ORYZA OIL & FAT CHEMICAL CO., LTD.

ORYZA PROTEIN
ORYZA PEPTIDE

Rich in nutrients, anti-obesity, muscle-building, beauty enhancement effects

- **ORYZA PROTEIN-P80**
  (Powder, Food Grade)
- **ORYZA PROTEIN-PC70**
  (Powder, Cosmetic Grade)
- **ORYZA PEPTIDE-P60**
  (Water-soluble powder, Food Grade)
- **ORYZA PEPTIDE-PC60**
  (Water-soluble powder, Cosmetic Grade)

ORYZA OIL&FAT CHEMICAL CO., LTD.
Ver. 2.3MK

ORYZA PROTEIN
ORYZA PEPTIDE

Diet and beauty enhancement foods and cosmetic ingredients

1. Introduction

Rice is the most common staple food in Japan and Japanese consume rice of an average of 60 kg per annum. Rice serves as an important source of carbohydrates as main energy source required for daily functions. Rice is loaded with health promoting bioactive components such as gamma-oryzanol, ferulic acid, tocotrienol (anti-oxidant and anti-metabolic), ceramide (excellent skin barrier function), and GABA (natural relaxant that lowers blood pressure).

In addition, rice is an important source of protein [1,2], although the protein constituent in rice is relatively low, 6.8% in brown rice and 6.1%[3] in white rice respectively. Rice contributed approximately 15% of Japanese dietary protein due to high consumption of rice as daily diet [4].

ORYZA PROTEIN-P80 is 100% derived from non-GMO rice with excellent nutritional profile. Meanwhile, ORYZA PEPTIDE-P60 is water-soluble rice peptide produced by enzymatic decomposition of rice protein.

ORYZA PROTEIN and ORYZA PEPTIDE are high quality functional ingredients suitable to be incorporated into a wide range of functional food, food and cosmetic applications. ORYZA PROTEIN and ORYZA PEPTIDE offer high quality protein with supportive research data on the prevention of weight gain and beauty enhancement.

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2. ORYZA PROTEIN
(1) Nutritional Value

According to Koo et al.[2], rice protein offer higher nutritional value compared to other plant proteins and rice protein demonstrated highest digestive efficiency compared with wheat, corn and oat. Similar study group reported that the protein efficiency of rice improved to 106% (while the protein efficiency of casein is 100%) upon addition of amino acids lysine and threonine.

Table 1 Digestive Efficiency of Plant Proteins[2]

	Rice	Wheat	Corn	Oat
Digestive Efficiency (%)	**93**	90	89	90

Digestive efficiency of Egg Protein : 100%.

Upon comparison with animal protein, Morita et al.[5], reported that the digestive efficiency of rice protein and casein was 87% and 97% respectively while their biological value* was 51% and 78% respectively. Murata et. al[1]., reported the relative nutritious value of rice protein is 51% of a whole egg.

Biological value = (nitrogen in the body)/(nitrogen absorbed into the body) X 100
The value is a ratio between amount of protein absorbed and the amount of protein in the body.

Table 2 Digestive Efficiency and Biological Value[5] of Rice Protein and Casein

	Rice	Casein
Digestive Efficiency (%)	**87**	97
Biological Value (%)	**51**	78

The content of essential amino acids of ORYZA PROTEIN-P70 is 15% higher than that of isolated soybean protein. In addition, it has been reported that the content of essential amino acids in white rice is higher than protein from soybean, wheat and pea and is equivalent to that of milk protein[6].

Rice protein offers excellent amino acid profile in spite of the low digestive efficiency and biological value. Moreover, ORYZA PROTEIN-P70 is easily digestible and has been maintaining Japanese health throughout centuries.

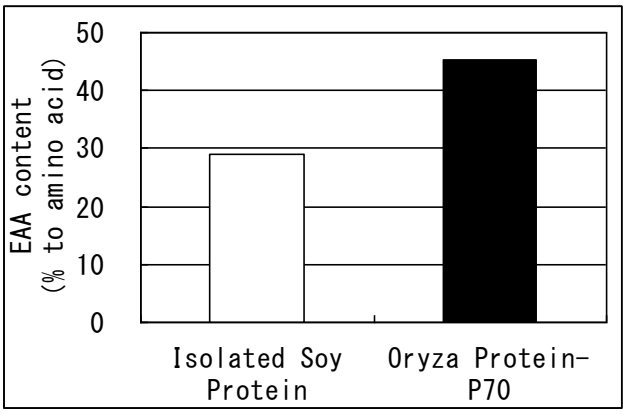


Fig. 1 Content of Essential Amino Acids of different sources

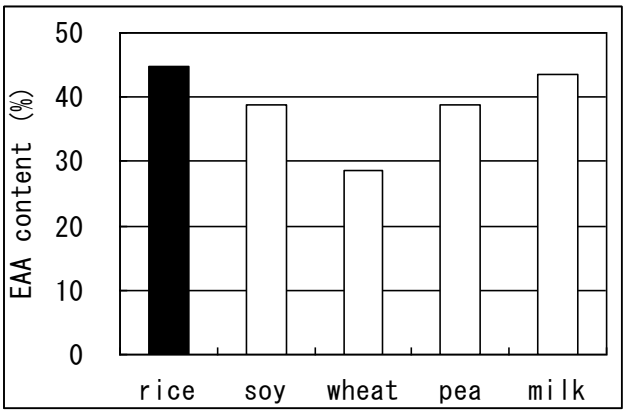


Fig. 2 Content of Essential Amino Acid in Plants and Milk

Table 3 Amino Acid Profile of ORYZA PROTEIN-P70

Amino Acid	Amino Acid Content g/100g
Arginine	6.29
Lysine	2.26
Histidine	2.05
Phenylalanine	4.25
Tyrosine	3.91
Leucine	6.54
Isoleucine	3.26
Methionine	2.35
Valine	4.62
Alanine	4.37
Glycine	3.46
Proline	3.70
Glutamic acid	14.00
Serine	3.92
Threonine	2.88
Asparagic acid	6.92
Tryptophan	1.10
Cystine	1.92

Essential amino acids are highlighted.

(2) Prevention of Weight Gain

In an *in vivo* experiment, oral administration of ORYZA PROTEIN-P70 in mice fed with high fat diet showed acceleration of fat metabolism and reduction of fat deposition. It is suggestive to prevent weight gain.

[Test Method]
Mice (ddy, male, 10-week old) were divided into 3 groups (n=7 or 8 in each group), one group fed with regular feed, 2 groups fed with high fat diet feed (High Fat Diet 32, CLEA Japan). Mice in one of the high fat diet group were given 1 g/kg of ORYZA PROTEIN-P70 orally once daily. Weight of mice was recorded over time and their organs were isolated 16 days later. RNA was extracted from each organ, cDNA was produced and gene expression was evaluated by RT-PCR.

