



ORYZA OIL & FAT CHEMICAL CO., LTD.

ORYZAPOLYAMINE

Promote hair growth, Repair of hair cuticle, Anti-glycation, Anti-ageing

- **ORYZA POLYAMINE-P**
(Water-soluble powder, food use)
- **ORYZA POLYAMINE-LC(BG30)**
(Water-soluble liquid, cosmetic use)



Oryza Oil&Fat Chemical Co., Ltd.

Ver.2.2 YF

Natural Ingredient for Healthy Hair and Repair of hair cuticle with Anti-ageing

ORYZA 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 protein synthesis¹⁾, 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.

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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.

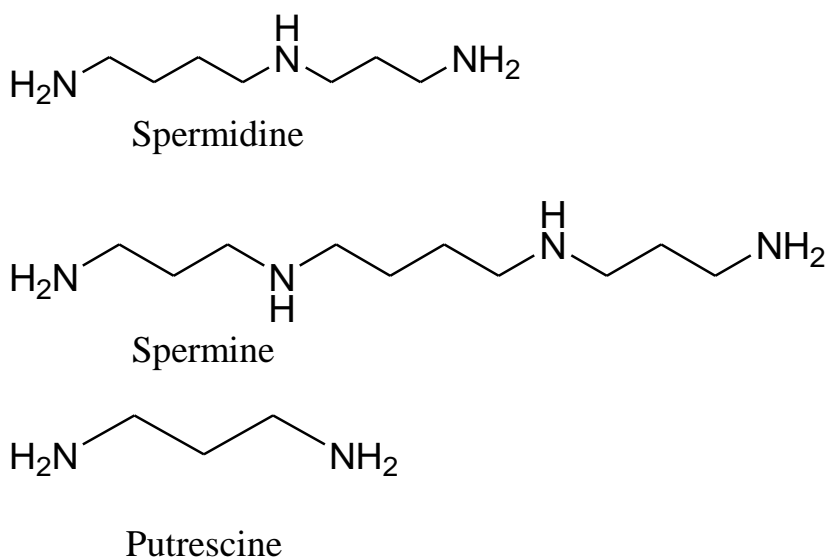


Fig. 1 Chemical Structure of Polyamines

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) and in a study conducted by Soda *et al.*, reported that long-term oral intake of Polyamine increases blood concentration of polyamine.¹⁴ (Fig. 4)

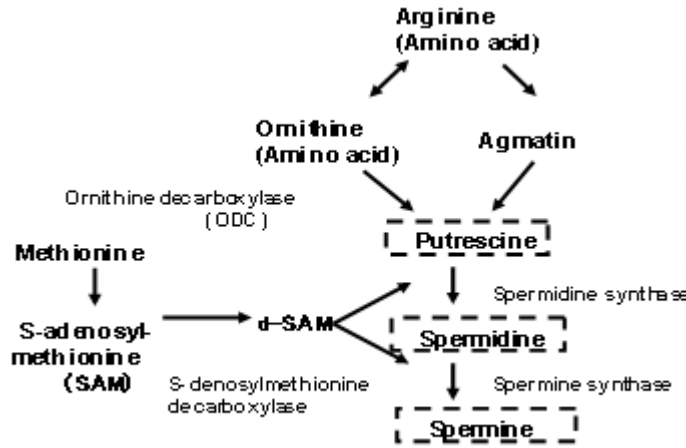


Fig. 2 Synthetic Pathway of Polyamines

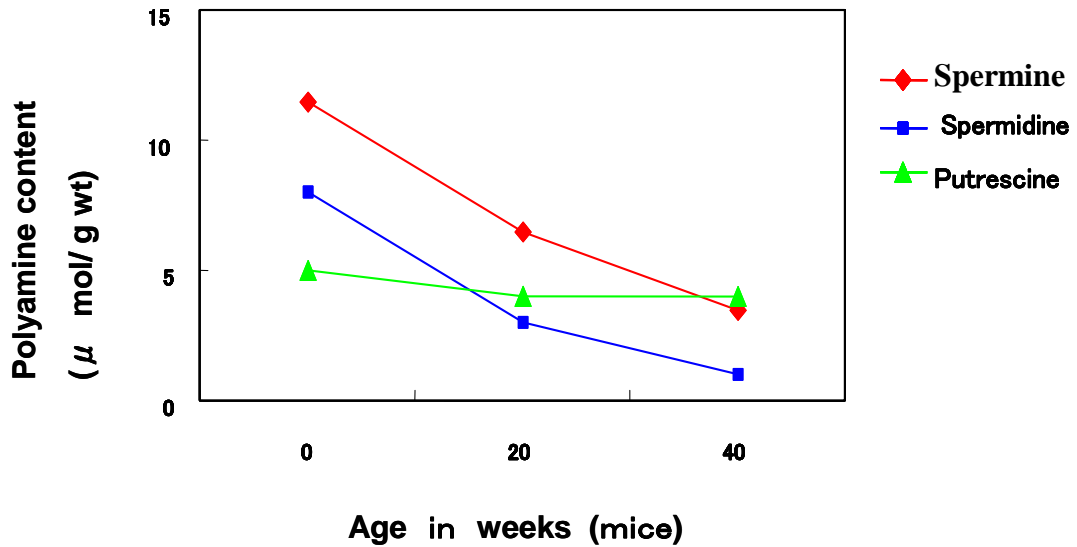


Fig. 3 Changes of Polyamine with age

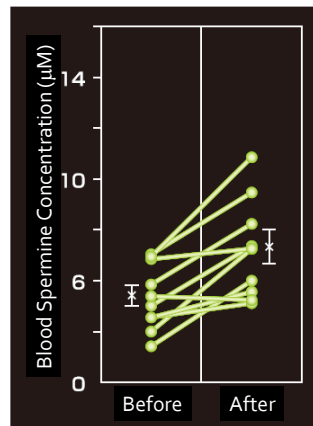


Fig. 4 The effect of polyamine-rich diet on blood polyamine concentration

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refeeding. *Exp. Gerontol.* 28, 565-72 (1993).

