 hours

[0064]

(Comparative measurement of the contained and accumulated amount of polyphosphoric acid)

After culturing the yeast in the conditions in Table 2, the contained and accumulated amount of polyphosphoric acid in yeast was evaluated. The yeast cells were suspended in distilled water with twice the weight of the cells originally put and the obtained suspension was subjected to heat treatment at a temperature of 80°C for 30 minutes. Thereafter, centrifugation was carried out in conditions of 10000 × g for 10 minutes and supernatant was collected to obtain an extract. To the extract, nitric acid was added so that the final concentration would be 3.4%, and by heat treatment at a temperature of 100°C for 30 minutes, polyphosphoric acid was completely hydrolyzed. Next, a vanadate-molybdate reagent was added to determine a phosphoric acid amount. A polyphosphoric acid amount was calculated from the determined phosphoric acid amount (Japanese Standard of Food Additives, Hirokawa Shoten Co., Method for determining potassium polyphosphate). The calculated results are shown in Table 3. From the results in Table 3, in Comparative Examples 1-1, 1-2, 1-3 and 1-4, the accumulated amount of polyphosphoric acid per g of yeast was 0.03 to 0.13 mmol by the number of moles when a phosphoric acid residue was considered as a unit.

Contrarily, it was found that the accumulated amount was large in Examples 1-5, 1-6 and 1-7 not using the broth A, which was 0.10 to 0.22 mmol. In Example 1-5, the contained and accumulated amount of polyphosphoric acid was highest and was 1.5 times or more that of Comparative Example 1-3. In addition, in Examples 1-5, 1-6 and 1-7, the culture time was also short, which was for 2 hours. Further, the broth was not required to be exchanged. It was found that in the production method of a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast, the production method of Example 1 was excellent as compared to the production method of Comparative Example 1 in terms of handling and costs.

[0065]

[Table 3]

Table 3

	Accumulated amount of polyphosphoric acid per g of yeast (mmol)
Comparative Example 1-1	0.03
Comparative Example 1-2	0.08
Comparative Example 1-3	0.14
Comparative Example 1-4	0.13
Example 1-5	0.22
Example 1-6	0.16
Example 1-7	0.10

Example 2

[0066]

(Evaluation of a method for extracting polyphosphoric acid)

The condition for extracting polyphosphoric acid from yeast was considered. Heat treatment was carried out so that the temperature (60 to 0°C) and time (0 to 60

min) of heat treatment would be conditions described in Table 4 and the extraction rate of polyphosphoric acid was compared. The broth of yeast was separated by centrifugation in conditions of 4000 × g for a minute to collect precipitates of yeast bodies. The collected yeast bodies were frozen at -20°C. Thereafter, the yeast cells were liquefied and suspended in distilled water again. The obtained suspension was dispensed to a microtube at each 50 μL. After dispensing and stirring, centrifugation was carried out in conditions of 8000 × g for 5 minutes and supernatant was collected as an extract. The obtained extract was used as a sample and electrophoresis was carried out using 15% polyacrylamide gel (150 V, 45 min). Thereafter, the gel was stained by 0.05% toluidine blue. The result of electrophoresis is shown in Fig. 1. When the molecular weight distribution of natural polyphosphoric acid extracted was confirmed by electrophoresis, it was found that molecular species with a large molecular weight decreased depending on heating time by heat treatment at 80°C for 40 minutes or more. This is because polyphosphoric acid has a possibility of being decomposed by heating for a long period of time. Because of this, it was found that the heat treatment at 80°C for approximately 30 minutes is preferred for the extraction of polyphosphoric acid.

[0067]

To the extract obtained by the above method, nitric acid was added so that the final concentration would be 3.4%, and by heat treatment at a temperature of 100°C for 30 minutes, polyphosphoric acid was completely hydrolyzed. Next, a vanadate-molybdate reagent was added to determine a phosphoric acid amount. A polyphosphoric acid amount was calculated from the determined phosphoric acid amount. The calculated results are shown in Table 4. From the results in Table 4, when heating at 80°C for 30 minutes, the greatest extraction amount of polyphosphoric acid was obtained.