As shown in Figure 3, weight gain in group fed with ORYZA PROTEIN-P70 was lower and better controlled compared with group fed with high fat diet only. In addition, the following findings was showed in group fed with ORYZA PROTEIN-P70:

- Weight of soleus muscle increase (Fig. 4)
- Reduced Perirenal fat (Fig. 5)
- Reduced Liver weight (Fig. 6)

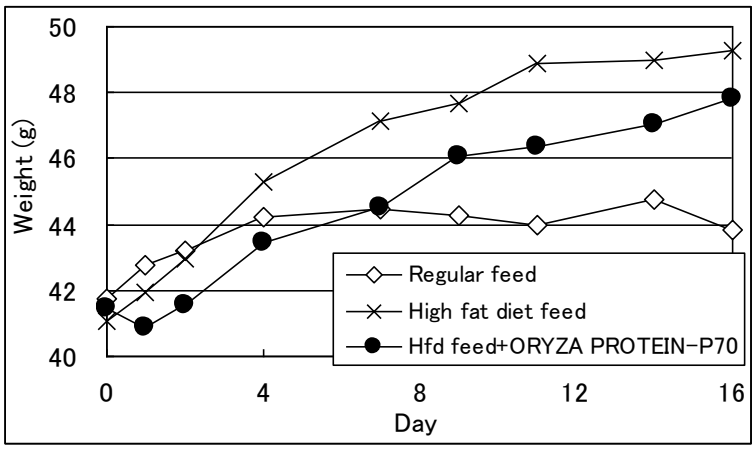


Fig. 3 Comparison on Weight Gain

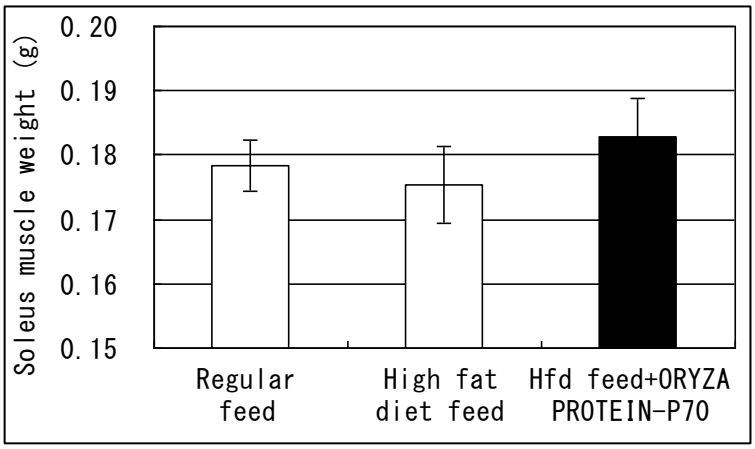


Fig. 4 Comparison on Weight of Soleus Muscle (average value±S.E.)

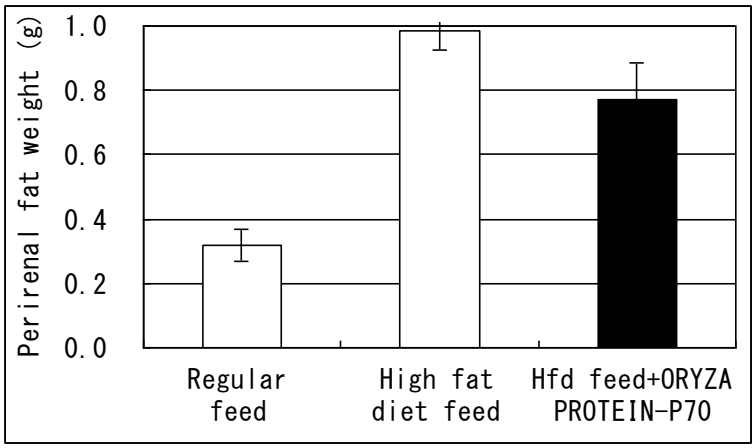


Fig. 5 Comparison on Weight of Perirenal fat (average value ± S.E.)

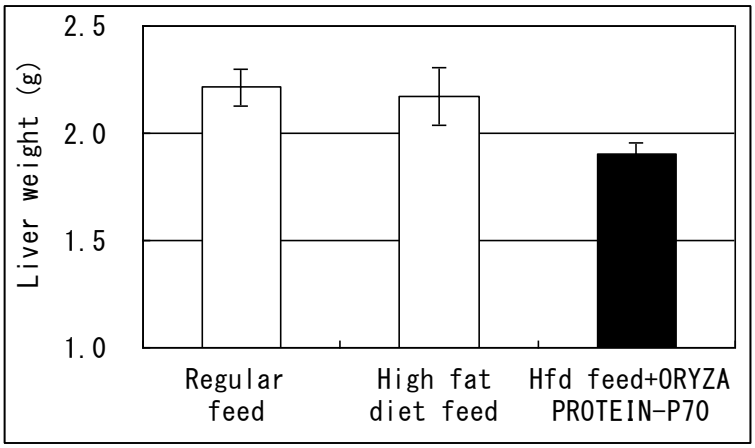


Fig. 6 Comparison on Liver Weight (average value ± S.E.)

The findings with RT-PCR indicated that the expression of CPT 1(carnitine palmitoyltransferase – an enzyme involve in beta oxidation) was significantly enhanced in the liver of mice fed with ORYZA PROTEIN-P70 and tended to be slightly promoted in soleus muscle of mice fed with ORYZA PROTEIN-P70.

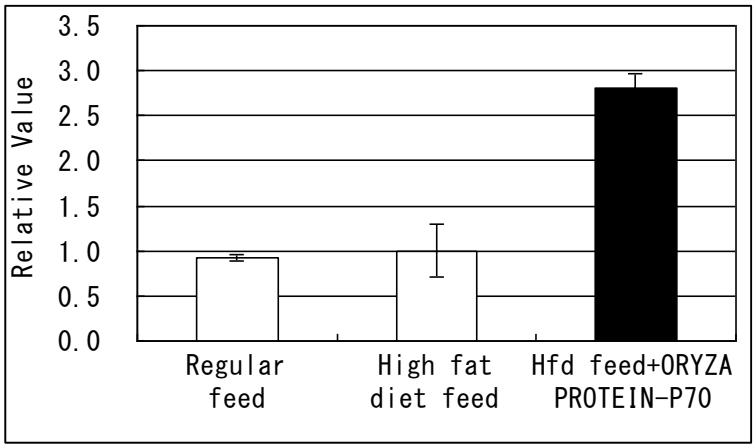


Fig. 7 Comparison on CPT1 Expression in Liver
(average value±S.E. , ** : $p < 0.01$)
β-actin as correction factor for gene expression