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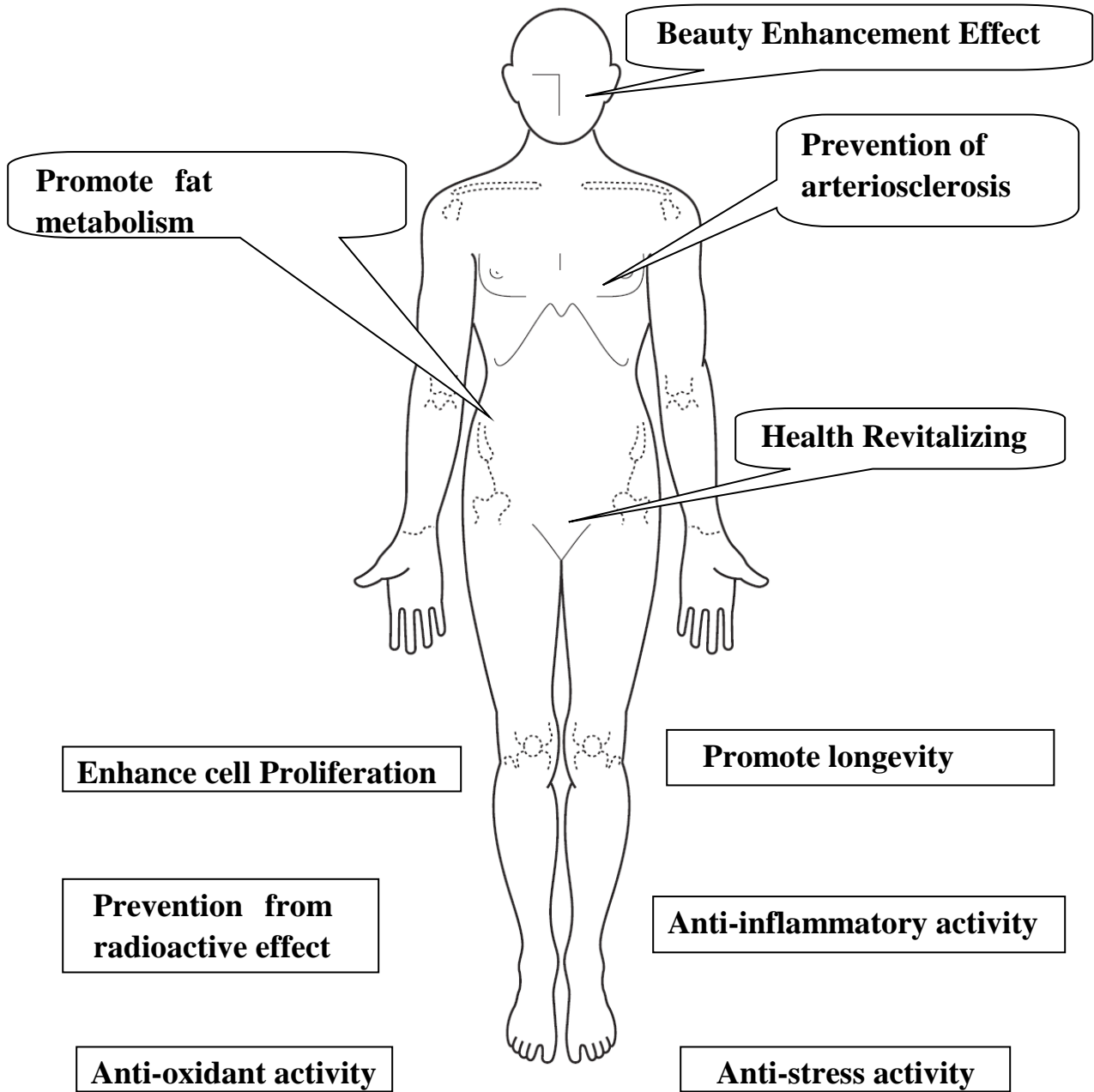


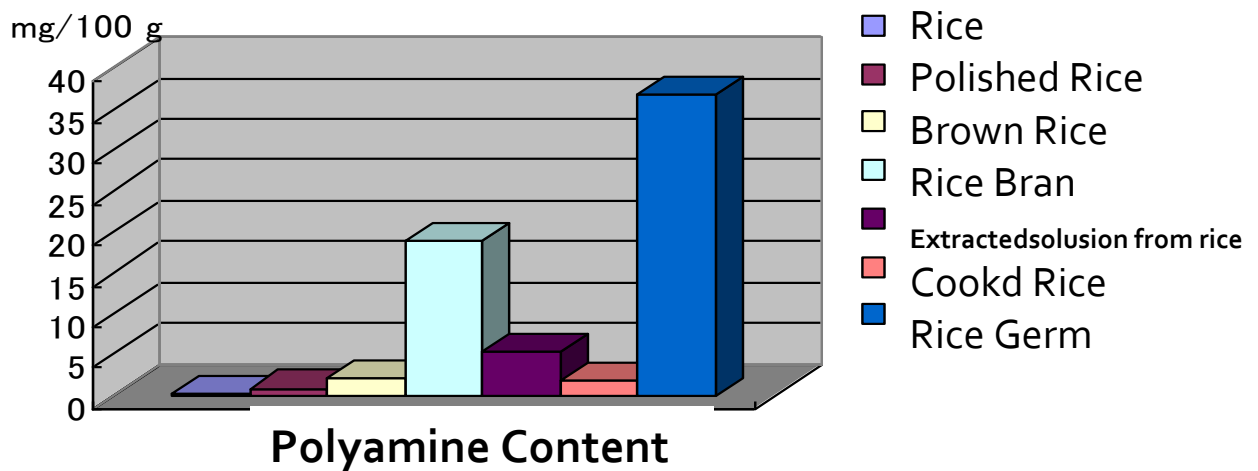
Fig. 5 Functional Effect of Polyamine on human body

3. Characteristics of Rice-derived Polyamines

(1) Comparison on Polyamine content

Polyamine content of different parts of rice was compared and results showed that rice germ contain highest amount of polyamine with a ratio of putrescine : spermidine : spermine = 2:3:5.

In general, spermine, physiologically is the most active compound among the polyamines, OrYZa Polyamine with high ratio of spermine is potentially recommended. (Fig. 6)



(Atiya A. M. *et al.*, Polyamines in foods: development of a food database. *Food Nutr. Res.* 5,3402 (2011))

Fig. 6 Comparison on Polyamine content

(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 showed 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.

Rice-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) Promote 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 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 follicles remain dormant. The cycle starts over when telogen phase finished.

The differentiation and proliferation of hair matrix cells is regulated by a numbers of molecular factors, namely, fibroblasts growth factors (FGF-7), Vascular Endothelial Growth Factors (VEGF), Insulin-like growth factor-1 (IGF-1), hepatocytes growth factors (HGF). (Fig. 7)

FGF-7	Epithelial cell-specific growth factor, predominantly exhibited in keratinocytes, directly influences cells in hair follicles which promote hair growth
VEGF	Vascular Endothelial Growth Factor, stimulates angiogenesis and vasculogenesis (capillary network) surrounding hair follicle supplying essential nutrition for hair growth.
IGF-1	Insulin-like Growth Factor-1, plays important role in growth. Suppress the transition period of catagen and telogen of hair cycle by inhibiting apoptosis.
HGF	Hepatocyte Growth Factor, regulates cell growth and cell motility. Enhance anagen phase of hair cycle.

