



ORYZA OIL & FAT CHEMICAL CO., LTD.

POLYAMINE

Natural Ingredient for Healthy Hair and Nail Treatment with Anti-ageing

- **POLYAMINE-P**
(Water-soluble powder, food use)



Oryza Oil&Fat Chemical Co., Ltd.

Ver.3.0 YF

Natural Ingredient for Healthy Hair and Nail Treatment with Anti-ageing Properties

POLYAMINE

1. Introduction

Polyamine is an organic compound consists of 2 or more amino groups (-NH₂). Polyamines are synthesized in cells and play essential role in the proliferation and development of mammalian cells. In addition, polyamines have been shown to exert antioxidant activity ^{2,3)}, anti-allergenic effect ^{4,5)}, and suppression on glycation process ^{6,7)}. Recently, there are increasing interests on the research of polyamines.

Polyamines are abundantly available in the liver of poultry, fermented soybeans, mushrooms and soybeans. The content of polyamines in the body declines with age regardless of the consumption of food rich in polyamines.

In Japan, there are increasing numbers of allergies cases which are believed to be caused by low consumption of food rich in polyamines (e.g. fermented soybeans and “tofu” bean curd) comparing with the past.

Lately, Polyamines has been reported to prevent arteriosclerosis ^{8,9)} and promotion of hair growth ^{10,11)} due to its anti-inflammatory properties and cell proliferative effect respectively. In view of rising interested in the nail and hair treatment in the industry, Oryza Oil & Fat Chemical Co., Ltd. has venture into the research and development of wheat-derived polyamine. Studies showed that wheat-derived polyamines promote the cornification of keratinocytes and the production of keratin.

Polyamines augmented the industry with new and natural ingredient for healthy hair and nail treatment coupled with anti-ageing properties.

References cited

- 1) Roseeuw D.I. *et al.*, Epidermal keratinocytes actively maintain their intracellular polyamine levels. *Cell Tissue Kinet.* 16, 493-504 (1983).
- 2) Ha H.C. *et al.*, The natural polyamine spermine functions directly as a free radical scavenge. *Proc. Natl. Acad. Sci. USA.*, 95, 11140-45 (1998).
- 3) Fujisawa S. *et al.*, Kinetic evaluation of polyamines as radical scavengers. *Anticancer Res.* 25, 965-70 (2005).
- 4) Soda K. *et al.*, Spermine, a natural polyamine, suppresses LFA-1 expression on human lymphocyte. *J. Immunol.* 175, 237-45 (2005).
- 5) Zhang M. *et al.*, Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells:a counterregulatory mechanism that restrains the immune response. *J. Exp. Med.* 185, 1759-68 (1997).
- 6) Gugliucci A. *et al.*, The polyamines spermine and spermidine protect proteins from structural and functional damage by AGE precursors: a new role for old molecules?*Life Sci.* 72, 2603-16 (2003).
- 7) Gugliucci A., Alternative antiglycation mechanisms: are spermine and f

- ructosamine-3-ki nase part of a carbonyl damage control pathway?. *Med. Hypotheses*. 64. 2603-16 (2005).
- 8) Soda K., Polyamine intake, dietary pattern, and cardiovascular disease. *Med. Hypotheses*. 75. 299-301 (2010).
- 9) de la Pena N.C. *et al.*, Inhibition of platelet aggregation by putrescine, spermidine, and spermine in hypercholesterolemic rabbits. *Arch. Med. Res.*,75. 299-301 (2000).
- 10) Ramot Y. *et al.*, Polyamines and hair: a couple in search of perfection. *Exp. Dermatol.* 19, 784-90 (2010).
- 11) Ramot Y., Spermidine promotes human hair growth and is a novel modulator of human epithelial stem cell functions. *Plos One*, 6,1-11(2011).

2. Polyamines

Polyamine, as its name implies it consists of 2 or more primary amino group (-NH₂). There are more than 20 type of polyamines present in the human body. Spermidine, spermine and putrescine are the most prevalent polyamine present in all living organisms (Fig. 1). The synthesis of polyamine is highest in cells of foetus and newborns due to its cell proliferative property. It was found that polyamines are loaded in breast milk.

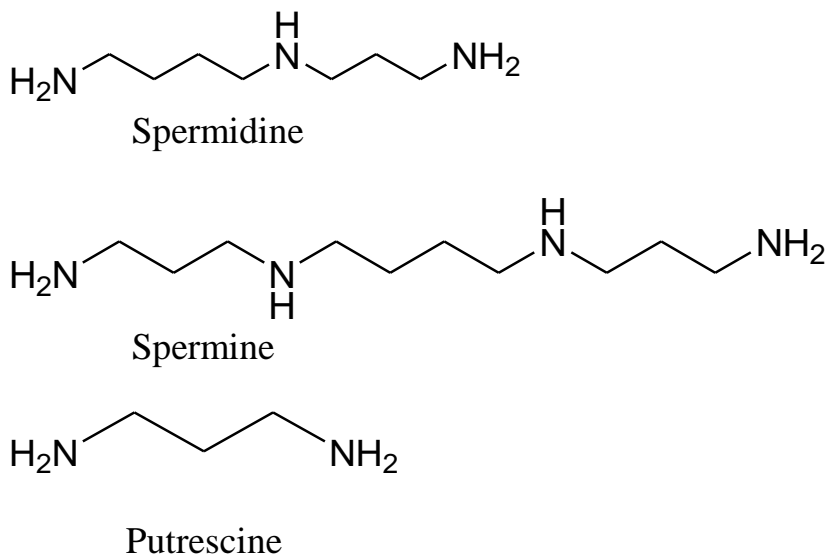


Fig. 1 Chemical Structure of Polyamines

Endogenously, polyamines are synthesized from the amino acid, arginine and converted into ornithine which will be converted to putrescine catalysed by the enzyme ornithine decarboxylase (ODC). Further to that, putrescine is converted to spermidine by spermidine synthase while spermidine is synthesized to spermine by spermine synthase (Fig. 2). The synthesis of polyamine declines with age due to the decline in enzyme catalyzing the reactions ^{12,13} (Fig. 3).