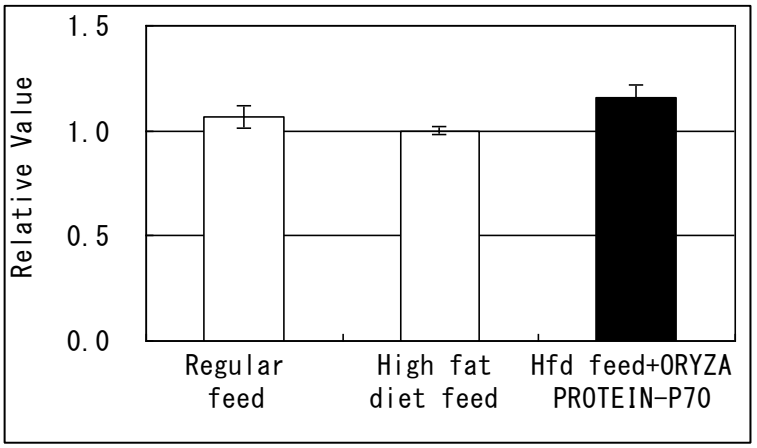


Fig. 8 Comparison on CPT1 Expression in Soleus Muscle
(average value±S.E.)
β-actin as correction factor for gene expression

Fatty acid is transported into the mitochondria by carnitine palmitoyltransferase for further metabolism in the beta oxidation pathway. Fat that is not being metabolized will be stored in the liver and adipose tissue. Yang et. al., [7] reported that liver triglyceride level reduced significantly in rats feed containing rice protein.
Enhancement of CPT expression and reduced liver triglyceride level as reported by Yang et. al., suggested that ORYZA PROTEIN is preventive from weight gain due to accelerated liver fat metabolism.

(3) Function to Lower Cholesterol Level

In the study conducted by Yang et. al. [7], 20% casein, isolated soy protein and rice protein was given to 7-week old male rats respectively together with high cholesterol feed. Meanwhile, 14% casein, isolated soy protein & rice protein was given to 20-week old rats respectively together with cholesterol free feed.
The following findings were reported in both cases:

- Serum cholesterol was lowered approximately 20% in groups consuming feed with rice protein compared to groups consuming feed with casein.
- The effect was equivalent to soy protein

Morita et. al.,[5] reported similar findings.

Table 4 Changes in Serum Lipid concentration when protein mixed feeds were given[5]

		Casein	Isolated Soy Protein	**Rice Protein**
7-week Old Rats	Total Cholesterol (mg/dL)	62.4±4.0[a]	44.4±4.4[b]	**50.2±1.7[b]**
	HDL-Cholesterol (mg/dL)	41.2±1.9[a]	31.4±1.4[b]	**43.9±1.6[a]**
	Neutral Fat (mg/dL)	21.9±2.6	22.2±2.2	**18.0±1.4**
20-week Old Rats	Total Cholesterol (mg/dL)	61.6±7.9[a]	46.2±1.4[b]	**47.7±3.0[b]**
	HDL-Cholesterol (mg/dL)	37.8±4.0[a]	30.0±1.8[b]	**39.1±2.3[a]**
	Neutral Fat (mg/dL)	43.2±7.5	33.5±3.4	**34.3±4.1**

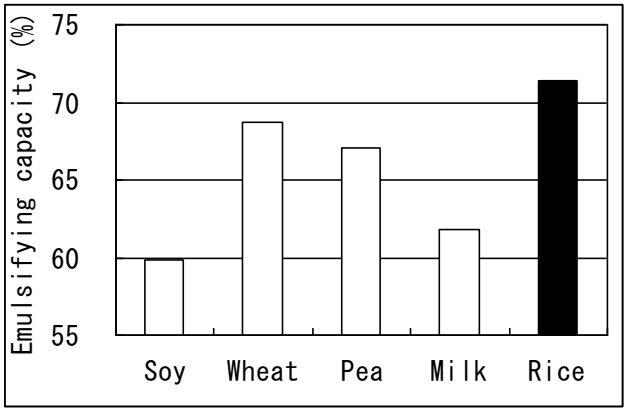
There is a significant difference between values marked with a letter (a, b) in each item. Average value ± S.E.

(4) Emulsifying Capacity

Emulsifying capacity of Rice Protein was evaluated and results revealed:

- Higher emulsifying capacity compared to soy protein, wheat protein and milk protein
- Higher emulsifying capacity than pea protein

ORYZA PROTEIN-P70 offers excellent emulsifying capacity, equivalent to or better than commonly used emulsifying proteins. ORYZA PROTEIN-P70 can improve the property of finished products.



[Test Method]
15mL of rice oil was added to 15mL of 3% sample protein solution and the mixture was homogenized at 17,500rpm at room temperature for 5 minutes. 10mL of emulsified sample was collected and centrifuged at 1,500rpm at room temperature for 5 minutes. Emulsifying capacity of each sample is determined by the following formula:
Emulsifying capacity(%): cubic volume of emulsified layer (mL)/10mL x 100

Fig. 9 Comparison on Emulsifying Capacity of Various Proteins

(5) Non Allergenic

In Japan, shrimp, crab, wheat, buckwheat, egg, milk and peanuts are classified as specific raw ingredients due to the allergenic nature. It is mandatory requirement to indicate the presence of specific raw ingredients in all processed food. On the other hand, no mandatory indication is required for food containing rice and rice protein. Rice Protein contains allergen, 16kDa molecular weight protein has been reported as the major allergen in rice protein.
However, Yamada et. al., [10] has reported that 14-16kDa allergen contained in rice protein is hardly absorbed by small intestine. Another report indicated that rice protein is considered hypoallergenic due to its gluten free nature[2]. Therefore, it is believed that rice protein is a safe source of protein.

(6) Other Functions

Ishii et. al.,[11] reported that bread consumption accelerates fat synthesis and deposit compared with rice consumption. Meanwhile, Chen et. al.,[12] reported that rice protein demonstrated positive effect on bone mass density (BMD) and bone mineral content (BMC) and the effect was better compared with casein.

3. ORYZA PEPTIDE
(1) Water Solubility and Nutritious Value

ORYZA PEPTIDE-P60 is easily water-soluble peptide powder produced by enzymatic decomposition of rice protein. The composition of amino acids and essential amino acids (in % to amino acid) are very similar to ORYZA PROTEIN-P70. ORYZA PEPTIDE-P60 is highly water soluble suitable to be incorporated into functional beverages.