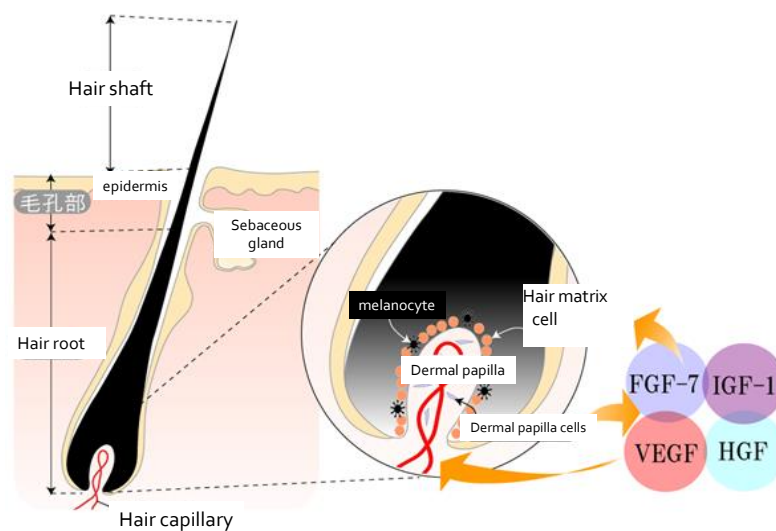


Fig. 7 Mechanism of Hair Growth

Ramot Y et al. ¹⁰⁾¹¹⁾ have reported that adding polyamine (spermidine) promotes the growth

of hair shaft. In their study, polyamine was reported to have an activity to prolong the anagen phase and accelerate the expression of the promoter of keratin 15 in hair follicles. Dermal papilla cells play important role in the process of hair growth and hair thickening. Larger dermal papilla cells helps in creating a thicker and stronger hair. The effect of Oryza Polyamine (rice-derived) on hair growth is examined. As illustrated in Fig. 8 below, using minoxidil (a prescribed medication for alopecia) 0.2µg/mL as positive control, Oryza Polyamine demonstrated a 40-60% improvement in the proliferation rate of dermal papilla cells. The growth rate of dermal papilla cells treated with 1µg/mL of Oryza Polaymine is equivalent to 1/5 of the concentration of minoxidil. (Fig. 8)

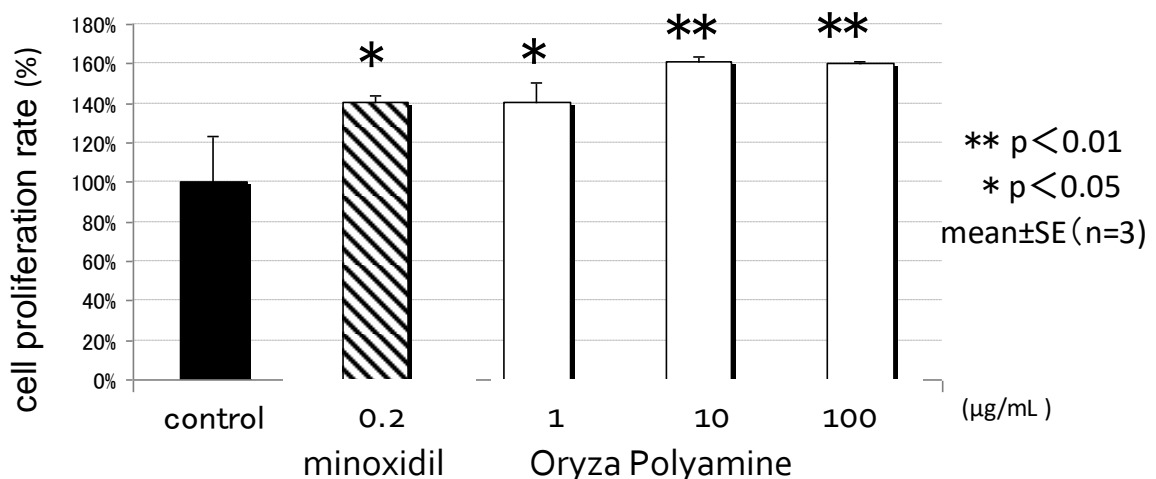


Fig. 8 The effect of Oryza Polyamine on the growth of dermal papilla cells

[Experimental Method]

Normal human dermal papilla cells were cultured and treated with trypsin. Cultured cells were placed in a 96-welled plate 5×10^3 cells/well continue incubation in a serum-free medium for 1 night. Then, serum-free medium was removed, 100µL of soluble Oryza Polyamine (rice germ extract containing 0.33% of polyamine) at concentration 1, 10, 100µg/mL was added to each cells and continue culture for 24 hours. Growth rate of dermal papilla cells was measured and determined by MTT assay.

Molecular factors such as FGF-7, VEGF, IGF-1, HGF are involved in the hair follicle growth. The expression of these factors in culture human dermal papilla cells was evaluated. The expression of HGF was up-regulated and showed significant difference. Hepatocytes growth factor (HGF) is a multifunctional polypeptide which was found to stimulate hair follicles growth during anagen phase. Meanwhile, the expression of FGF-7 was up-regulated in samples containing Oryza Polyamine (without binder) 10, 100µg/mL, the up-regulation of VEGF and IGF-1 was expressed at concentration of Oryza Polyamine 100µg/mL (Fig. 9). Above finding clearly suggested that Oryza Polyamine promote hair follicles growth with up-regulation of hair growth promoting factors – HGF, FGF-7, VEGF and IGF-1 respectively.