[0068]

[Table 4]

Table 4

Extraction conditions	Heating temperature (°C)	Heating time (min)	Polyphosphoric acid concentration in yeast cell (mM)
Example 2-1	90	10	66.09
Example 2-2	80	0	7.24
Example 2-3	80	10	88.68
Example 2-4	80	20	86.13
Example 2-5	80	30	93.17
Example 2-6	80	40	91.92
Example 2-7	80	50	85.46
Example 2-8	80	60	85.20
Example 2-9	70	0	11.49
Example 2-10	70	10	72.14
Example 2-11	70	20	78.15
Example 2-12	70	30	83.13
Example 2-13	70	40	80.49
Example 2-14	70	50	68.89
Example 2-15	70	60	77.60
Example 2-16	60	10	19.65
Example 2-17	60	30	31.83
Example 2-18	60	60	48.18

Example 3

[0069]

(Shapes of polyphosphoric acid extracted from yeast)

A polyphosphoric acid composition containing polyphosphoric acid extracted from yeast was freeze-dried to obtain dry powder of a polyphosphoric acid extract. The fine structure of the dry powder was observed by a scanning electron microscope. As shown in Fig. 2, a large number of particles with a diameter of 1000 nm or less were

confirmed along with structures which are believed that constituents of yeast cells were dried and solidified. This could confirm that polyphosphoric acid extracted from yeast existed as particles with a diameter of the order of nanometers.

Example 4

[0070]

(Promotion of fibroblast proliferation by a polyphosphoric acid composition extracted from yeast)

The effect of a polyphosphoric acid composition containing polyphosphoric acid extracted from yeast to fibroblast proliferation was evaluated by MTS method (CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit manufactured by Promega KK). Yeast was cultured by the procedure in Examples 1-5. For extraction, these suspensions of fungal bodies were dispensed at 0.2 mL each to an Eppendorf tube and heat treatment was carried out at 80°C for 30 minutes. Centrifugation was carried out in conditions of 10000 × g for 10 minutes and supernatant was collected to obtain an extract P. The obtained extracts P each were sterilized by filtration with a 0.45 μm sterilization filter and the protein concentration and the phosphoric acid amount were measured. As a result of the measurement of the phosphoric acid amount, the polyphosphoric acid concentration in the extract P was 91.1 mM. In addition, as a result of the measurement of the protein concentration, the protein concentration contained in the extract P was 15.89 mg/ml.

[0071]

Comparative Example 2

Yeast cultured only in a broth not containing phosphoric acid (broth A used in the procedure in Comparative Examples 1-3) for 15 hours was subjected to heat treatment in the same conditions as the extraction conditions of the extract P to prepare an extract Q which hardly contained polyphosphoric acid. The background level polyphosphoric acid concentration in the extract Q was 0.65 mM and the protein

concentration was 9.84 mg/ml.

[0072]

(Comparison of promotion of fibroblast proliferation)

Next, these extracts P and Q were added to a broth of dermal cells in normal human breast (HDF, manufactured by Cell Applications, Inc. USA) and the effect of promoting cell proliferation was evaluated by MTS method (CellTiter 96 Aqueous One Solution Cell Proliferation Assay kit, manufactured by Promega KK). The cells were seeded in a 96-well plate at 3000 cells/well and cultured in D-MEM medium with 10% FBS (fetal bovine serum) for 24 hours. Thereafter, the cells were cultured in D-MEM with 1% FBS overnight. The cell broth was exchanged with D-MEM medium supplemented with each extract containing polyphosphoric acid. The MTS reaction was carried out at 24, 48 and 69 hours and OD_{492} nm was measured at 95 minutes from the onset of the reaction by a plate reader. Each extract was adjusted so that the protein concentration would be 0.174 mg/ml. In this case, the polyphosphoric acid concentration in the extract P was 1 mM in the medium. The polyphosphoric acid concentration in the extract Q was 0.0115 mM in the medium. The polyphosphoric acid concentration of the medium supplemented with the extract P is a concentration approximately 87 times the polyphosphoric acid concentration of the medium supplemented with the extract Q. In addition, a sample in which the concentration of polyphosphoric acid extracted from yeast was 0.2 mM in the extract was also evaluated.