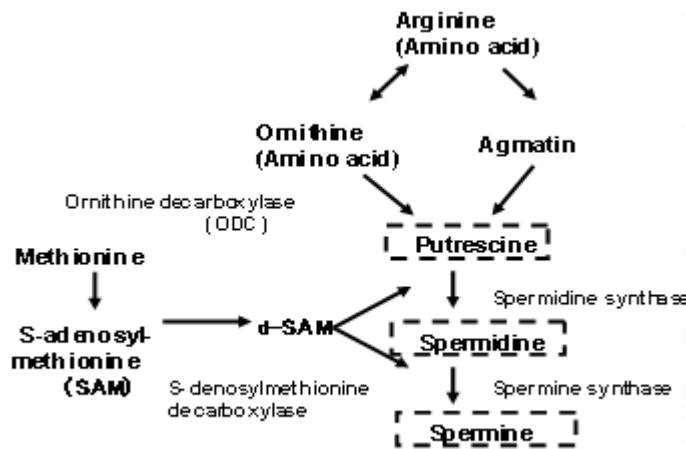


Fig. 2 Synthetic Pathway of Polyamines

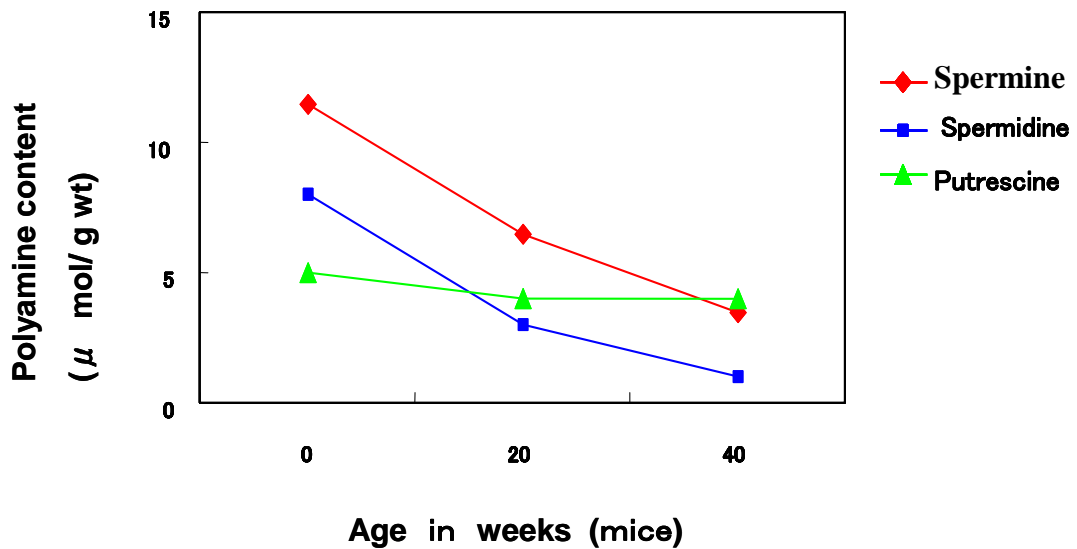


Fig. 3 Age-related Changes in Polyamine Concentration

- 12) Yoshinaga K. *et al.*, Age-related changes in polyamine biosynthesis after fasting and refeeding. *Exp. Gerontol.*28, 565-72 (1993).
- 13) Scalabrino G. *et al.*, Polyamines in mammalian ageing: an oncological problem, too? A review., *Mech. Ageing Dev.*, 26,149-64 (1984).

Fig. 4 illustrated the physiological functions of polyamine on the human body (Fig. 4).

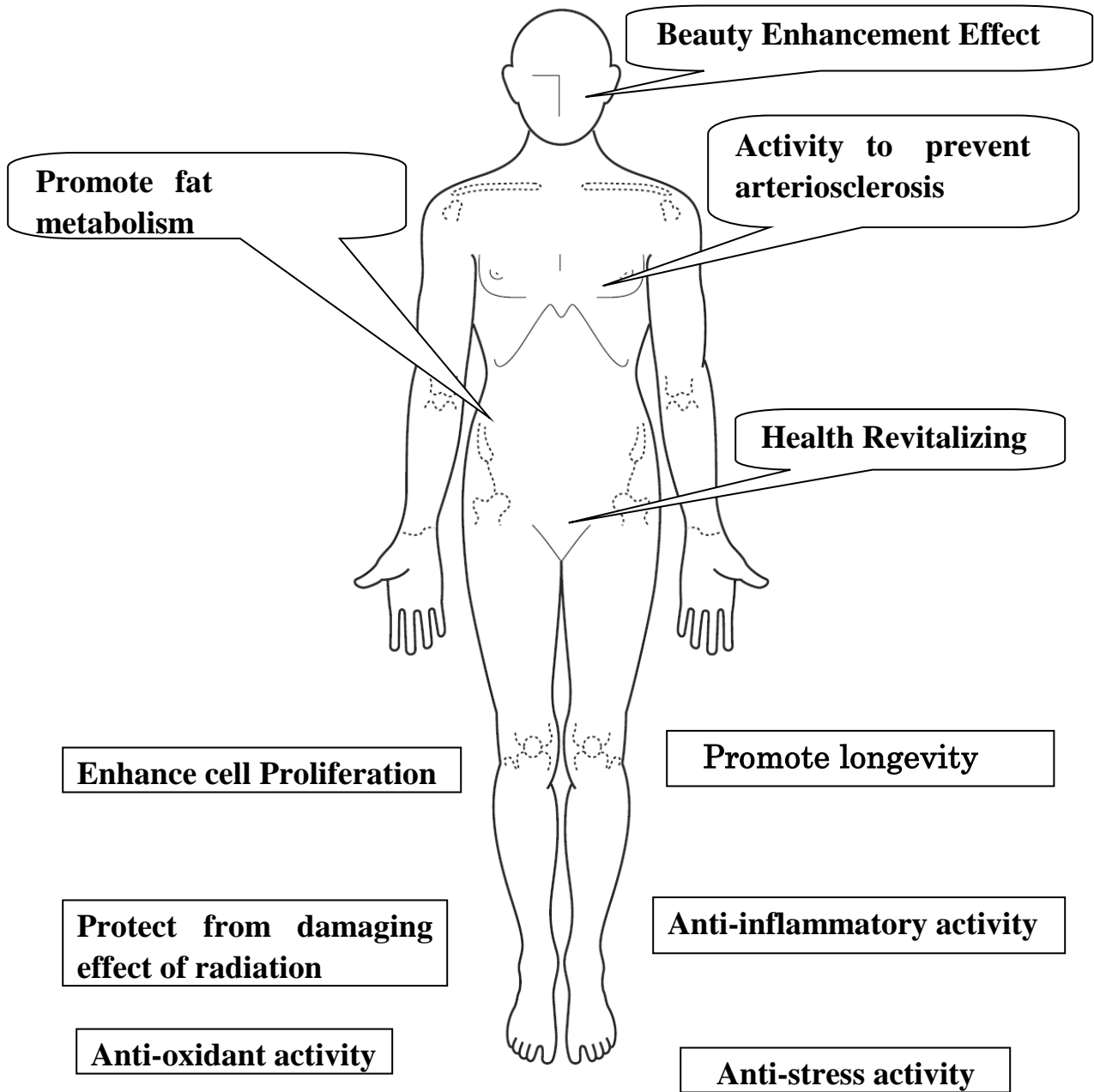
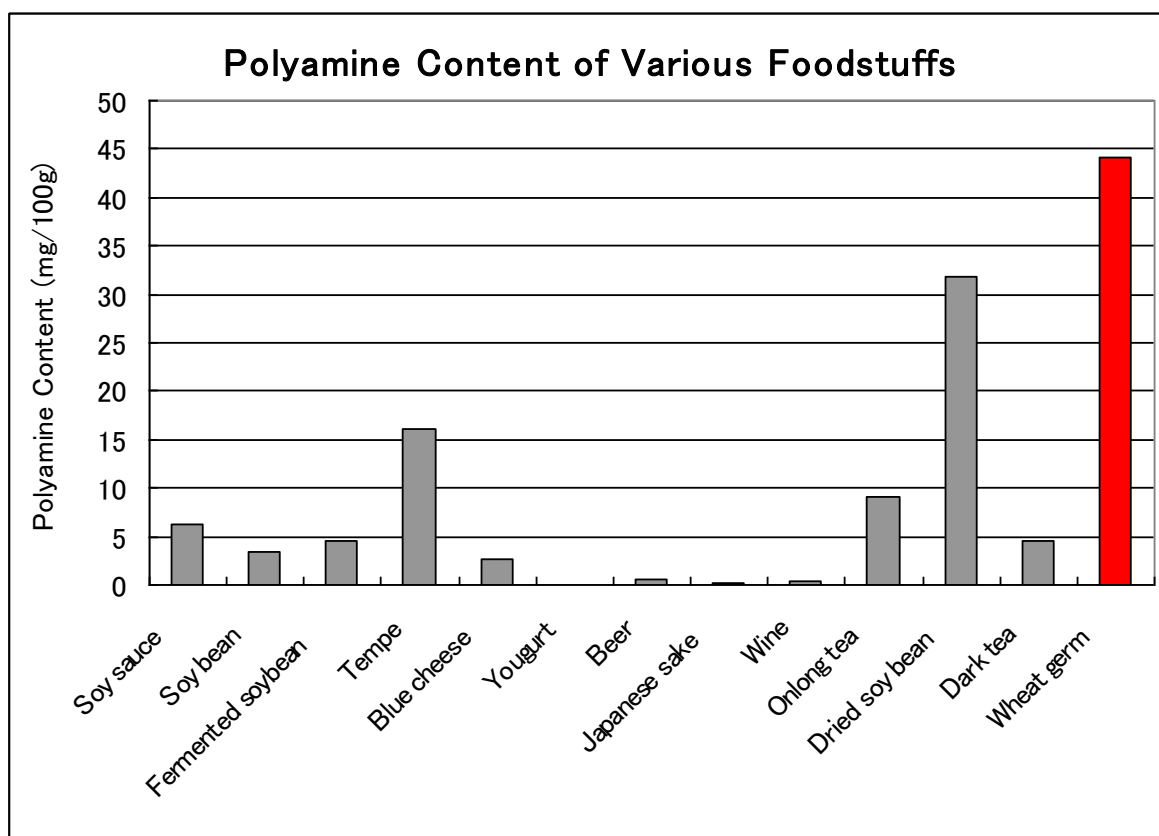


Fig. 4 Active points of Polyamines

3. Characteristics of Wheat-derived Polyamines

(1) The content of Polyamine – Comparison with different food sources

The content of Polyamine among different food sources were compared and wheat germ has the highest content of polyamines compared with fermented soybean or soybean paste which are high in polyamine (Fig. 5).