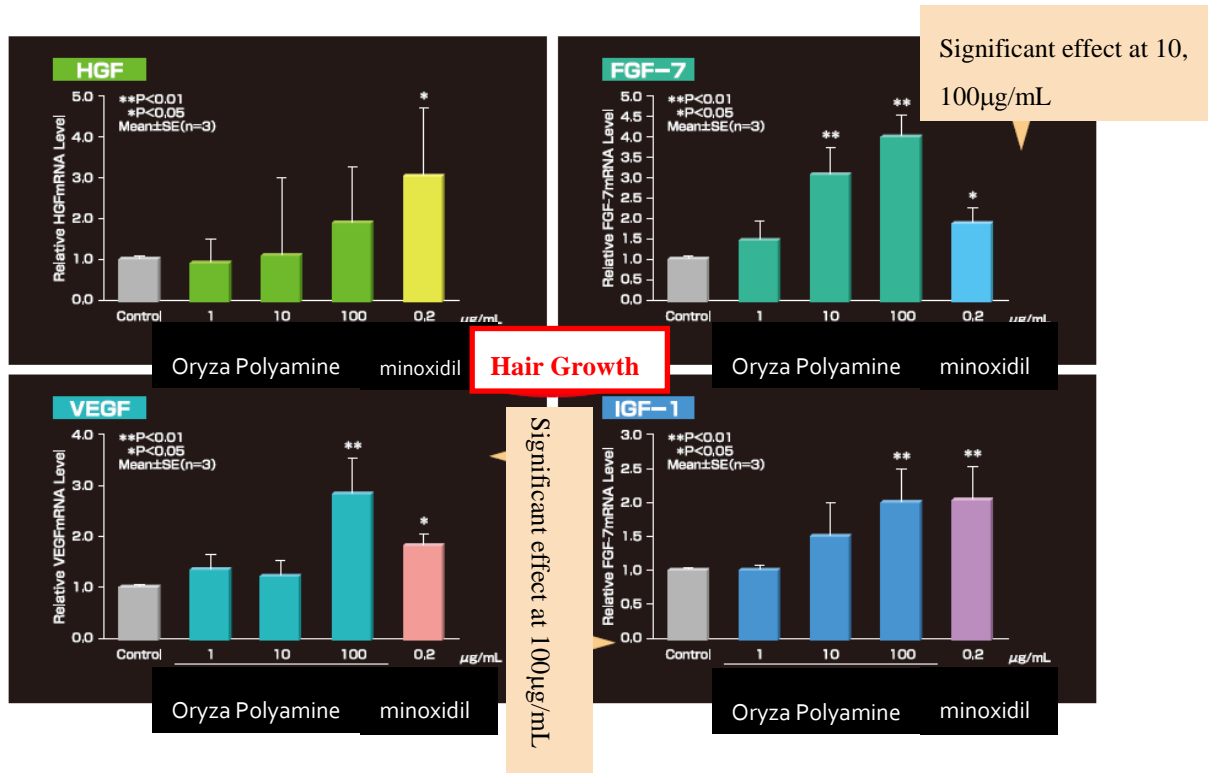


Fig. 9 The effect of Oryza Polyamine on hair growth promoting factors

[Experimental Method]

Normal human dermal papilla cells were cultured and treated with trypsin . Cultured cells were placed in a 96-welled plate 5x10⁴ cells/well continue incubation in a serum-free medium for 1 night. Then, serum-free medium was removed, 500µL of soluble Oryza Polyamine (rice germ extract containing 0.33% of polyamine) at concentration 1, 10, 100µg/mL was added to each cells and continue culture for 1-2 hours. Last, PCR analysis was carried out by collecting cells for total RNA extraction and recombination of cDNA.

(2) Repair of Hair Cuticle

Next, the topical effect of Oryza Polyamine on human hair cuticle was examined. A bundle of hair treated with shampoo, perming and bleaching was prepared and immersed in a solution containing 0.5% rice germ extract (containing 0.00165% of polyamine). The hair was scanned and observed using electron microscope. As illustrated in Fig. 10, a lifting-up effect (cuticle repair) was observed on damaged hair upon treatment with Oryza Polyamine.

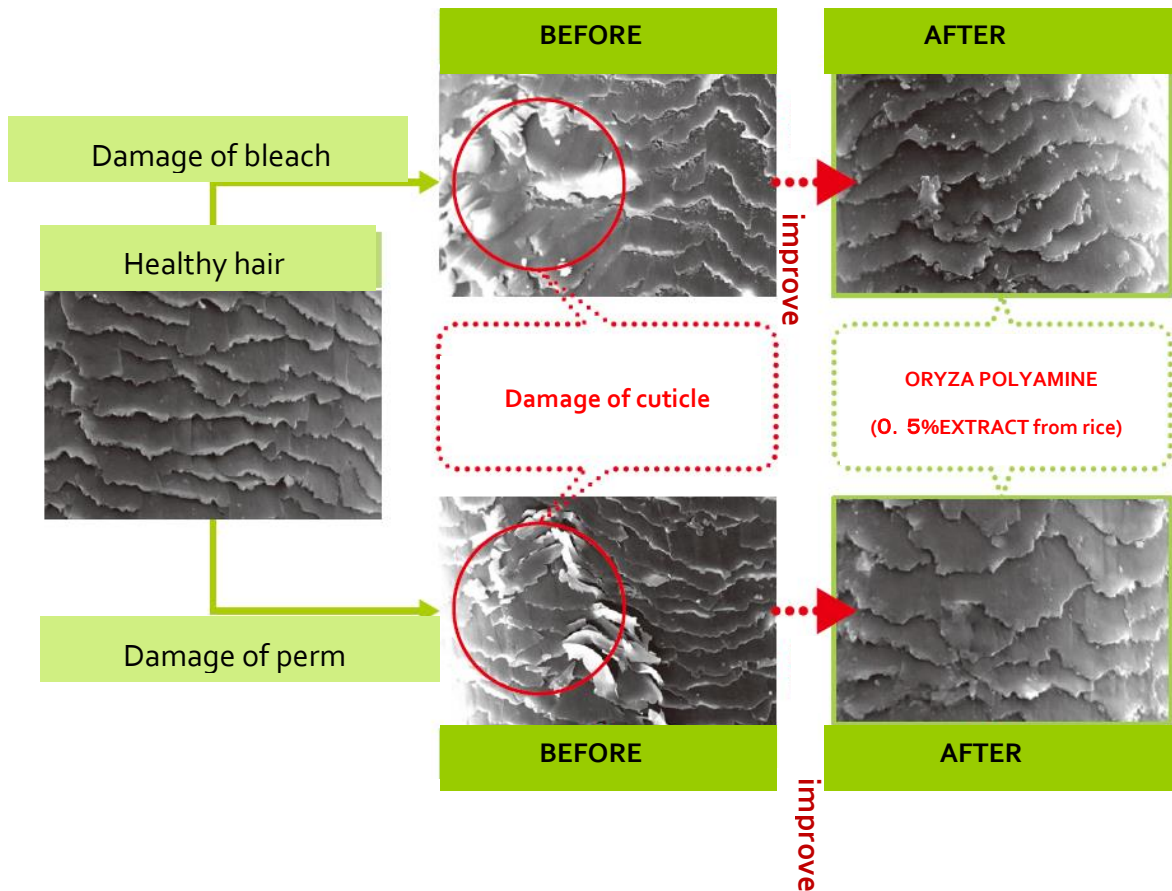


Fig. 10. The effect of Oryza Polyamine on hair growth promoting factors

(3) Improve hair texture

The effect of Oryza Polyamine on the texture and quality of hair was evaluated on human monitor test:

Healthy individuals (10 female subjects, aged 20-60) were given hair treatment product containing Oryza Polyamine (content of polyamine: 0.005%) for daily use for a duration of 2 weeks. The content of the hair treatment is listed in Table 1. A questionnaire survey was conducted to assess the quality of the hair treatment product containing Oryza Polyamine. As shown in Fig. 11, there was 87% improvement on hair texture reported which include improvement in hair luster, shine of hair.