[0073]

As comparative examples, a case in which polyphosphoric acid was not added to a medium and a case to which medium-chain sodium polyphosphate which was not derived from yeast and chemically synthesized (chemically synthesized polyphosphoric acid, the average polymerization number of phosphoric acid is approximately 60) was added at a concentration of 1 mM were evaluated. The promotion of fibroblast proliferation is compared and the results are shown in Table 5.

[0074]

[Table 5]

Table 5

Time/Group	Example 4-1	Example 4-2	Comparative Example 2-1	Comparative Example 2-2	Comparative Example 2-3
Details	1 mM Polyphosphoric acid extracted from yeast (extract P)	0.2 mM Polyphosphoric acid extracted from yeast (extract P)	Extract which hardly contains polyphosphoric acid (extract 0)	1 mM Chemically synthesized polyphosphoric acid	No treatment
24	0.3448 (1.17)	0.3117 (1.05)	0.3311 (1.12)	0.2913 (0.99)	0.2958 (1.00)
48	0.4334 (1.50)	0.3533 (1.22)	0.3279 (1.13)	0.3594 (1.24)	0.2892 (1.00)
69	0.532 (2.12)	0.4818 (1.92)	0.4107 (1.64)	0.4346 (1.73)	0.2507 (1.00)

Numbers in parentheses show proliferation rates at the time when a value of no treatment (Comparative Example 2-3) at each time is considered as 1.

[0075]

Table 5 shows the details of each example and comparative example and cell proliferation rates thereof (absorbance at 490 nm). Fig. 3 is a figure in which the values are shown by a graph. It was found that Example 4-1 had the highest proliferation rate and an extract containing polyphosphoric acid extracted from yeast at 1 mM had a very high activity of promoting cell proliferation. It was also found that the extract in Example 4-2 containing polyphosphoric acid extracted from yeast at 0.2 mM also had a relatively high activity of promoting cell proliferation. It was found that its ability to promote proliferation depended on a polyphosphoric acid content. In Comparative Example 2-1 which hardly contained polyphosphoric acid, an ability to promote proliferation was low as compared to samples containing polyphosphoric acid. It was also found that when polyphosphoric acid extracted from yeast was added, a proliferation promotion rate was approximately 1.5 times to twice higher than when the same concentration of chemically synthesized polyphosphoric acid was added. From the above, it was found that polyphosphoric acid extracted from yeast had a more excellent ability to promote cell proliferation than that of conventional polyphosphoric acid which was chemically synthesized.

Example 5

[0076]

(Permeability of polyphosphoric acid extracted from yeast into artificial skin)

The permeability of chemically synthesized polyphosphoric acid and polyphosphoric acid extracted from yeast was compared using artificial skin (TESTSKIN LSE-high, TOYOBO CO., LTD). To the horny layer surface of artificial skin, 0.1 ml of a 20 mM aqueous solution of polyphosphoric acid was added, and 1.2 ml of TBS (20mM Tris-HCl pH 7.5, 137 mM NaCl) was added to the lower part of artificial skin and temperature was retained under conditions at 37°C for 24 hours. Thereafter, the amount of polyphosphoric acid permeated into the lower part of artificial skin (under the dermal layer) and merged in TBS was determined. In a determination method, the phosphoric acid amount was determined by a vanadate-molybdate reagent in the same manner as in Example 1 and the polyphosphoric acid amount was calculated from the value. The permeability into artificial skin is compared and the results are shown in Table 6. The results in Table 6 confirmed that in artificial skin supplemented with polyphosphoric acid extracted from yeast, polyphosphoric acid extracted from yeast had permeation with approximately 2.1 times the amount of chemically synthesized polyphosphoric acid. Therefore, it was found that polyphosphoric acid extracted from yeast more easily permeated into skin than chemically synthesized polyphosphoric acid.