Okamoto A. *et al.*, Polyamine content of ordinary foodstuffs and various fermented foods. *Biosci.Biotech.Biochem.*61,1582-4 (1997)

Fig. 5 The content of Polyamine – Comparison with different food sources

The content of polyamine was compared with different wheat-derived products. As tabulated in Table 1, content of polyamine in wheat germ is greater than that of albumin or other wheat-derived food such as bread and noodles (Table 1).

The level of polyamines in our body is maintained by endogenous biosynthesis, intestinal microorganisms and through exogenous supply through the diet. As mentioned earlier, synthesis of polyamine declines with age and consumption of food rich in arginine and

ornithine will not increase the content of polyamine *in vivo*. Similarly, studies indicated that level of spermine and spermidine decreased with age ^{12,13}). Thus, dietary polyamines is important for the maintenance of optimal level of polyamine and physiological functions of various organs in the elderly. Wheat-derived polyamine is highly recommended due to its high spermine and spermidine content.

Table 1. Comparison on the content of Polyamine among various wheat and wheat-derived products.

Raw Material /Product	Spermidine	Spermine	Putrescine	TOTAL (mg/kg)
Wheat germ	243	134	64	441
Albmen	5	1	3	9
Bread	3	4	1	8
Wheat noodle	0.4	0.4	0.4	1.2

(2) Absorption of Polyamine

Polyamine has a molecular mass of ≤ 250 , similar to that of low molecular mass amino acids, it is absorbed through the digestive tract and transferred to the bloodstream to be utilized. Study showed that majority of luminal polyamines are degraded in the gut before reaching systemic circulation indicating that polyamine is absorbed, distributed in and utilized by the body.

Diamine oxidase, the enzyme that breaks down putrescine is present in the intestine resulting in a lower absorption of putrescine. However, spermine and spermidine are well absorbed in the GI tract in view of the higher molecular mass of spermine and spermidine resulting in the absence of enzymes that is capable of breaking down the compound.

Wheat-derived polyamine with high content of spermine and spermidine is recommended as dietary polyamines for maintenance of optimal health.

4. Functional effect of Polyamines

(1) The effect of Polyamines on keratinocytes

1) The effect of polyamines on expression of keratin1

The effect of spermidine, spermine and putrescine on the expression of keratin 1 was studied and experimented using human origin keratinocytes. Results showed that expression of keratin 1 mRNA was up regulated under the treatment of polyamines with a ratio of spermidine, spermine and putrescine = 7:2:1. (Fig. 6)

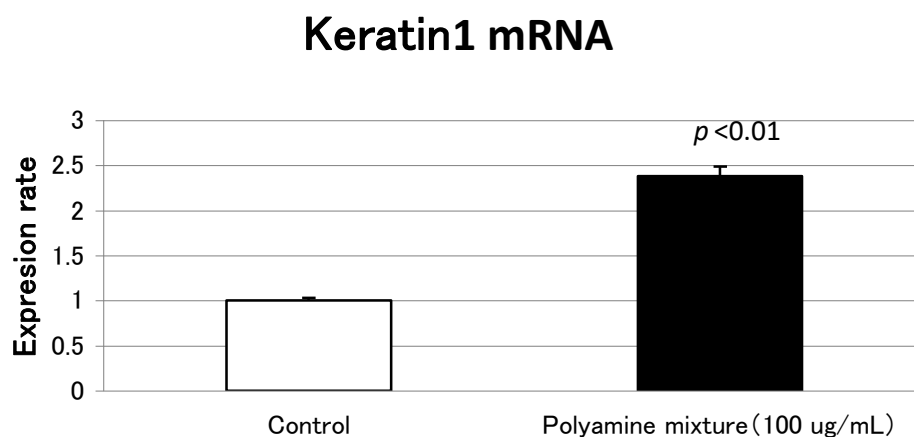


Fig. 6 The effect of Polyamines on the Expression of Keratin 1 mRNA on human keratinocytes
(The expression rate was corrected by β -actin. means \pm S.D., n=4)

[Method of experiment]

100 μ g/mL of polyamines (with ratio of spermidine, spermine and putrescine = 7:2:1) was added to human breast keratinocytes and cultured for 3 days. Upon completion of the cultured, cells were collected for extraction of RNA and expression of keratin 1 was determined.

2) The Effect of polyamines on keratin1 protein

Further study was prompted on the effect of spermidine, spermine and putrescine on the expression of keratin 1 protein using human keratinocytes. As shown in Fig. 7, expression of keratin 1 protein was up regulated in samples treated with spermidine and spermine at concentration 1mg/mL.

The results described in sections 1) and 2) suggest that wheat-derived polyamines accelerate the synthesis of proteins and nucleic acid and help the regeneration of nails and hair.

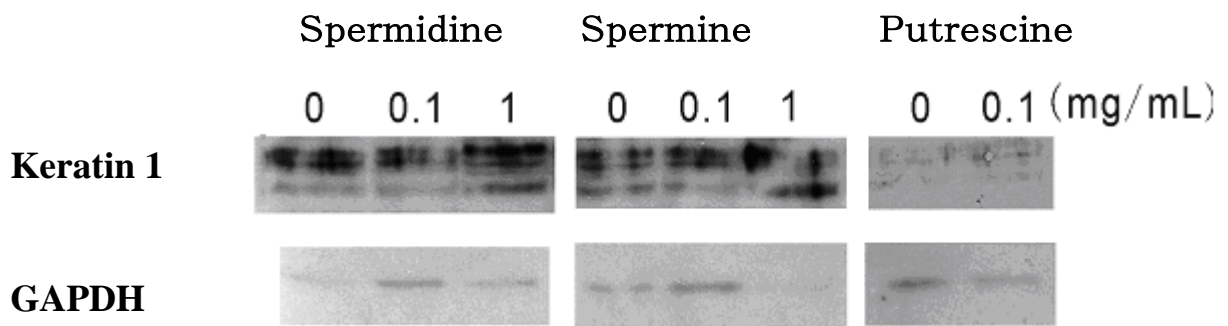


Fig.7 The effect of Spermidine, Spermine, and Putrescine on the expression of keratin1 protein.

[Method of experiment]

Human breast keratinocytes (3×10^4 cells/mL) was insemiated on a plate and cultured for 24 hours. Samples of polyamines, namely, spermidine, spermine and putrescine at concentration 01.mg/mL and 1mg/mL was added to the pre-cultured cells and continued cultured for 3 days. Expression of keratin 1 protein was determined at the end of the experiment by Western Blotting method.

Based on above finding, wheat-derived polyamines up-regulated the expression of keratin 1 and keratin 1 protein *in vitro*. It is suggestive that wheat-derived polyamines accelerate the synthesis of proteins and nuclei acid, similar to most findings described in published journals. Thus, wheat-derived polyamine is potentially beneficial on the regeneration of hair and nails.

(2) The Effect on Nail Formation

Polyamines are abundance in the epidermal keratinocytes essential for cell growth and proliferation. The nail is hyperkeratotic epithelial tissue which consist of the nail plate, nail matrix, nail wall and nail bed. The nail matrix is responsible for the production of cells which forms the nail plate. The nail plate or body of nail (*corpus unguis*) is the actual nail where keratinocytes differentiate, proliferate and cornified (Fig. 8). The strength of the nail plate is determined by the by its thickness and flexibility. A thicker nail plate is stronger with sufficient flexibility to withstand external forces.