Table 1. Formulation of Hair Treatment Product containing Oryza Polyamine
(polyamine content: 0.005%)

Product name	Major application	Mixture ratio (%)
Polyoxy ethylene alkyl ether	hydrophilic emulsifier	2.0
Alkylammonium salt	softener • conditioning	8.0
Cetanol	alcohol • emulsion stabilizer	5.0
Fatty acid ester	polish and dispersant of cream	4.0
Polyalcohol fatty acid ester	base material of oiliness	6.0
Olive squalene	moisturizing agent	2.0
Propyl parahydroxy benzoate	moisturizing agent	0.1
Methyl paraben	preservation	0.1
Butylene Glycol	preservation	4.0
Citric acid	moisturizing agent	6.0
Soylecithin derivative	pH adjuster	0.1
Purified water	emulsifier	61.18
Oryza Polyamine (without binder)	polyamine contents in Oryza polyamine (without binder) :0.33 %	1.52 (polyamine contents0.005%)

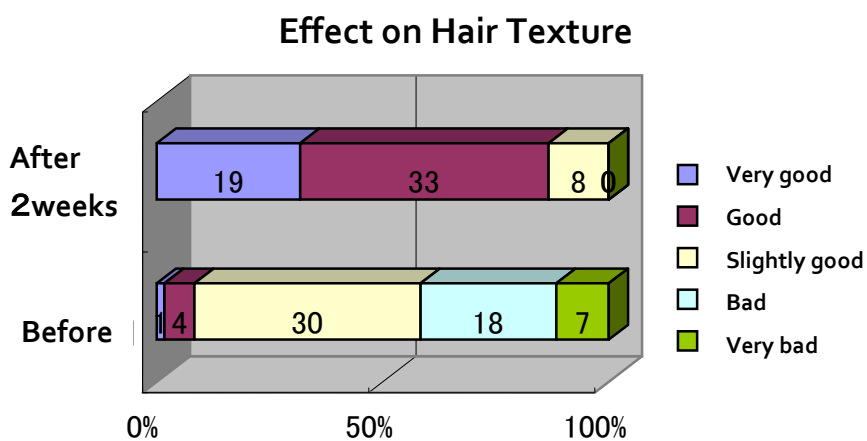


Fig. 11. The effect of Oryza Polyamine on hair texture (questionnaires)

Based on above findings, Oryza Polyamine up-regulated the expression of molecular factors (HGF, IGF-1, FGF-7 and VEGF) which promote hair follicles growth and thus exert hair thickening effect. Ultimately, hair cuticle repair and improvement in hair texture is observed.

(4) Anti-glycation

Promote the formation of collagen matrix

Fibroblasts form collagen lattice when culture in a collagen containing medium. This process is suppressed when a glycation agent (glyoxal) is added to fibroblasts in the system. The effect of Oryza Polyamine on glycation process was examined using fibroblast cells treated with glyoxal.

As shown in Fig. 12, in samples treated with Oryza Polyamine 10 and 100µg/mL effectively inhibited glycation process while formation of collagen lattice is observed. Oryza Polyamine is suggested to prevent glycation of fibroblasts while effectively maintaining extracellular collagen matrix.

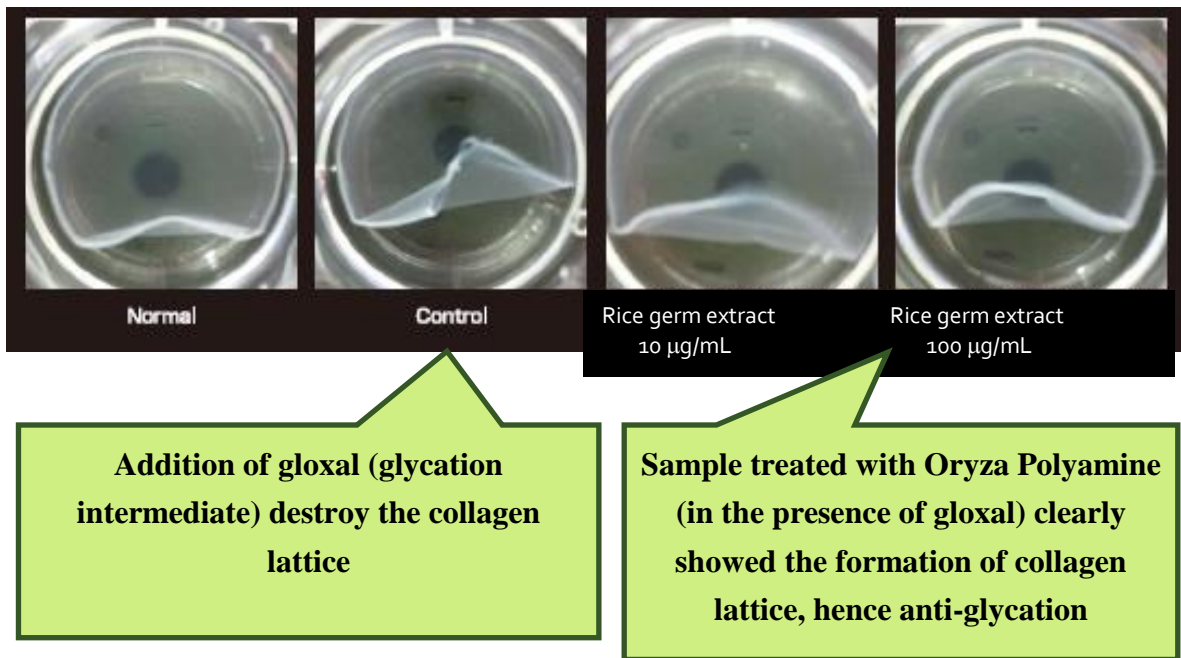


Fig12. The effect of Oryza Polyamine on glycated fibroblasts and collagen lattice formation

(5) Clinical trials

- Effect of Oryza Polyamine on the Fingernail Conditions -

Recently, interest in not only the skin beauty but also “the nail beauty” has been growing. Fingernails reflect a lot of our health conditions. For instance, age-related vertical ridges, horizontal ridges reflecting bad health condition and external stimuli, and hangnails caused by excessive dryness are typical sign (Table 2).

Table 2 Fingernail problems and causes

Fingernail problem	Cause
Vertical ridges	Aging
Horizontal ridges	Bad health condition, external stimuli
Hangnails	Dryness

To evaluate the effect of Oryza Polyamine on fingernail problems, we conducted a clinical trial. Subjects were randomly divided into two groups (placebo or Oryza Polyamine group). After ingestion of test sample (50 mg/day) for two weeks, the conditions of fingernails were evaluated by a microscope. As shown in Fig.13, the oral intake of Oryza Polyamine decreased the vertical ridge on nail surface and improved the hangnails.

Next, in order to assess whether Oryza Polyamine affect the ceramide content, we measured the amount of ceramide in fingernails. Ceramide is known to play a critical role in the barrier function and water retention in nails. As a result, while the amounts of total ceramides and ceramide 5 tended to decrease after 2-month ingestion in placebo group, the Oryza Polyamine group increased these contents in fingernails. Moreover, trans epidermal water loss (TEWL) was measured to assess the effect of Oryza Polyamine on water retention in fingernails. In the placebo group, the TEWL value increased by 28% and 53% after 1- and 2-month ingestion, respectively (Fig.15). In contrast, in the Oryza Polyamine group, the value decreased by 14 and 25% after 1- and 2-month ingestion, respectively. These results suggest that oral intake of Oryza Polyamine prevent the water loss from fingernails.

In summary, the oral intake of Oryza Polyamine increases the amount of ceramide and prevent water loss from fingernails. These results indicate that Oryza Polyamine improves the fingernail conditions to support “nail beauty”.