Fig. 10
Solubility of ORYZA PEPTIDE-P60
Left: 1 % solution (pH 3.8)
Right: 3 % solution (pH 3.9)

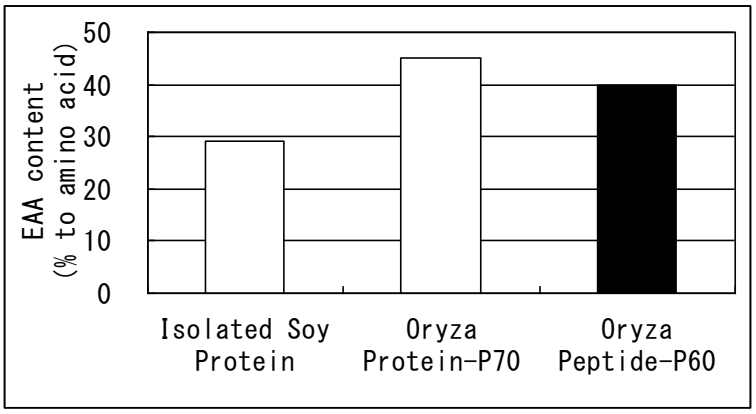


Fig. 11 Comparison on Content of Essential Amino Acid

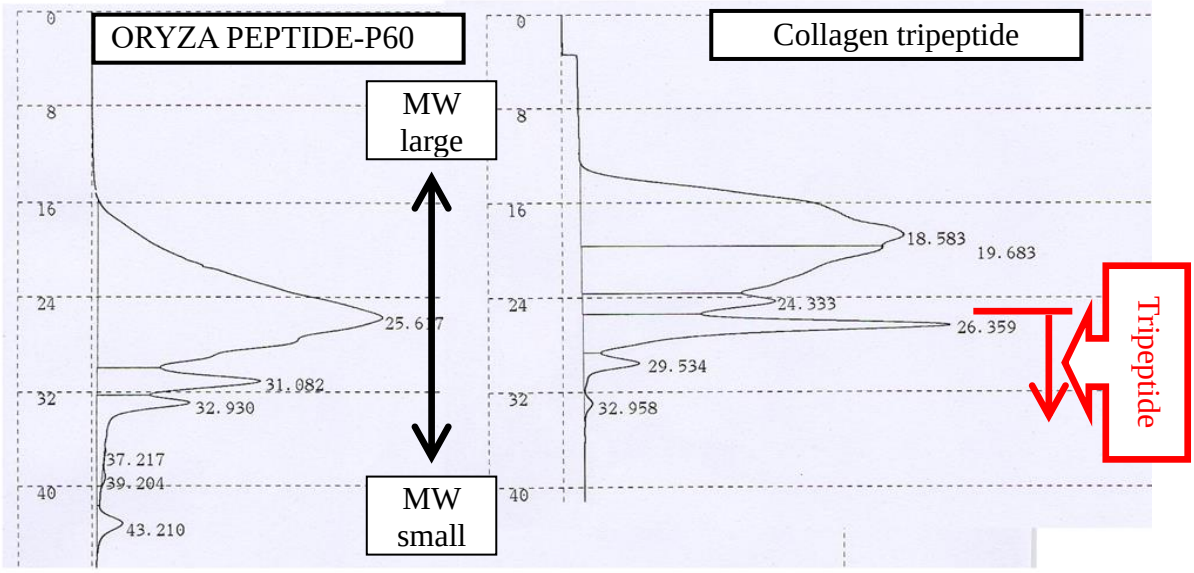
Table 5 Amino Acid Composition of ORYZA PEPTIDE-P60

Amino Acid	Amino Acid Content g/100g
Arginine	6.73
Lysine	2.14
Histidine	1.81
Phenylalanine	3.09
Tyrosine	2.49
Leucine	4.45
Isoleucine	2.16
Methionine	0.88
Valine	3.33
Alanine	3.31
Glycine	2.77
Proline	2.79
Glutamic acid	11.20
Serine	3.11
Threonine	2.08
Asparagic acid	5.85
Tryptophan	0.39
Cystine	0.79

Essential amino acids are highlighted.

(2) Average Molecular Weight

It is expected that the average molecular weight (AMW) of ORYZA PEPTIDE-P60 was small because of high water solubility of that, so we compared the AMW of ORYZA PEPTIDE-P60 and Collagen tripeptide.
Results indicated that the AMW of ORYZA PEPTIDE-P60 was smaller than the one of Collagen tripeptide. It was suggested that the tripeptide content of ORYZA PEPTIDE-P60 was approximately 50 % though the one of Collagen tripeptide was less than 20 %. Therefore, it was suggested that ORYZA PEPTIDE-P60 excelled in bioavailability and beauty effect.



(3) Anti-Obesity Function
(3-1) Prevention of Weight Gain

In an *in vivo* experiment, oral administration of ORYZA PEPTIDE-P60 with high fat diet feed showed enhancement of fat metabolism and reduction of fat deposition. It is believed to prevent weight gain.

[Test Method]
Mice (ddy, male, 10-week old) were divided into 3 groups (n=7 or 8 in each group. One group of mice was fed with regular feed only, two groups fed with high fat diet (High Fat Diet 32, CLEA Japan). ORYZA PEPTIDE-P60 (1g/kg) once a day was orally given to one of the group fed with high fat diet feed. Weight of mice was measured over time and their organs were isolated 16 days later. RNA was extracted from each organ, cDNA was produced and gene expression was quantified by RT-PCR.

Results indicated the following observations in group consuming ORYZA PEPTIDE-P60:

- Weight gain was prevented

- Weight of soleus muscle increased
- Weight of perirenal fat significantly reduced
- Weight of liver significantly reduced

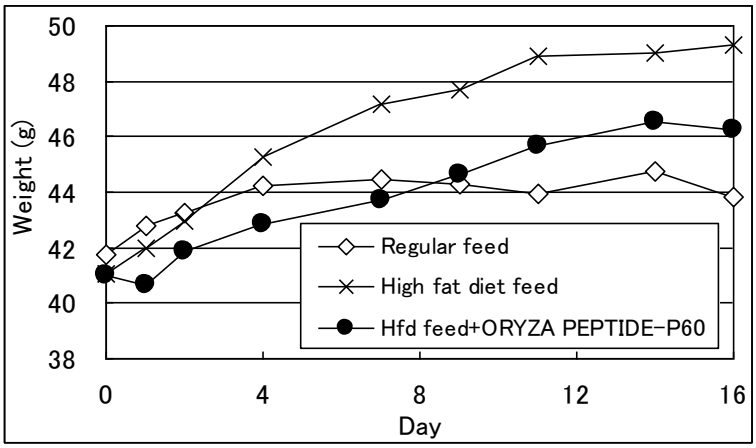


Fig. 12 Comparison on Weight Gain

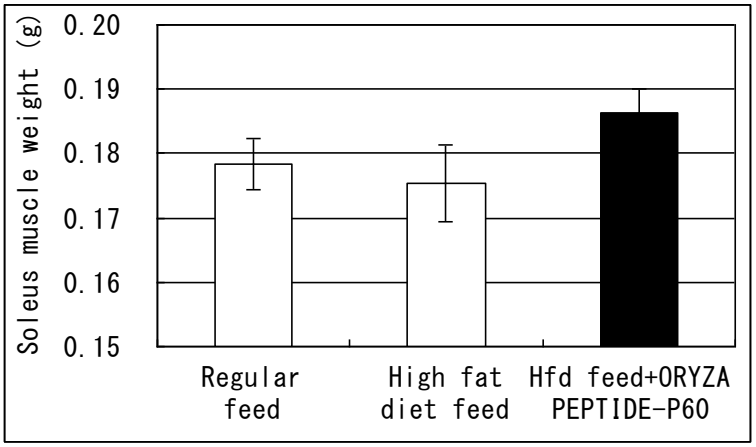


Fig. 13 Comparison on Weight of Soleus Muscle (average value ±S.E.)