The nail plate is hard as it is formed by hard keratin instead of its high calcium content. Approximately 15% of nail lipids consist of ceramide which plays important role in maintaining moisture of the nail.

The following experimental findings reveal the effect of polyamine on nail formation on the rear feet of mice.

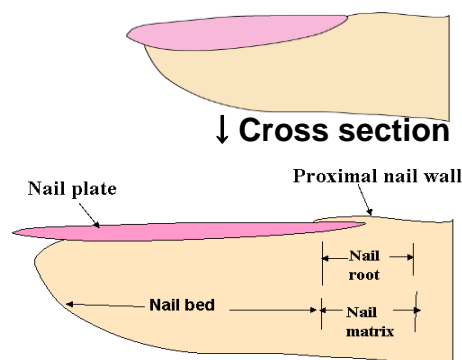


Fig.8 Structure of Nail

1) The effect of polyamine on the expression of keratin near the nails on the rear feet of mice (polyamine mixture)

Mice were fed 10mg/kg of polyamines containing spermidine, spermine and putrescine in a ratio of 7:2:1 for nine days. Expression of keratin 1 near the nails on the rear feet of mice was determined. As illustrated in Fig. 9A, expression of keratin 1 mRNA near the nails on the rear feet of mice was up-regulated after oral administration of polyamines. This findings was confirmed by Western Blot method where the band appear thicker in group of mice treated with polyamines, indicating an up-regulation of expression of keratin 1 protein (Fig. 9B).

Meanwhile, hematoxylin-eosin (HE)-stained images showed that the area being stained in purple-red (area surrounded by dotted line) increased in group of mice treated with polyamines (Fig. 10). Thus, nail formation from the surface layer to the keratin transitional layer being enhanced by polyamines.

(A)

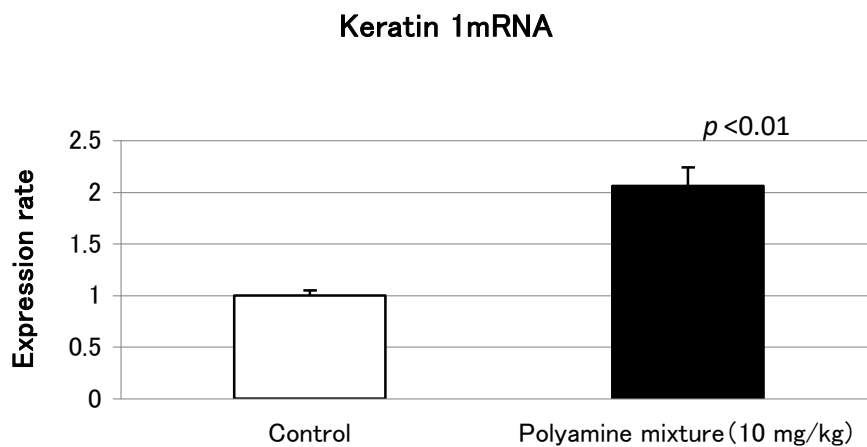


Fig 9A.The Effect of Polyamines on the expression of keratin 1 near nails of rear feet of mice. (The expression rate was corrected by β -actin. Average value \pm standard error, n=3-5)

(B)

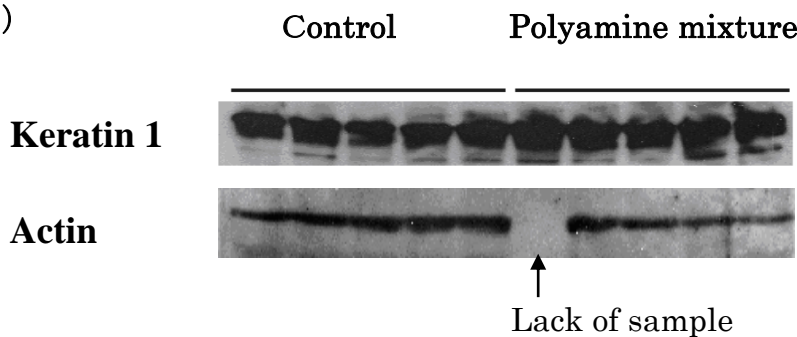
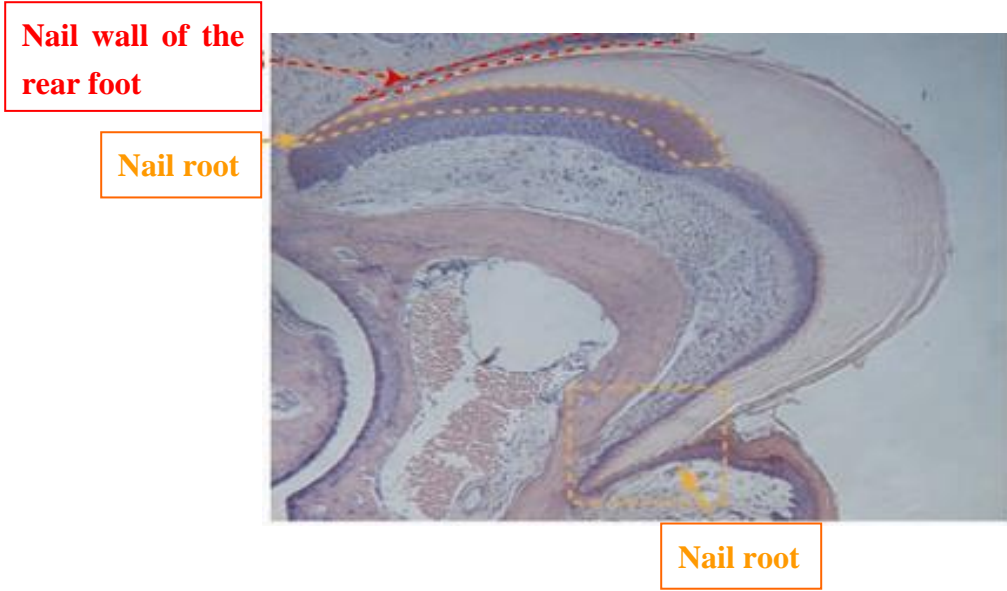


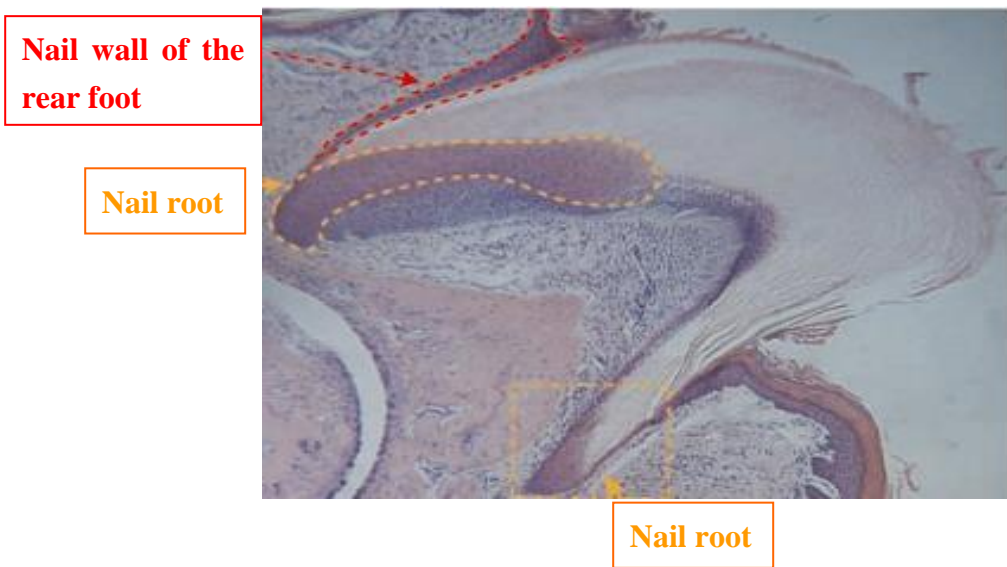
Fig 9B.The Effect of Polyamines on the expression of keratin 1 near nails of rear feet of mice.