	Before intake	After intake (1 month)	After intake (2 months)
Placebo group <Subject 1>			
No change			
Placebo group <Subject 2>			
No change			
Placebo group <Subject 3>			
No change			
Oryza Polyamine group <Subject 1>			
Decreased the vertical ridge on nail surface			
Oryza Polyamine group <Subject 2>			
Improved hangnails			
Oryza Polyamine group <Subject 3>			
Improved nail cracking			

Fig.13 Effect of Oryza Polyamine on the fingernail problems (microscopic image)

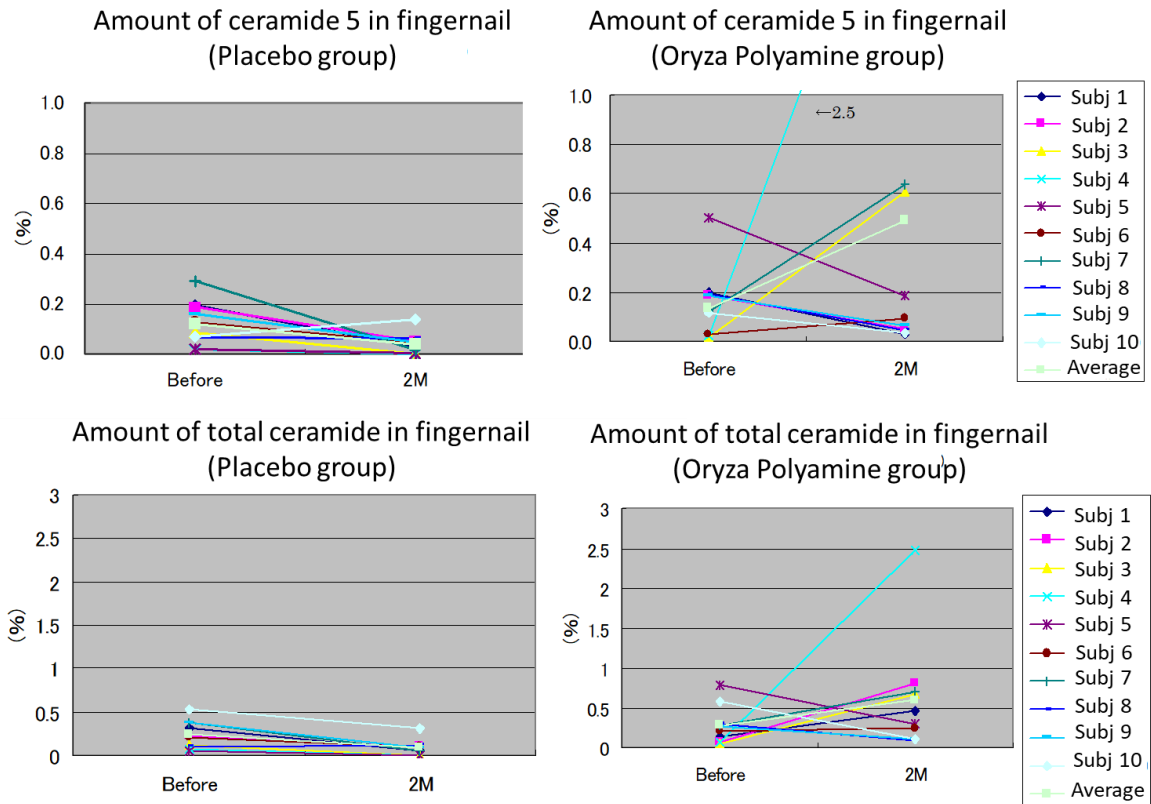


Fig.14 Oryza Polyamine increase the ceramide amount in fingernails

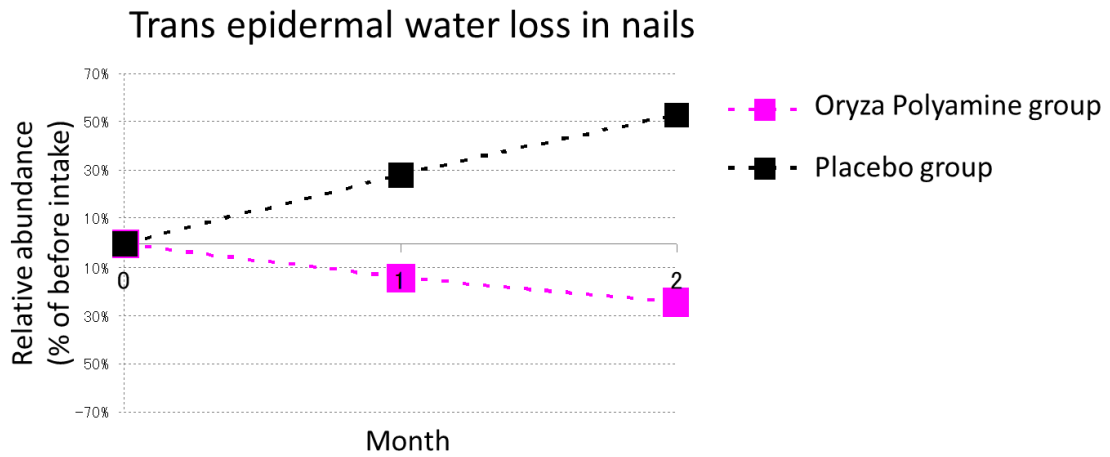


Fig.15 Oryza Polyamine decreases the trans epidermal water loss in fingernails

(6) Clinical trials (2)

- Effect of Oryza Polyamine on the Eyelash Cuticle -

We conducted a clinical trial to evaluate the effect of Oryza Polyamine on eyelashes cuticle. Test sample was the eyelash serum which contains Oryza Polyamine (Hereinafter referred to as “Oryza Polyamine serum”). The composition of Oryza Polyamine serum is shown in Table 3 (Oryza Polyamine-LC: 1%, content of polyamine: 0.005%). Eight female subjects used this serum for two months, and then eyelash conditions were evaluated by a scanning electron microscope (SEM). Many women use an eyelash curler and mascara in daily life to lift up eyelashes. This process will damage the eyelash cuticle, causing eyelash breakage. As shown in Fig.16, the cuticle damage of eyelashes improved by using the Oryza Polyamine serum.

Moreover, subjects answered the questionnaire about the eyelash conditions (length, damage, polish, thickness, density and stiffness). As a result, the score of all item increased by using Oryza Polyamine serum (Fig.17). These results indicate that the Oryza Polyamine improves the eyelash cuticle and ameliorate the eyelash problems.