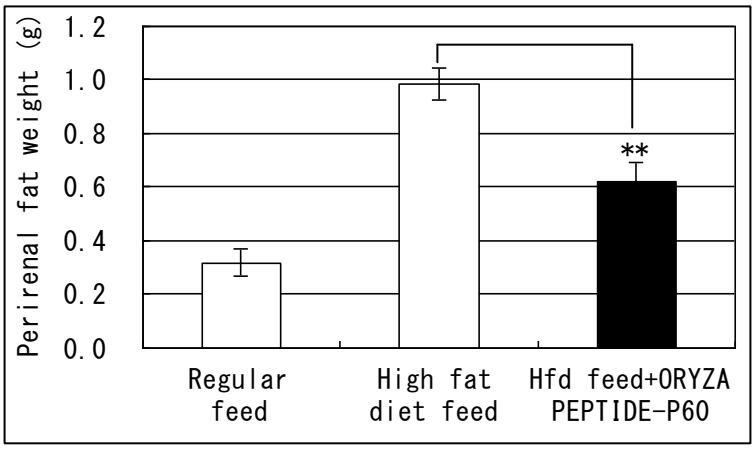


Fig. 14 Comparison on Weight of Perirenal Fat (average value±S.E. , ** : $p < 0.01$)

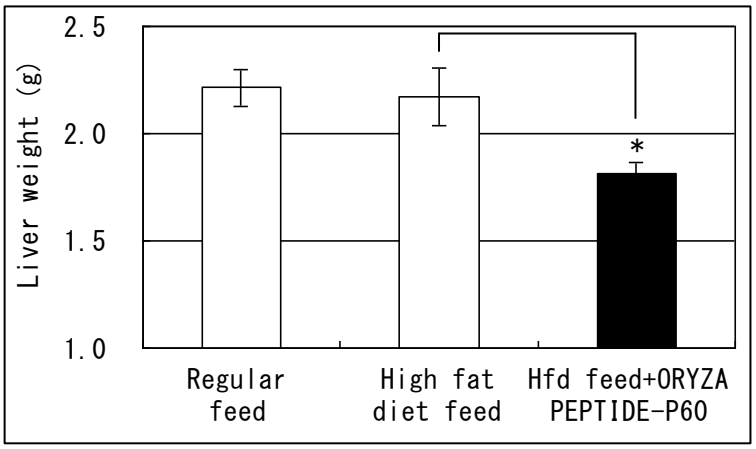


Fig. 15 Comparison on Liver Weight (average value±S.E. , * : $p < 0.05$)

Quantification of RT-PCR indicated that the expression of CPT1 (carnitine palmitoyl transferase- an enzyme involves in beta-oxidation) was significantly enhanced in mice liver fed with ORYZA PEPTIDE-P60.

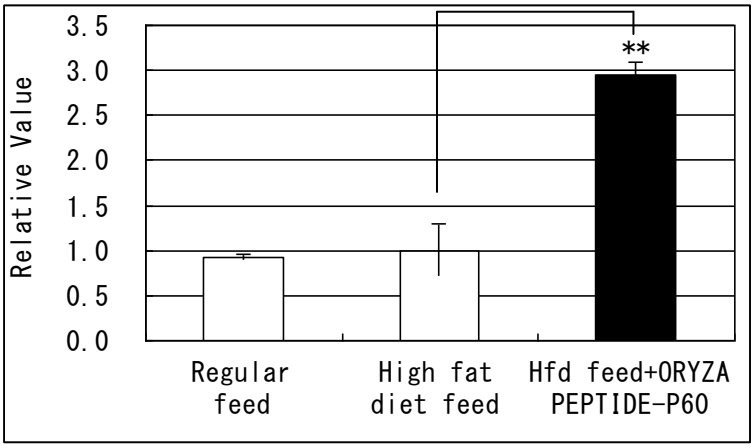


Fig. 16 Comparison on CPT1 Expression Rate in Liver (average value ± S.E. , ** : $p < 0.01$)
β-actin as correction factor for gene expression

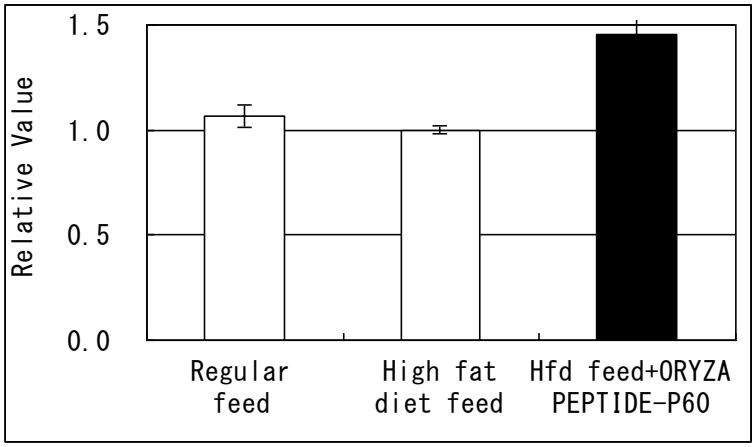


Fig. 17 Comparison on CPT1 Expression Rate in Soleus Muscle
(average value ± S.E.)
β-actin as correction factor for gene expression

In addition, results showed that ORYZA PEPTIDE-P60 significantly prevented weight gain of perirenal fat in spite of consumption of high fat diet. Therefore, it is suggestive that ORYZA PEPTIDE-P60 prevents weight gain by enhancing fat metabolism and decreasing fat deposition.

(3-2) Human Clinical Test

To examine the effect of body fat reduction by Oryza Peptide-P60 coupled with light walking (1.3 times as much as normal) in a double blind human trial.

[Test Method]
Dosage: 1g of Oryza Peptide-P60 is blended in 1 bottle of beverage drink and being administered to test subjects.
Test subjects: female with BMI >25, average aged 40years old
Duration of trial: 8-week

After 8-week of trial, weight loss and decreased in visceral fat was observed in group consuming breakfast drink containing Oryza Peptide-P60. In addition, results showed that there is a reduction in circumference of upper arm and thigh in group consuming Oryza Peptide-P60. Therefore, Oryza Peptide-P60 is effective and healthy in weight and body fat management.

Weight

Weight reduction in group consuming Oryza Peptide-P60 was 1.4kg while 1kg reduction observed in placebo group. Therefore, Oryza Peptide-P60 is preventive on weight gain.

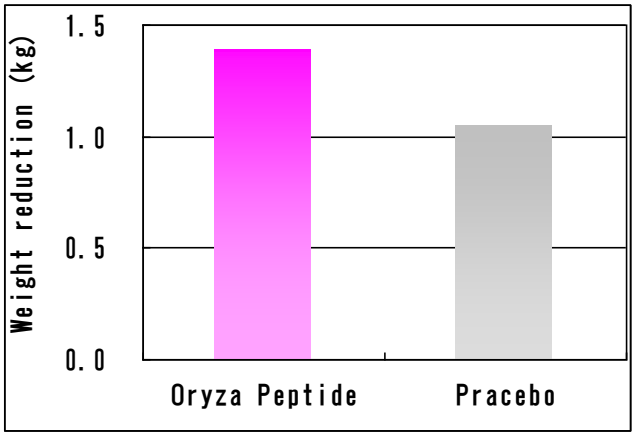
Visceral Fat Area
Result showed a significant reduction in visceral fat area in both groups. However, the reduction observed was greater in group consuming Oryza Peptide-P60.