< H.E. staining >

Control



Polyamine mixture



The purple-red stained area surrounded with a dotted line clearly increased as compared to the control.



This indicates that the nail formation from the surface layer to the keratin transitional layer was accelerated.

Fig.10 The effect of polyamines on nail formation near nails of rear feet of mice by HE Staining

[Method of experiment]

Mice (male, ICR, 5-week old) were fed with samples containing 10mg/kg of polyamines containing spermidine, spermine and putrescine in ratio of 7:2:1 for nine days. Condition of the first joint of the 3rd toe of the left rear foot of mice was evaluation by Western-Blot method. Test samples was collected from the 4th toe for hematoxylin-eosin (HE) staining test to observe the cross section of the nail.

2) The Effect of polyamine (without binder) on nail formation

Further experiment was carried out to study the oral effect of Polyamine on nail formation in mice. Upon comparison on the HE-Staining of nail root, results showed that polyamine enhanced nail formation from the surface layer (layer of blue granulated powder) to keratin transitional layer (purple red layer surrounded by black dotted line) (Fig. 11A).

Meanwhile, HE-Staining clearly showed that expression of keratin 1 and keratin 16 are up-regulated in group consuming polyamine (dark-brown stained area surrounded by black dotted line). Besides, results from Western-Blot method showed that the band of group of mice consuming polyamines appeared thicker indicating the up-regulation of the expression of keratin 1 protein (Fig. 11B). Thus, above findings suggested that polyamines enhances the production and cornification of keratin 1.

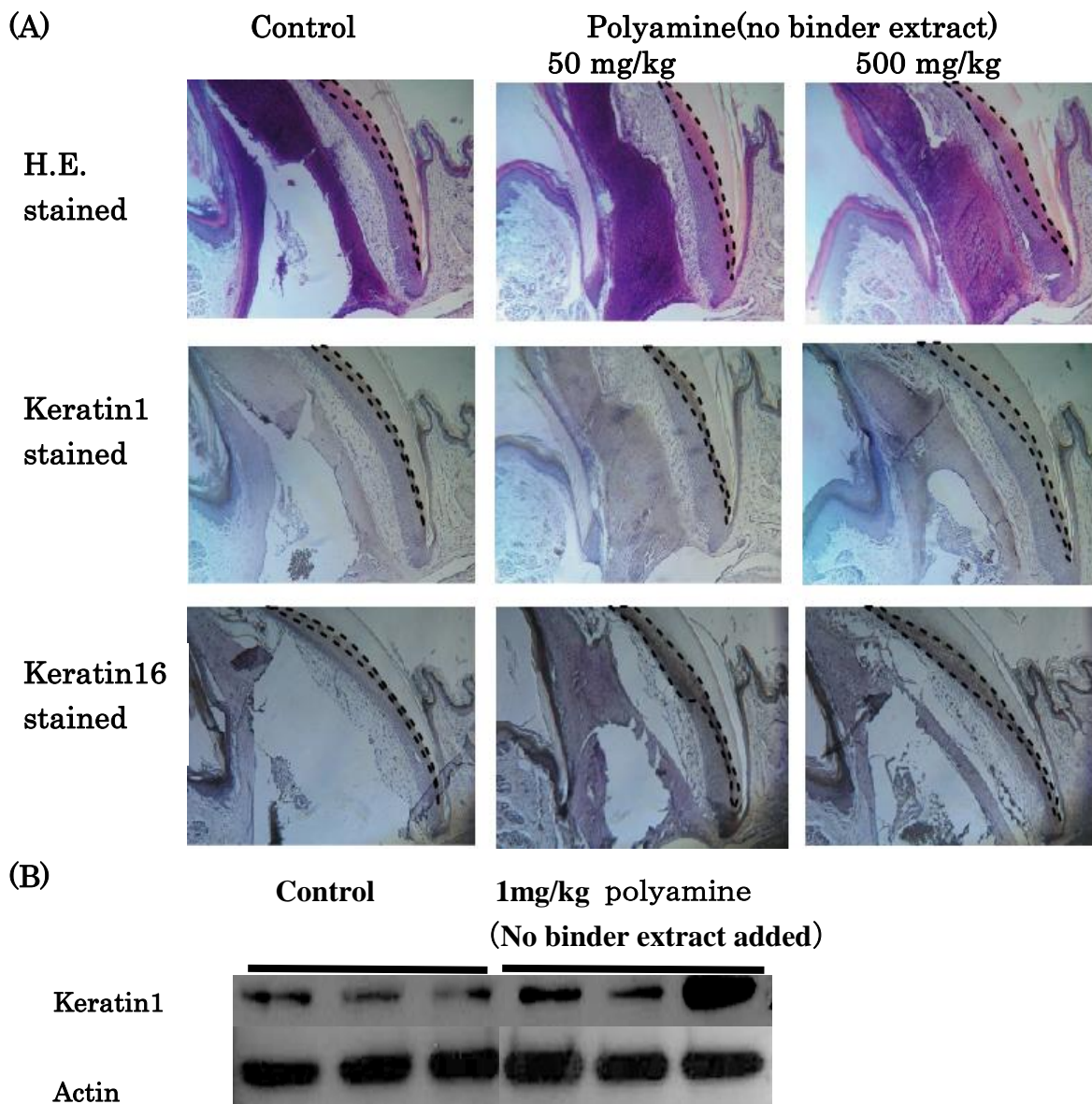


Fig. 11 The Effect of Polyamines on Nail Formation on Mice

[Method of experiment]

POLYAMINE(without binder) 50mg/kg and 500mg/kg (containing 0.1mg/kg and 1mg/kg as pure polyamines) of polyamines was orally given to mice for 12 days. Condition of the first joint of the 3rd toe of the left rear foot was examined by HE-Staining and Keratin 1 stained samples.

(3) The Effect of Polyamines on Cell Proliferation

Polyamines are indispensable for cell growth, stabilization of DNA/RNA and modulation of DNA replication/transcription ¹⁴). The effect of wheat-derived polyamines on cell proliferation was studied using human fibroblasts. Results from MTT assay showed that cells proliferated in samples containing polyamines of 0.01% to 0.1% as illustrated in Fig. 12.

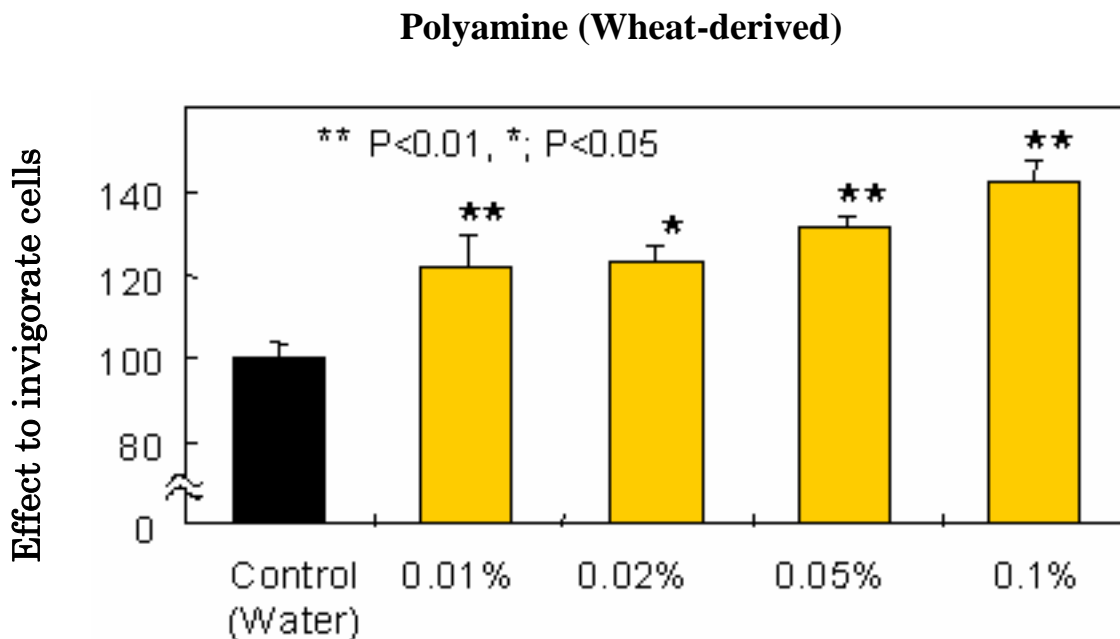


Fig. 12 The Effect of Wheat-derived Polyamines on Human Fibroblasts. (n=4-8)