[0077]

[Table 6]

Table 6

	Permeation amount (μM)
Chemically synthesized polyphosphoric acid	0.053
Polyphosphoric acid extracted from yeast	0.109

Example 6

[0078]

(Effect of polyphosphoric acid extracted from yeast on cell proliferation)

To the horny layer surface of artificial skin (TESTSKIN LSE-high, TOYOBO CO., LTD), 0.1 ml of 20 mM or 100 mM each aqueous solution of polyphosphoric acid was added. Contrarily, 0.1 ml of TBS was added to the horny layer surface of artificial skin as a comparative example. To the lower part of the artificial skin, 1.2 ml of TBS was added, and temperature was retained at 37°C for 70 hours and the survival rate of artificial skin cells was then evaluated by MTT method. MTT[3-(4,5-dimethlthiazol-2-yl)-diphenyl tetrazolium bromide] was dissolved in TBS to 0.333 mg/ml and 1.2 ml of lower layer liquid of artificial skin was exchanged with TBS having MTT dissolved therein. Temperature was retained at 37°C for 4 hours and the formation of insoluble formazan was then confirmed. The results are shown in Fig. 4. Further, artificial skin was then collected and formazan precipitated in the artificial skin was dissolved in 0.3 ml of isopropanol and absorbance at 595 nm was measured. The results are shown in Table 7.

[0079]

[Table 7]

Table 7

sample	Absorbance
100 mM polyphosphoric acid extracted from yeast	0.8089
20 mM polyphosphoric acid extracted from yeast	0.1499
100 mM chemically synthesized polyphosphoric acid	0.1911
20 mM chemically synthesized polyphosphoric acid	0.0963
TBS	0.0208

[0080]

Fig. 4 is photographs substitute for drawings which show the precipitation amount of formazan formed by the reduction ability of mitochondria of living cells by MTT method. In artificial skin to which 20 mM or 100 mM polyphosphoric acid extracted from yeast was added and which was cultured, the formulation of formazan was confirmed with the naked eye. In a sample treated with 100 mM polyphosphoric acid extracted from yeast, the formation of formazan was confirmed more remarkably than the others. From the results in Table 7, the formulation amount of formazan was 0.1499 in a sample treated with 20 mM polyphosphoric acid extracted from yeast. The formulation amount of formazan was 0.8089 in a sample treated with 100 mM polyphosphoric acid extracted from yeast. This confirmed that a sample treated with 100 mM polyphosphoric acid extracted from yeast had a high cell survival rate. Contrarily, in a sample treated with chemically synthesized polyphosphoric acid for 70 hours, the formation of formazan was hardly observed with the naked eye (Fig. 4). In addition, the formation amount of formazan in a sample treated with 20 mM chemically synthesized polyphosphoric acid was 0.0963. The formation amount of formazan in a sample treated with 100 mM chemically synthesized polyphosphoric acid was 0.1911. It was found that since the formation amount of formazan on artificial skin treated with TBS was 0.0208, the cell survival rate slightly increased even by chemically synthesized polyphosphoric acid, but the survival rate was lower than those of samples treated with polyphosphoric acid in yeast cells.