Table.3 The composition of Oryza Polyamine serum (Oryza Polyamine-LC; 1 %)

Materials	Characteristics	Composition (%)
Carbopol 1382 (polymer)	Emulsion stabilizer / thickening	2.0
Purified water	—	73.2
Hydroxylated lecithin	Emulsifier / Solubilizer	3.0
Ascorbyl tetraisopalmitate	O/W emulsion base	8.0
Glycerin	Moisturiser	12.0
Phenoxyethanol	Preservative	0.8
Oryza Polyamine-LC (T-218)	The content of polyamine 0.005 %	1

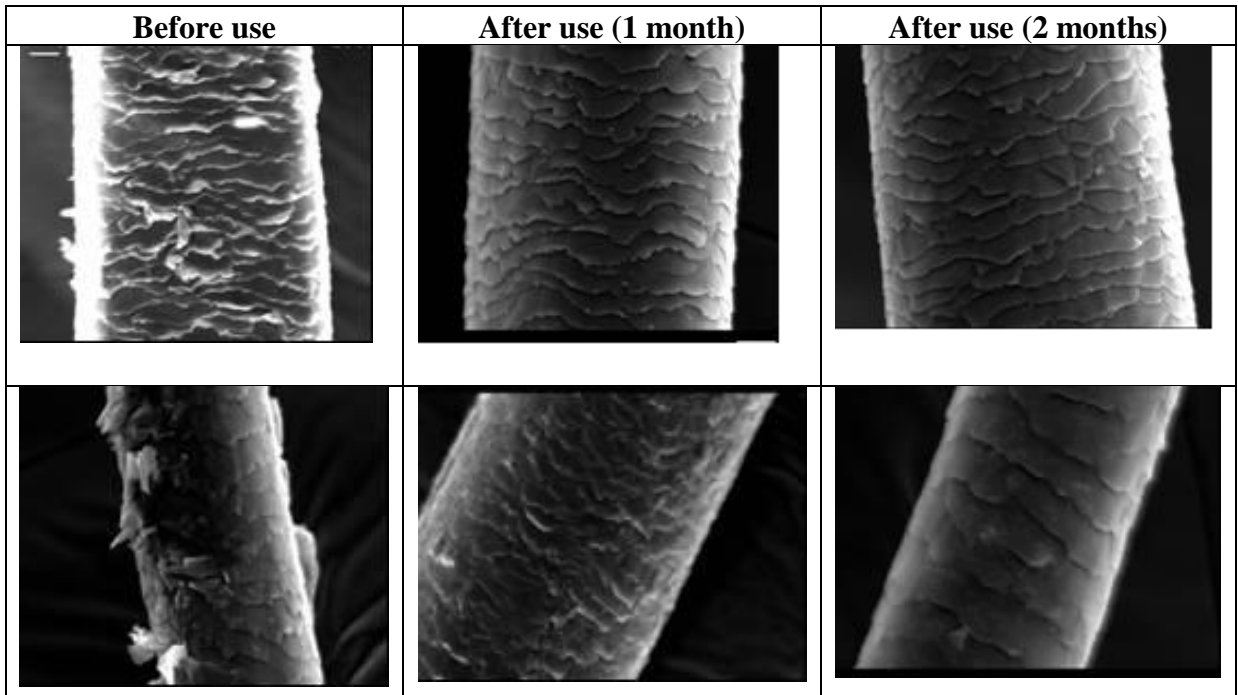
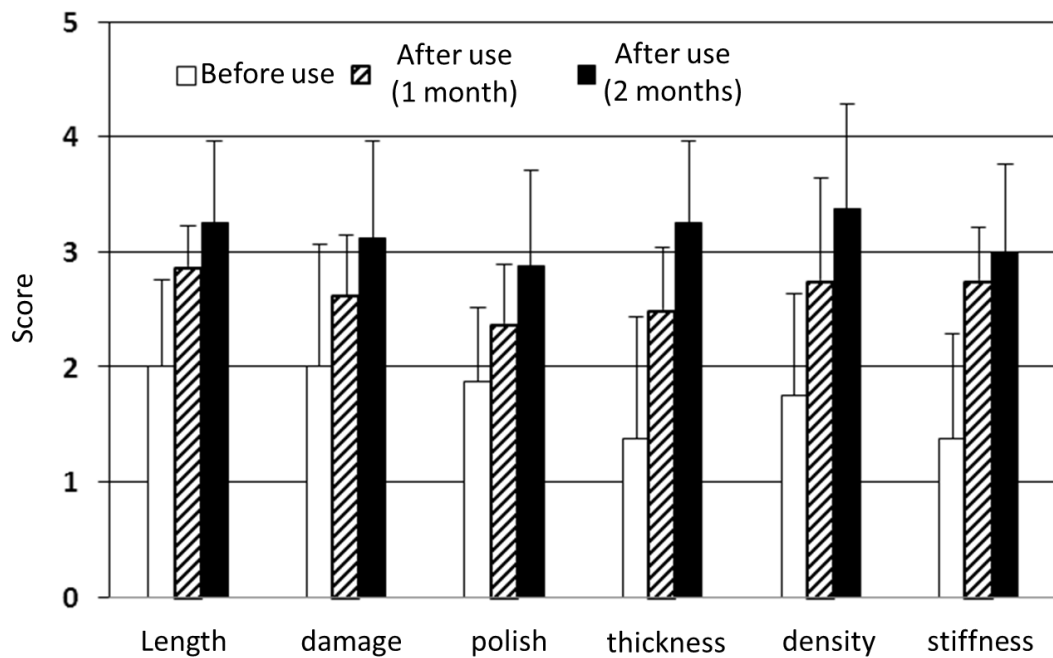


Fig.16 Oryza Polyamine improve the eyelash cuticle (scanning electron microscope)



-Score- Very good: 4, Good: 3, Neither good nor bad: 2, bad: 1, Very bad: 0

Mean ± SD (n=8)

Fig.17 Questionnaire results about the eyelash conditions

- (5) Effect on keratinocytes (Improve keratinocytes turnover)**
- (6) Promote nail formation**
- (7) Anti-aging**
- (8) Promote fertility (support reproductive system)**
- (9) Promote Longevity**
- (10) Anti-Inflammatory**
- (11) The Effect of Polyamine on Arteriosclerosis**
- (12) The Effect of Polyamine on radiation**

* Please refer to Polyamine (wheat) brochure for further details

5. Heat Stability

The decomposition of Oryza polyamine (without binder) upon heating was examined. As illustrate in Fig. 13, 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. Approximately 10% reduction of polyamine is observed upon heating at 100°C for long period of time and amount loss must be taken into consideration.