[Method of experiment]

Fibroblasts cells 4x10⁴ cells/ml (1x10⁴ cells/250µl) (CAI: 106-05, Lot:1314) was prepared in DMEM medium containing FCS 1% followed by insertion into a 48-well plate per unit of 250µl (1x10⁴ cells). Cells were cultivated overnight prior to addition of 1.25 to 2.5µl of test samples (concentration of 0.5 to 1%) to each well and continue cultured for 3 days. Cell proliferation was examined and determined by MTT assay.

(4) The Effect of Polyamines on collagen production

Further to the positive effect on cell proliferation, the effect of polyamines on collagen production was studied. As illustrated in Fig. 13, wheat-derived polyamines demonstrated a dose-dependent enhancement on collagen production.

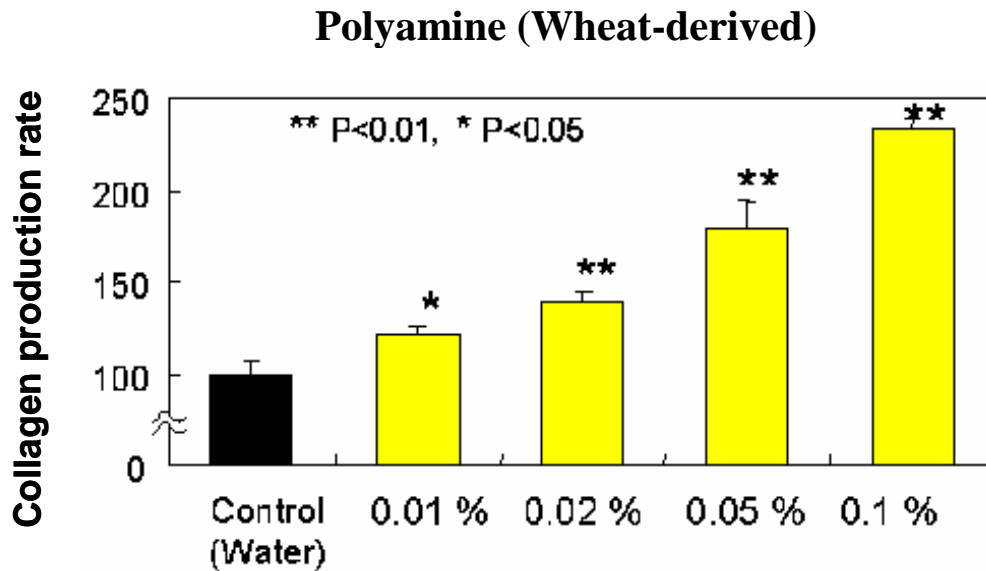


Fig. 13 The effect of wheat-derived Polyamines on Collagen Production (n=4-8)

[Method of experiment]

Fibroblasts cells (CAI:106-05, Lot: 1314) was cultivated over night adopting experimental method used for examining cell proliferation above. Fibroblasts cells was prepared 4×10^4 cells/ml (1×10^4 cells/250 μ l) in DMEM medium containing FCS 1%. Samples and 250 μ l of serum-free DMEM were added and cultured for 3 days. Collagen content in the medium was measured.

14) Igarashi K., Physiological functions of polyamines and regulation of polyamine content in cells. *YAKUGAKU ZASSHI*, 126, 455-71 (2006).

(5) The Effect of Polyamine on Hair Growth

Hair grows in cycle with 3 distinct and concurrent phases, namely anagen, catagen and telogen phases. The anagen phase is also known as growth phase when hair follicles actively divide to form hair. Meanwhile, catagen phase begins when anagen phase ends, it is the transitional period when hair follicles undergo apoptosis, disintegrating and cutting of the hair strand from nourishing blood supply. Last, entering into telogen phase, or resting phase when the hair and hair follicles remain dormant. The cycle starts over when telogen phase finished.

Ramot Y et al.^{15,16} reported that spermidine promotes human hair growth and it is a novel modulator of human epithelial stem cell functions. Fig. 14 showed that administration of spermidine resulting in >20% increase in hair shaft production after 6 days in culture. In addition, all doses of spermidine investigated showed an effect on increasing the percentage of hair follicles in anagen phase while decreasing that of catagen phase (Fig. 15). Anagen maintenance requires constant production of hair follicles keratinocytes from resident epithelial stem cells which are thought to migrate towards the hair matrix. Fig. 16 showed that spermidine up-regulates expression of epithelial stem cell-associated keratin K15 (flourescence –stained area increased with addition of polyamine).

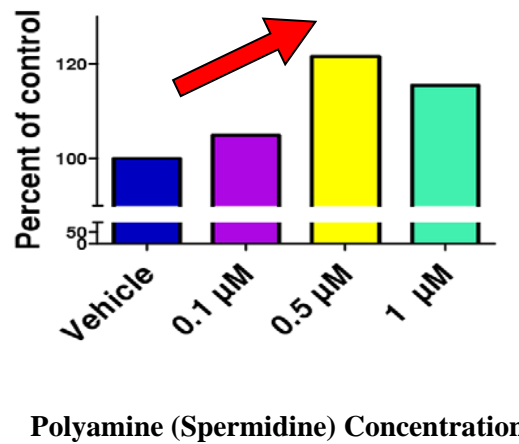


Fig. 14 Effect on Hair Shaft when Polyamine (Spermidine) is Added

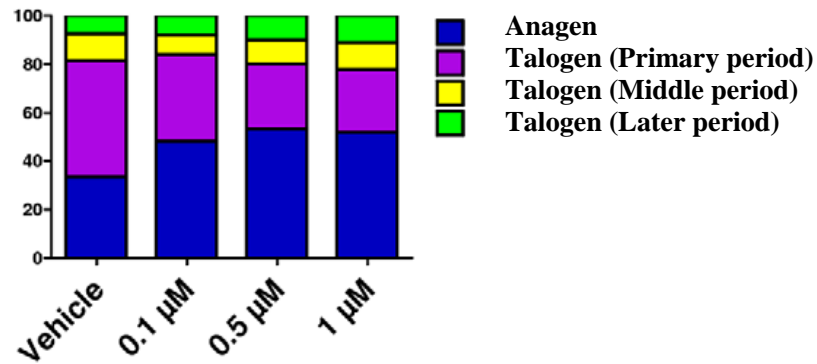


Fig. 15 Effect of Adding Polyamine (Spermidine) on Hair Growth Cycle

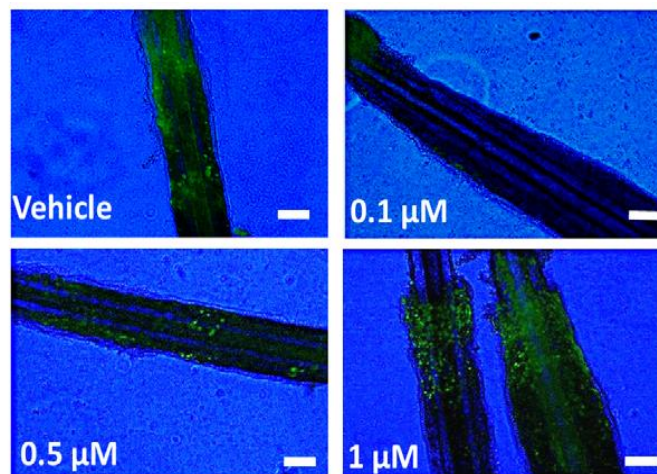


Fig. 16 The effect of polyamine on expression of epithelial stem cell-associated keratin K15.