Example 7

[0081]

(Effect of promoting hair elongation by organ culture of hair follicles)

The effects of chemically synthesized polyphosphoric acid and polyphosphoric acid extracted from yeast on promoting hair elongation were compared. A single hair follicle tissue was isolated from human scalp tissues collected at the time of the operation of hair implantation and the isolated hair follicle was cultured in a broth

containing medium to long-chain polyphosphoric acid (5 mM) with an average chain length of 60 or more phosphate residues and polyphosphoric acid extracted from yeast (5 mM) for 12 days. During the culture, the length of hair was measured with time and the whole length of the hair was shown in Fig. 5. From the results in Fig. 5, it was found that hair follicles cultured in a broth containing polyphosphoric acid extracted from yeast had a greater degree of elongation of hair than that of hair follicles cultured in a broth containing medium to long-chain polyphosphoric acid.

Example 8

[0082]

(Evaluation of the percentage of hair follicles in the growth phase by organ culture of hair follicles)

Using hair follicles, the percentage of hair follicles in the growth phase of hair was evaluated. A single hair follicle tissue was isolated by the same method as in Example 7 and the isolated hair follicle was cultured in a broth containing chemically synthesized polyphosphoric acid (5 mM) and polyphosphoric acid extracted from yeast (5 mM) for 12 days. Thereafter, as shown in Fig. 6, a sample in which hair growth could be confirmed by organ culture as hair follicles in the growth phase, a sample in which hair growth could not be observed and hair was falling out from hair follicles as hair follicles in the regression phase and a sample in which changes were not observed at all as unchanged hair follicles were distinguished. The changes in the percentage of each hair follicle treated with various types of polyphosphoric acid were examined. The evaluation of the elongation phase, regression phase and unchanged phase of hair follicles is shown in Fig. 6. The resulting percentages were shown in Fig. 7. In the hair follicles treated with polyphosphoric acid extracted from yeast, the percentage of the elongation phase was high, in which the elongation phase was 87% until the 4th day of culture and the elongation phase was 62% even on the 6th day, while in the hair follicles treated with chemically synthesized polyphosphoric acid, the percentage of hair

follicles in the elongation phase decreased with time, in which the elongation phase was 62% on the 2nd day, 62% on the 4th day and the elongation phase was 50% on the 6th day. Therefore, it was found that the elongation phase was retained long in the hair follicles treated with polyphosphoric acid extracted from yeast and hair follicles were activated.

Example 9

[0083]

(Increase in the production amount of polyphosphoric acid by mixed culture with a lactic acid bacterium)

A lactic acid bacterium (Lactobacillus Casei) was mixed with yeast (Oriental yeast, regular, Oriental Yeast Co., Ltd.) in a percentage of 2.5% of the weight of yeast (Example 9-1) or 5% (Example 9-2) and the accumulation treatment of polyphosphoric acid was carried out by the method in Example 1-5. A sample in which polyphosphoric acid was accumulated with only yeast (Comparative Example 9-1) and a sample in which polyphosphoric acid was accumulated with only a lactic acid bacterium were prepared as comparative examples and the amounts of polyphosphoric acid produced by extraction of polyphosphoric acid from the samples were compared. The results were shown in Table 8. Compared to Comparative Example 9-1, the accumulated amount of polyphosphoric acid increased, which was 106.3% in Example 9-1 which was mixed with the lactic acid bacterium and cultured and 121.7% in Example 9-2. Since the accumulation of polyphosphoric acid in the sample of only the lactic acid bacterium (Comparative Example 9-2) was not more than the limit value of measurement, it is believed that an ability to accumulate polyphosphoric acid of yeast themselves was increased by adding the lactic acid bacterium. Consequently, an ability to produce polyphosphoric acid can be increased by mixing yeast with a lactic acid bacterium and culturing the obtained mixture.

[0084]

[Table 8]

Table 8

		Accumulated amount of polyphosphoric acid per g of yeast (mmol)	Production efficiency (%)
Comparative Example 9-1	only yeast	0.221	100
Comparative Example 9-2	only lactic acid bacterium	not more than the limit of detection	—
Example 9-1	yeast + 2.5% lactic acid bacterium	0.235	106.3
Example 9-2	yeast + 5% lactic acid bacterium	0.269	121.7