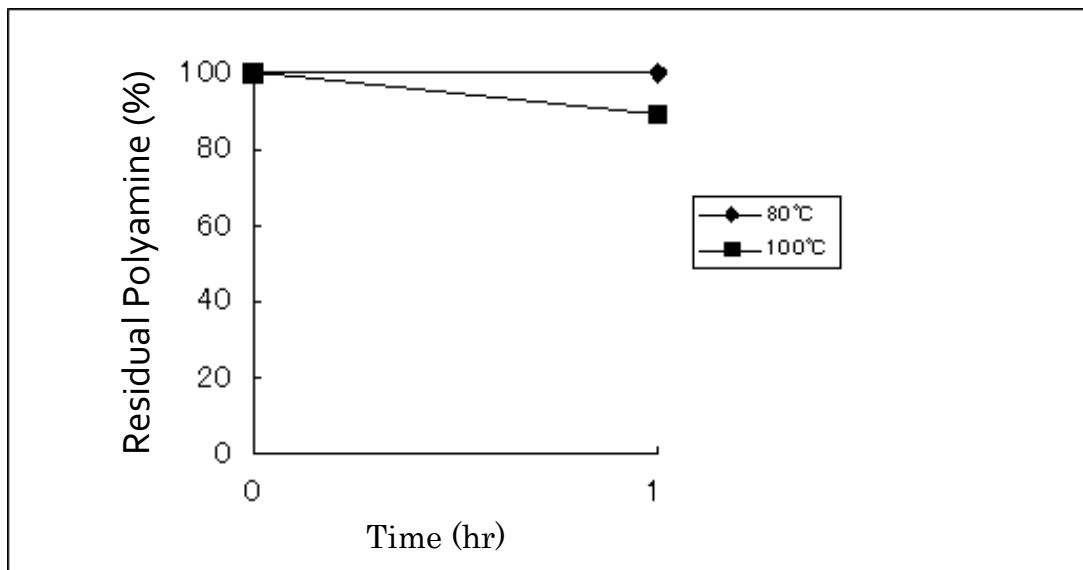


Fig13. Heat Stability of Oryza Polyamine

6. pH Stability

The pH stability of Oryza Polyamine(without binder) was conducted. Oryza Polyamine was dissolved in distilled water at different pH condition and stored at room temperature in darkness for 1 week. As showed in Fig. 14, content of polyamine remain stable at both acidic and alkaline condition.

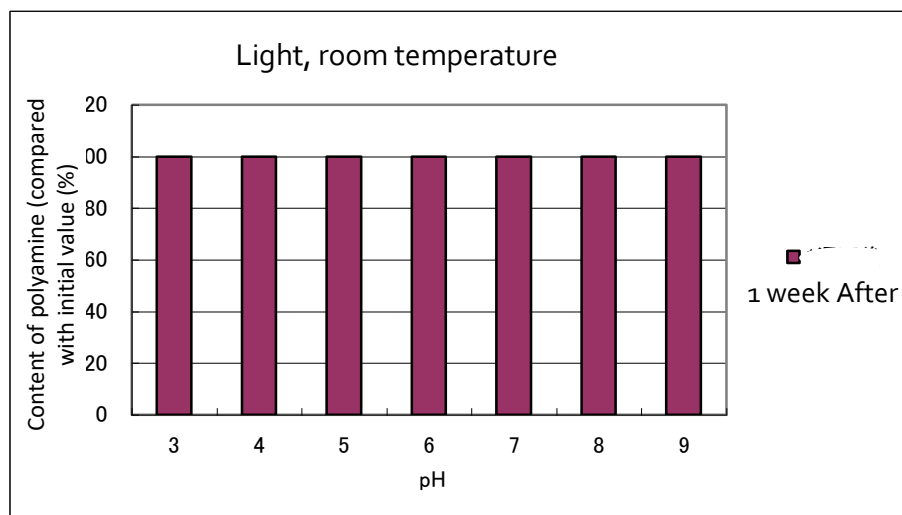


Fig. 14 . pH stability of Oryza Polyamine

7. Recommended Dosage

The recommended daily dose of Oryza Polyamine-P is 50-100mg/day

8. Nutritional Profile

Description	Oryza Polyamine-P (per 100 g)	Analysis Method
Water	6.3 g	Heat drying at atmospheric pressure
Protein	9.0 g	Combustion method
Fat	1.0 g	Acid degradation
Ash	34.4g	Direct incineration
Carbohydrate	40.8g	Calculation: 100-(water + protein + fat + ash)
Energy	225 kcal	Modified Atwater method
Food Fiber	8.5g	Prosky method
Sodium	68.8 mg	Atomic absorption spectrophotometry

Energy conversion factor: protein 4, fat 9, carbohydrate 4, food fiber 2

Oryza Polyamine-P is the calculated value from the value of nutritional ingredients analysis.

Test trustee: Japan Food Research Center Foundation

Date of analysis: May 17, 2012

Test No.: 120508128-001-0

9. Safety Profile

(1) Residual Agricultural Chemicals

Oryza Polyamine was screened and analysed for 535 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: May 17, 2012

(2) Mutagenicity (Ames test)

Ames test was conducted to evaluate the mutagenicity of Oryza Polyamine (without binder) using *Salmonella typhimurium* TA98 and TA100. No increased in the number of colonies observed(19.5 – 2,500µg/plate) with or without metabolic activation, Oryza Polyamine is non-mutagenic.

10. Applications

Applications	Examples
Health foods	Soft-capsule, Tablet, Hard-capsule, etc.
Foods	Candy, Gum, Cake, Cookie, Wafer, Drink, Nutritional oil, etc.
Cosmetics	Shampoo, rinse, hair tonic, foundation, cream, emulsion, toner, cleanser, lipstick, lotion etc.

11. Packaging

Oryza Polyamine-P (water soluble powder, food grade)

5kg Interior Packaging: Aluminium bag
Exterior Packaging: Cardboard

Oryza Polyamine-LC(BG30) (water soluble liquid, cosmetics grade)

5kg Interior Packaging: Cubic polyethylene container
Exterior Packaging: Cardboard

12. Storage

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

13. Expression

< **Food** > Please follow regulations in your country.

Product name	Expression
Polyamine-P	Oryza Sativa (Rice) Extract , Dextrin, Citric acid

< **Cosmetics** >

Product name	Expression
Polyamine-LC(BG30)	Water, Butylene Glycol , Sodium Citrate , Oryza Sativa (Rice) Extract , Citric acid

PRODUCT STANDARD

PRODUCT NAME

ORYZA POLYAMINE – P

FOOD

This product is water-soluble powder extracted from rice germ (*Oryza sativa* Linne) with citric acid solution. It guarantees minimum of 0.2% polyamine.

<u>Appearance</u>	Pale yellow to pale brown powder with light unique smell.	
<u>Polyamine</u>	Min. 0.2 %	(HPLC)
<u>Loss on Drying</u>	Max. 10 %	(Analysis for Hygienic Chemists, 1g, 105 °C, 2 hr)
<u>Purity Test</u>		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As ₂ O ₃)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
<u>Standard Plate Counts</u>	Max. 1×10 ³ cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Rice Germ Extract	53 %
	Maltodextrin	30 %
	Citric acid	17 %
	Total	100 %

<u>Expiry date</u>	2 years from date of manufacturing.
<u>Storage</u>	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.

PRODUCT STANDARD

PRODUCT NAME

ORYZA POLYAMINE – LC(BG30)

COSMETIC

This product is extracted from rice germ, germ of *Oryza sativa* Linne, with citric acid solution and is dissolved in a aqueous 1,3-butylene glycol.
It guarantees minimum of 0.004% polyamine.

<u>Appearance</u>	Slightly yellow liquid with slight unique smell.	
<u>Polyamine</u>	Min. 0.004 %	(HPLC)
<u>Purity Test</u>		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As₂O₃)	Max. 1 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
<u>Standard Plate Counts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Max. 1×10 ² cfu/g	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>	<u>Ingredient</u>	<u>Content</u>
	Water	68.09 %
	Butylene Glycol	30.00 %
	Sodium Citrate	1.05 %
	Oryza Sativa (Rice) Extract	0.65 %
	Citric acid	0.21 %
	Total	100 .00 %

Expiry date 1 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:

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