- 15) Ramot Y. *et al.*, Polyamines and hair: a couple in search of perfection. *Exp. Dermatol.* 19, 784-90 (2010).
- 16) Ramot Y. *et al.*, Spermidine promotes human hair growth and is a novel modulator of human epithelial stem cell functions. *Plos One*, 6, e22564 (2011).

(6) The effect of polyamine on aging skin

Studies showed that intercellular communication through epidermal GAP junctions and gap junction communication in human keratinocytes declines with age, UV radiation etc which affects normal tissue functions^{17,18}). Rapid cell proliferation is associated with extensive GAP junction communication. Shore L. *et al.*, reported that addition of polyamine will increase the activity of ornithine decarboxylase resulting in the increased level of GAP

junction communication between various epithelial cells, thus enhancing tissue functions.

- 17) Ishimura H., Improvement of intercellular communication through epidermal GAP junction by seaweed plasmodesmata extract and its application to cosmetics. *FRAGRANCE J.*, 31(12), 100-5 (2003).
- 18) Provost N. *et al.*, Ultraviolet A radiation transiently disrupts gap junctional communication in human keratinocytes. *Am. J. Physiol. Cell Physiol.*, 284 C51-9 (2003).
- 19) Shore L. *et al.*, Polyamines regulate gap junction communication in connexin 43-expressing cells. *Biochem. J.* 357, 489-95 (2001).

(7) The effect of Polyamine on Fertility (support reproductive ability)

The history of polyamines began in 1678 with the discovery by Leeuwenhoek of the crystallization of spermine from human semen. Studies showed that these ubiquitous compounds, namely spermine, spermidine and putrescine are essential for reproduction process as well as embryonic development. In a study conducted by Lefevre P. L. *et al.*, shown that polyamines expression correlates with stages of spermatogenesis and enhance motility of sperms in male reproduction system. Meanwhile, polyamine plays important role in the implantation of embryo and formation of placental ²⁰⁾. Polyamines are suggested as nutritious agent for maintenance of healthy reproduction system.

- 20) Lefevre P. L. *et al.*, Polyamines on the reproductive randscape. *Endocr. Rev.* 26, 2011-12(2011).

(8) The Effect of Polyamine on Longevity

Minois N *et al.*, reported that changes in polyamine levels have been associated with aging and diseases where polyamines declines continuously with age ²¹⁾. In the study conducted by Minois N *et al.*, polyamine-enhanced diet (containing spermine 0.0075% and spermidine 0.0223%) prolong life expectancy in model organisms. Besides, Soda K. *et al.*, reported that survival rate of mice fed with high polyamine chow was significantly higher while inhibiting the progression of age-associated pathologies ²²⁾.

- 21) Minois N. *et al.*, Polyamines in aging and disease., *Aging*, 3, 1-17 (2011).
- 22) Soda K. *et al.*, Polyamine-rich food decreases age-associated pathology and mortality in aged mice. *Exp. Gerontol.* 44, 727-32 (2009).

(9) The Effect of Polyamine on Inflammation

Zhang M et al., reported that spermine effectively inhibited the synthesis of pro-inflammatory cytokines tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, MIP-1 α and MIP-1 β in human peripheral blood mononuclear cells stimulated by lipopolysaccharides (LPS). Local administration of spermine in vivo protected mice against acute footpad inflammation induced by carageenan²³⁾.

In another study conducted by Soda K. et al., indicated that polyamine, especially spermine, decreases LFA-1 expression on human lymphocytes and adhesion capacity of peripheral blood mononuclear cells (PBMC)²⁴⁾. Chronic inflammation are contributory to age-related diseases such as Alzheimer's, chronic arthritis, osteoporosis etc. Polyamines are believed to counteract on those symptoms²⁵⁾.

- 23) Zhang M. *et al.*, Spermine inhibits proinflammatory cytokine synthesis in human mononuclear cells: a counterregulatory mechanism that restrains the immune response. *J. Exp. Med.* 185, 1759-68 (1997).
- 24) Soda K. *et al.*, Spermine, a natural polyamine, suppresses LFA-1 expression on human lymphocyte. *J. Immunol.* 175, 237-45 (2005).
- 25) Soda K. *et al.*, Polyamines' anti-aging effects. *Food style* 21, 10(10), 43-54 (2006).

(10) The Effect of Polyamine on Arteriosclerosis

Platelet hyper-aggregation is one of the predisposing factors to arteriosclerosis. In a study conducted by De la Pena NC et al., revealed that polyamines putrescine, spermidine, and spermine antagonised platelet aggregation indicating an important role of polyamine in platelet aggregation under normal and hypercholesterolemic conditions. The potency order of polyamine action was spermine > spermidine > putrescine on the inhibition of platelet aggregation in rabbit fed with cholesterol-enriched diet²⁷⁾.

Meanwhile, Soda K et al., similarly reported that increased polyamine intake may prevent cardiovascular disease due to suppression on pro-inflammatory cytokines and leukocyte function-associated antigen-1²⁶⁾.

- 26) Soda K. *et al.*, Polyamine intake, dietary pattern, and cardiovascular disease. *Med. Hypotheses*.75.299-301 (2010).
- 27) De la Pena N.C., Inhibition of platelet aggregation by putrescine, spermidine, and spermine in hypercholesterolemic rabbits. *Arch. Med. Res.*75, 299-301 (2000).

(11) The Effect of Polyamine on radiation

Polyamines have been reported to prevent DNA from radiation-induced strand breaks and crosslinks to protein. Thierry D et al., reported that the polyamine-mediated protection against radiation-induced DNA degradation is due to the compaction of DNA structure^{28, 29}.

- 28) Thierry D. *et al.*, Protection against radiation-induced degradation of DNA bases by polyamines., *Radiation Res.* 153,29-35(2000).
- 29) Waters R.L. *et al.*, Radioprotection of human cell nuclear DNA by polyamines: radiosensitivity of chromatin is influenced by tightly bound spermine. *Radiation Res.* 151, 354-62(1999).

5. Heat Stability

The decomposition of polyamine (without binder) upon heating was examined. As illustrated in Fig. 17, content of polyamine remain stable upon heating at normal food processing temperature 80°C for 60 min. However, degradation begins when heating temperature increased to 100°C for 60 min. Heating below 100°C is recommended if it is required for application.

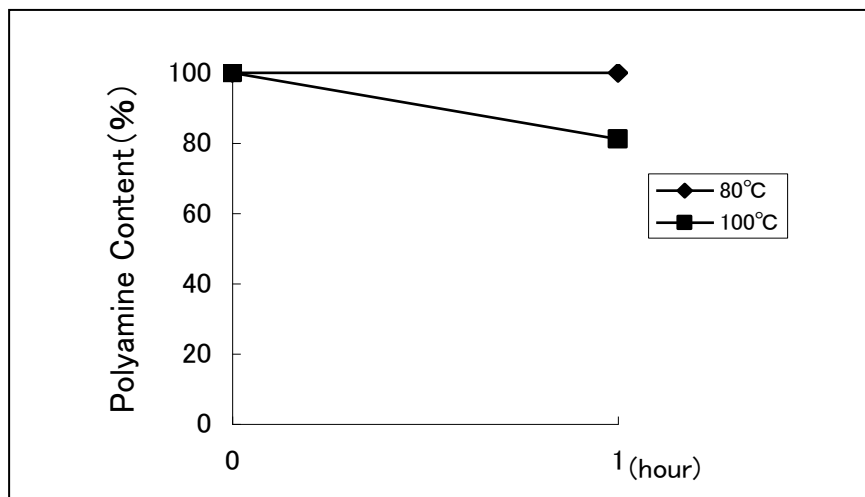


Fig. 17 . Thermostability of Polyamine

6. pH Stability

The pH stability of Polyamine was conducted. Polyamine was dissolved in distilled water at different pH condition and stored at room temperature in darkness for 1 week. As showed in Fig. 18, content of polyamine remain stable at pH range between 3-8.