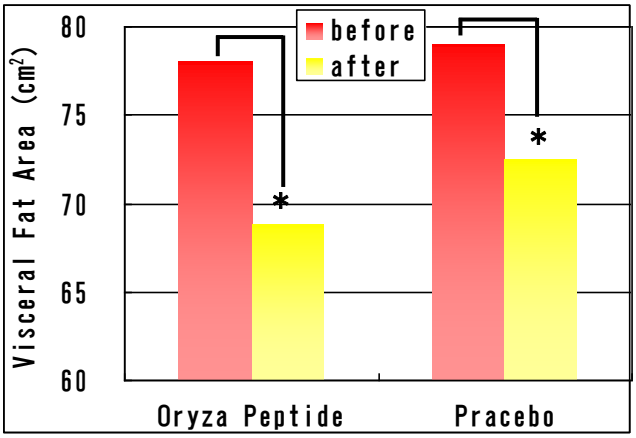
Thigh Circumference

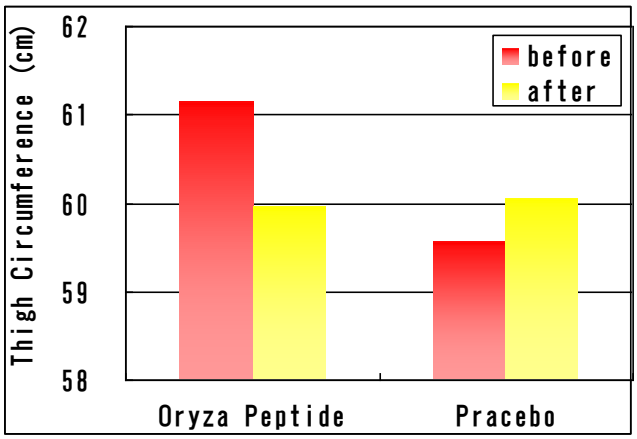
Result showed that reduction in thigh circumference observed only in group consuming Oryza Peptide-P60.

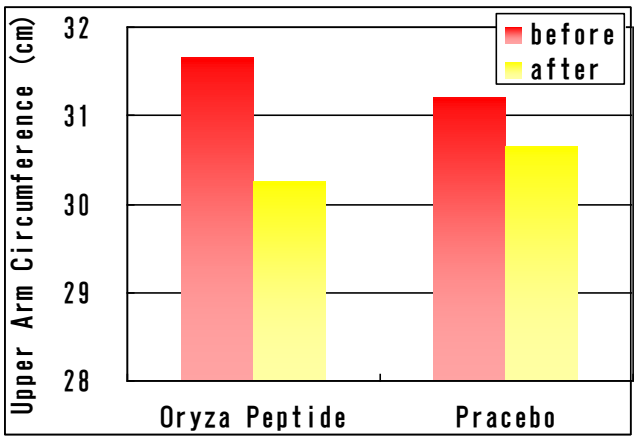
Upper Arm Circumference

Group consuming Oryza Peptide-P60 demonstrated 1.4cm reduction in upper arm circumference while 0.5cm reduction observed in placebo group. Therefore, Oryza Peptide-P60 helps to reduce upper arm circumference.









Before

After

	Fat Area (cm²)	
	Before Trial	End of Trial
Whole body	343.81	220.28
Subcutaneous	267.11	171.20
Visceral	76.70	49.08

(4) Beauty Enhancement Function

(4-1) Function to Inhibit of Melanin Production

The effect of ORYZA PEPTIDE-P60 on melanin production was evaluated *in vitro* using B16 melanoma cells model. As shown in Table 6, ORYZA PEPTIDE-P60 demonstrated dose-dependent inhibition on melanin cells production.

Table 6 The Effect of ORYZA PEPTIDE-P60 on B16 melanoma cells production

Concentration (μg/mL)	0	1	3	10
Melanin Production Rate (%)	100±4.7	94.3±0.3	91.0±2.5	86.8±1.5

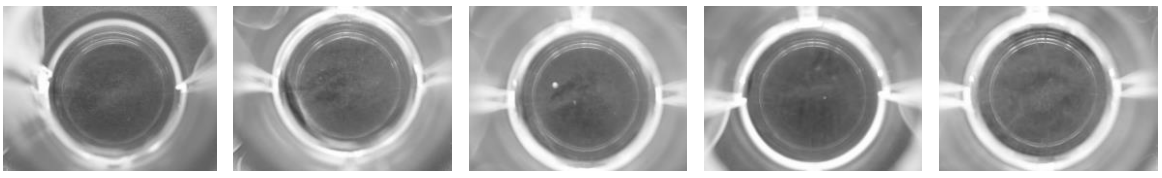
[Test Method]
B16 melanoma cells ($1x10^5$ cells/mL) were suspended in 2mM theophylline containing α-MEM medium (containing 10% FCS, penicillin/streptomycin) and 200μL of the suspension was inseminated into a 48-well plate. Sample(20μL) of ORYZA PEPTIDE-P60 was added to the cells and cultured for 3 days. Absorbance at wavelength 415nm was determined by microplate reader after ultrasonic fragmentation (1N NaOH) of cultured cells.

(4-2) Promotive Effect to Form Collagen Lattice

Collagen synthesized from fibroblast forms extra cellular matrix in skin. Fibroblasts exist in the matrix and maintain skin homeostasis. The *in vitro* collagen lattice model is used to evaluate the affinity of fibroblast to collagen matrix. In this study, we compared the ability of Oryza Peptide and Collagen tripeptide on formation of collagen lattice.

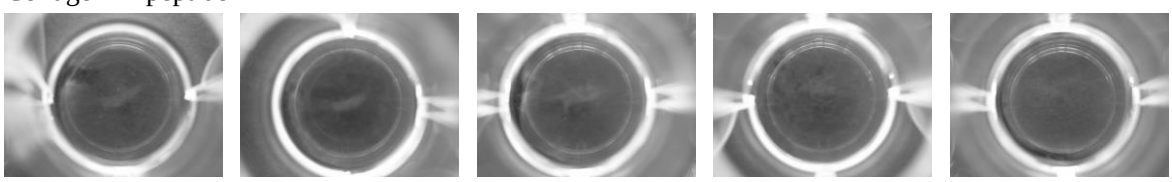
Collagen lattice was observed macroscopically as white gel-like insoluble floater in the well. Oryza peptide enhanced the lattice formation 10 and 100 μg/mL and 10 mg/mL. Collagen tripeptide enhanced the lattice formation more than 100 μg/mL.
Thus, both peptides were found to enhance collagen lattice formation. Oryza Peptide enhanced at lower concentration (10 μg/mL) compared to Collagen Tripeptide.

Oryza Peptide



Control 10 μg/mL 100 μg/mL 1 mg/mL 10 mg/mL

Collagen Tripeptide



Control 10 μg/mL 100 μg/mL 1 mg/mL 10 mg/mL

(4-3) Promotive Effect to Produce Skin Collagen

We compared the effect of two peptides on the amount of skin collagen in mice.

[Test Method]
The mice were divided into 5 groups and were given orally the peptide (500 or 1000 mg/kg) once a day for 28 days. After final administration of the peptides, back skin was removed. The skin was kept at -65 ºC by determination of collagen. The stocked skin was thawed and was treated with pepsin for 7 days followed by digested with elastase (1 day) to solubilize the collagen. The amount of collagen in skin was determined by Mouse Type 1 Collagen Detection Kit (Chondrex, Inc, WA, USA).

As shown in bellow table, skin collagen was increased by oral administration of ORYZA PEPTIDE-P60 in dose-dependent manner. On the other hand, Collagen peptide increases skin collagen at 1000 mg/kg without significance. Hence, both peptides were found to increase skin collagen in mice. The effect of ORYZA PEPTIDE-P60 was more potent than Collagen peptide.

Table 7 Effect of Peptides on the Amount of Skin Collagen in Mice

	Dose mg/kg	Collagen in skin μg/mg-skin
Control	-	40.0±2.2
ORYZA PEPTIDE-P60	500	44.9±1.5
	1000	50.2±2.2 *
Collagen peptide	500	40.8±3.5
	1000	46.2±2.0

n = 6
Each value represents mean±SEM.
An asterisk denotes significant difference from the control at *: $p<0.05$.

(4-4) Suppression on genetic expression of ceramidase & hyaluronidase

The effect of ORYZA PEPTIDE-P60 on the genetic expression of ceramidase and hyaluronidase was evaluated *in vivo* using hairless mice (Hos: HR-1, female, 5-week old).

[Test Method]
Hairless mice (Hos: HR-1, female, 5-week old) were kept for 3 days. ORYZA PEPTIDE-P60 (50, 100, 200mg/kg) once daily was orally given to the mice for 7 days. mRNA of skin was prepared to transcribed into cDNA for genetic expression evaluation by RT-PCR.

Ceramides are family of lipid molecules consists of sphingosine and a fatty acid. It is predominantly found in the epidermal intracellular lipid bilayer. Ceramides play important role in maintaining skin moisture and skin barrier function. The synthesis of ceramide is catalyzed by the enzyme serine palmitoyl transferase and degraded by ceramidase.

In an *in vivo* experiment, ORYZA PEPTIDE-P60 (50, 100, 200mg/kg) was orally given to hairless mice for 7 days and genetic expression of ceramide-metabolizing enzymes in skin was determined by RT-PCR. Results showed that genetic expression of ceramidases, namely, neutral ceramidase, alkaline ceramidase and phyto-ceramidase was suppressed at all doses of ORYZA PEPTIDE-P60. With exception, genetic expression of acidic ceramidase was not suppressed.

Meanwhile, hyaluronic acid is an important component of the extracellular matrix and plays important role in skin wound healing and skin moisture retention. Hyaluronic acid is degraded by family of enzymes called hyaluronidases. Similarly, an in vivo experiment was conducted to evaluate the effect of ORYZA PEPTIDE-P60 on the genetic expression of hyaluronidases. ORYZA PEPTIDE-P60 was orally given to hairless mice for 7 days. Genetic expression of hyaluronidases was determined by RT-PCR. Results indicated that genetic expression of hyaluronidase 1, hyaluronidase 2, hyaluronidase 3 and hyaluronidase 4 was suppressed by ORYZA PEPTIDE-P60 at a dose of 200mg/kg.

Based on the above results, it is suggestive that ORYZA PEPTIDE-P60 is potentially beneficial in preventing the breakdown of ceramide and hyaluronic acid and thus improve skin condition and skin moisture retention.

Table 8 The effect of ORYZA PEPTIDE-P60 on the genetic expression of Ceramidases

	Acid ceramidase	Neutral ceramidase	Alkalin ceramidase	Phyto-Ceramidase
Control	1.00±0.03	1.00±0.02	1.00±0.02	1.00±0.03
50 mg/kg	1.25±0.02	**0.80±0.01**	**0.81±0.02**	**0.76±0.01**
100 mg/kg	1.18±0.01	**0.73±0.01**	**0.72±0.03**	**0.67±0.01**
200 mg/kg	1.07±0.02	**0.67±0.01**	**0.62±0.01**	**0.77±0.01**

Table 9 The effect of ORYZA PEPTIDE-P60 on the genetic expression of Hyaluronidases

	Hyaluronidase 1	Hyaluronidase 2	Hyaluronidase 3	Hyaluronidase 4
Control	1.00±0.03	1.00±0.03	1.00±0.01	1.00±0.01
50 mg/kg	**0.78±0.01**	**0.63±0.01**	0.92±0.01	1.07±0.02
100 mg/kg	**0.65±0.01**	**0.54±0.01**	**0.76±0.01**	0.92±0.01
200 mg/kg	**0.72±0.01**	**0.60±0.02**	**0.78±0.01**	**0.78±0.01**

(5) Inhibitory Effect to elevate blood pressure

It is well known that angiotensin converting enzyme (ACE) plays an important role to control blood pressure in Renin-Angiotensin System. It is also reported that some peptides derived from various materials have blood pressure blood-pressure-lowering effect and sardine peptide is known as an ingredient to inhibit ACE activity and lower blood pressure.
We evaluated the effect to inhibit ACE activity of ORYZA PEPTIDE-P60 and found that the IC50 value of ORYZA PEPTIDE-P60 is the lowest among various peptides. It is suggested that ORYZA PEPTIDE-P60 has blood-pressure-lowering effect and the effect of ORYZA PEPTIDE-P60 may be stronger than sardine peptide.

Table 10 Comparison of IC50 value to inhibit ACE activity

Sample	IC_{50} (μg/mL)
ORYZA PEPTIDE-P60	31.6
Sardine peptide	34.4
Wheat peptide	102.2
Soya peptide	610.7
Milk peptide	776.1

4. Reference Literatures

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5. Stability

(1) Storage Stability

ORYZA PROTEIN-P70 is highly stable upon storage for long period of time. As shown below, the protein content remained unchanged after 6 months storage at 40°C/RH 80%.