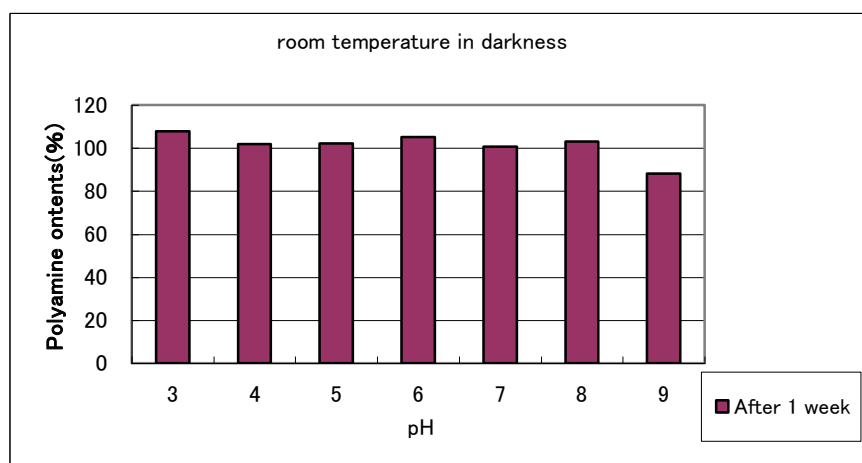


Fig. 18 . pH stability of Polyamine

7. Storage Stability

The storage stability of polyamine was assessed at 5°C, 25°C and 40°C for 4 months respectively. Results showed that content of polyamine remain stable at 5°C and 25°C. However, there was approximately 8% decreased in the content of polyamine stored at 40°C.

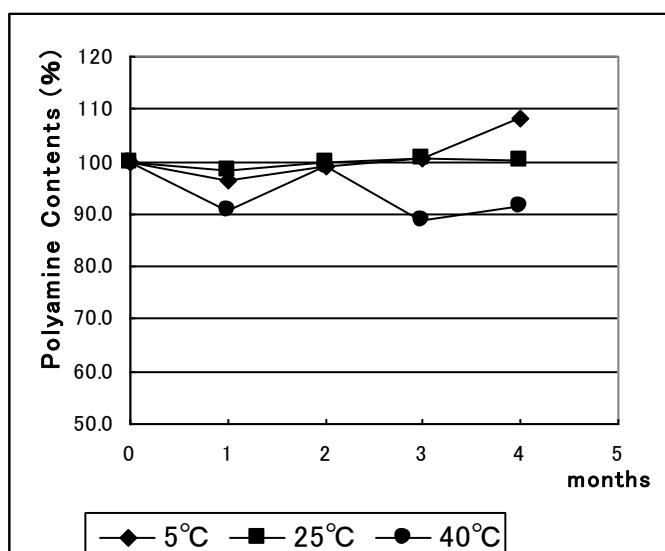


Fig. 19. Storage stability of Polyamine

8. Recommended Dosage

The recommended daily dose of Polyamine-P is 70mg/day.

(Dosage recommended according to the experimental results on The Effect of Polyamine on Nail Formation)

9. Nutritional Composition

Description	Polyamine-P	Remark	Analysis Method
Water	1.3 g/100g		Heat drying at atmospheric pressure
Protein	9.0 g/100g	1	Combustion method
Fat	0.1 g/100g		Acid degradation
Ash	17.5 g/100g		Direct incineration
Carbohydrate	72.1 g/100g	2	
Energy	336 kcal/100g	3	Modified Atwater method
Food Fiber	5.2 g/100g		Prosky method
Sodium	3654 mg/100g		Atomic absorption spectrophotometry
NaCl equiv.	9.31 g/100g		Sodium equiv. value

- 1) Nitrogen, protein conversion factor: 6.25
- 2) Calculation: $100 - (\text{water} + \text{protein} + \text{fat} + \text{ash})$
- 3) Energy expression standard Conversion factor: Protein 4, fat 9, sugar 4,

Test trustee: Japan Food Research Center Foundation

Date of analysis: August, 24, 2011

Test No.:201108100038

10. Safety Profile

(1) Residual agricultural chemicals

Polyamine was screened and analysed for 529 items of residual agricultural chemicals stipulated under the Food Sanitation Act and Pesticides Control Act. Results indicated that

Polyamine confirms to the standards stipulated.

Test Trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis

Date: September 5, 2011

Report No. 48667

(2) Acute toxicity (LD₅₀)

Acute Toxicity on Polyamine was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where Polyamine (without binder) 2000mg/kg was orally given to mice (ddy, male and female, 5-week old, weight approximately 30g) for 14 days. During the test period, the mice were housed at 23±2°C at 50±10% humidity with free access to feed and drinking water. No abnormalities and fatal event observed at 2000mg/kg. No abnormalities of organs observed under macroscopic examination upon autopsy. Thus, LD₅₀ of Polyamine is deduced to be >2000mg/kg.

(3) Mutagenicity (Ames test)

Ames test was conducted to evaluate the mutagenicity of Polyamine (without binder) using *Salmonella typhimurium* TA98 and TA100. No increased in the number of colonies observed, Polyamine is non-mutagenic.

11. Applications

Applications	Examples
Health foods	Soft-capsule, Tablet, Hard-capsule, etc.
Foods	Candy, Gum, Cake, Cookie, Wafer, Drink, Nutritional oil, etc.

12. Packaging

Polyamine-P (water soluble powder, food grade)

5kg Interior Packaging: Aluminium bag

Exterior Packaging: Cardboard

13. Storage

Store in cool, dry and dark place. Avoid places with high humidity and direct heat, and store it in a closed container.

14. Expression

<Food> Please follow regulations in your country.

Product name	Expression
Polyamine-P	Polyamine content wheat germ extract or Wheat germ extract , Dextrin, Citric acid , Sodium citrate

PRODUCT STANDARD

PRODUCT NAME

POLYAMINE – P

FOOD

This product is powder extracted from wheat germ (*Triticum aestivum*) with citric acid solution. It guarantees minimum of 0.15 % Polyamine.

This product is water-soluble.

Appearance

Pale yellow to pale brown powder with light unique smell.

Polyamine

Min. 0.15 % (HPLC)

Loss on Drying

Max. 10 % (Analysis for Hygienic Chemists, 1g, 105 °C, 2 hr)

Purity Test

(1) Heavy Metals (as Pb)

Max. 20 ppm (Sodium Sulfide Colorimetric Method)

(2) Arsenic (as As₂O₃)

Max. 1 ppm (Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)

Standard Plate Counts

Max. 1×10³ cfu/g (Analysis for Hygienic Chemists)

Moulds and Yeasts

Max. 1×10² cfu/g (Analysis for Hygienic Chemists)

Coliforms

Negative (Analysis for Hygienic Chemists)

Composition

<u>Ingredient</u>	<u>Content</u>
Wheat germ extract	44 %
Maltodextrin	40 %
Sodium citrate	16 %
Total	100 %

Expiry date

2 years from date of manufacturing.

Storage

Store it in a cool, dry, ventilated area with desiccant.

Keep it away from high temperature and sunlight, and store it in a closed container.

ORYZA OIL & FAT CHEMICAL CO., LTD. striving for the development of the new functional food materials to promote health and general well-being.

From product planning to OEM - For any additional information or assistance, please contact :

ORYZA OIL & FAT CHEMICAL CO., LTD.

Headquarters:

No.1, Numata Kitagata-cho, Ichinomiya-city, Aichi-pref.,
493-8001 JAPAN

TEL : +81 (0) 586 86 5141

FAX : +81 (0) 586 86 6191

URL/ <http://www.oryza.co.jp/>

E-mail : info@oryza.co.jp



Tokyo sales office:

5F Diamant-building 1-5 Kanda-suda-cho
Chiyoda-ku, Tokyo, 101-0041 JAPAN

TEL (03)5209-9150 FAX (03)5209-9151

E-mail: tokyo@oryza.co.jp

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