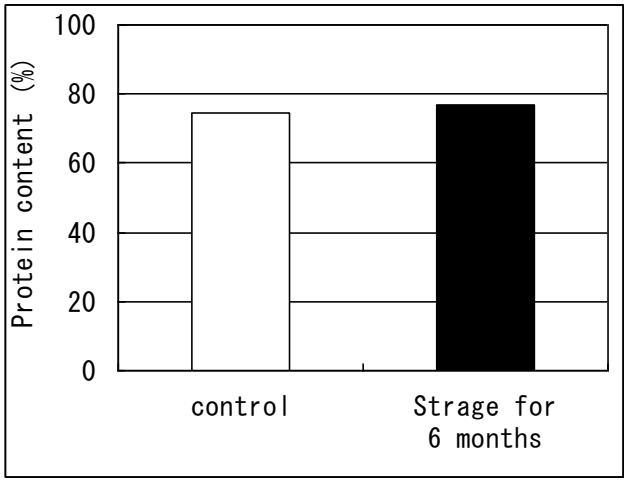

Fig. 18 Stability of ORYZA PROTEIN-P70 upon storage for 6 months

[Test Method]
ORYZA PROTEIN-P70 was stored in dark at 40°C/RH 80% for 6 months. The protein content was determined before and after 6 months by Kjeldahl method.

(2) pH Stability

As shown in Fig. 19, ORYZA PEPTIDE-P60 is highly stable under acidic and neutral condition. Meanwhile, minimal degradation of peptide is observed under alkaline condition.

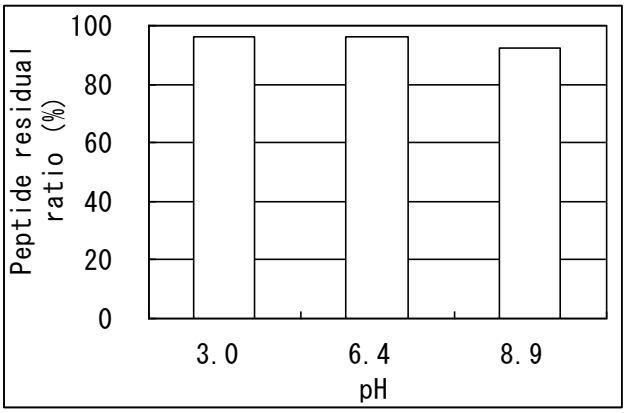

Fig. 19 pH Stability of aqueous ORYZA PEPTIDE-P60

[Test Method]
ORYZA PEPTIDE-P60 was dissolved in water. Storage pH was adjusted with hydrocholic acid for acidic condition and diluted alkali for alkaline condition. ORYZA PEPTIDE-P60 was stored under different pH condition at room temperature for 3 days. The peptide content was determined to evaluate its pH stability.

6. Nutrition Information

Description	Amount per 100g		Analytical Method
	ORYZA PROTEIN -P80	ORYZA PEPTIDE -P60	
Energy	371 kcal	371 kcal	Atwater Method (Revised) *1
Protein	87.8 g	65.8 g	Combustion Method *2
Fat	0.5 g	0.1 g	Acid degradation
Carbohydrate	3.9 g	26.7 g	100 g – (water + protein + fat + ash) *3
Sodium	13.3 mg	36 mg	Atomic absorption spectrophotometory
Sodium	0.003 g	0.1 g	Sodium Equiv. value
Moisture	5.2 g	2.8 g	Heat-drying at atmospheric pressure
Ash	2.6 g	4.6 g	Direct Incineration
Dietary fiber	-	1.0 g	Prosky Method

*1 Energy expression standard (Ministry of Health and Welfare's announcement No. 176)
Conversion factor: Protein 4, fat 9, sugar 4; dietary fiber 2
*2 Nitrogen, protein conversion factor: 5.95
*3 Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)

ORYZA PROTEIN-P80 is the value calculated from ORYZA PROTEIN-P70.
【ORYZA PROTEIN-P70】
Test trustee: SRL, Inc / Date of analysis: July 30, 2009 / Test No.: 200907160028
【ORYZA PEPTIDE-P60】
Test trustee: SRL, Inc / Date of analysis: September 25, 2009 / Test No.:200909090044

7. Product Safety Profile

(1) Residual Agricultural Chemicals

As confirmed by test trustee, analysis results of **ORYZA PROTEIN-P70** is conformed to the regulation stipulated in the Food Sanitation Act on 517 residual agricultural compounds.

Test trustee：Masis Co. Ltd.
Data：May 7, 2009
Report No.：30318

As confirmed by test trustee, the analysis results of **ORYZA PEPTIDE-P60** is conformed to the regulation stipulated in the Food Sanitation Act on 518 residual agricultural compounds.

Test Trustee : Masis Co., Ltd.
Date : Sept 25, 2009
Report No. : 33400

(2) Acute Toxicity (LD_{50})

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where **ORYZA PROTEIN-P70** 2000 mg/kg was orally given to starved mice (male & female ddy, 5 weeks old, weight ~30 g) for 14 days. No abnormalities and fatal event observed at 2000 mg/kg. No abnormalities of organs observed under macroscopic examination upon autopsy. Thus, LD_{50} of ORYZA PROTEIN-P70 is deduced to be >2000 mg/kg.

Similarly, ORYZA PEPTIDE-P60 5000 mg/kg was orally given to starved mice (male & female ddy, 5 weeks old, weight ~30 g) for 14 days. No abnormalities and fatal event observed at 5000 mg/kg. No abnormalities of organs observed under macroscopic examination upon autopsy. Thus, LD_{50} of ORYZA PROTEIN-P70 is deduced to be >5000 mg/kg.

(3)Mutagenicity

Ames test was conducted regarding ORYZA PEPTIDE-P60 and finding was Negative. Thus, ORYZA PEPTIDE-P60 is non-mutagenic.

(4)Melamine

The presence of melamine is monitored, ORYZA PROTEIN-P70 was tested according to the analysis method laid down by Ministry of Health, Labour and Welfare. As confirmed by test trustee, ORYZA PROTEIN-P70 conformed to the permitted level stipulated.

Test trustee: Qsai Analysis and Research Center Co., Ltd.
Date: April 27, 2009
Report No.: 2009041509-01-1

8. Recommended Daily Dosage

Products	Claims	Recommended daily dosage
Oryza Protein-P80	Nutritional support Anti-obesity Muscle building	500～1,000 mg/day
Oryza Peptide-P60	Nutrient Anti-obesity Muscle building	500～1,000 mg/day
	Beauty enhancement	100～200 mg/day

9. Applications

	Applications	Claims	Examples
Foods	Nutritional support food, Beauty food, Anti-obesity food	1) Nutritional support 2) Anti-obesity 3) Beauty enhancement	Beverages Hard & soft capsules, tablets Candies, chewing gums, chocolates, wafers, jellies Ham, sausage, etc.
Cosmetics	Beauty cosmetic		Body lotions, body gel etc.

10. Packaging

Products	Weight	Packaging
Oryza Protein-P80 (Powder, for food) Oryza Protein-PC70 (Powder, for cosmetic)	Interior packaging： Aluminum bag Exterior packaging： Cardboard	1 kg 5 kg
Oryza Peptide-P60 (Water-soluble powder, for food) Oryza Peptide-PC60 (Water-soluble powder, for cosmetic)	Interior packaging： Aluminum bag Exterior packaging： Cardboard	1 kg 5 kg

11. Storage

Store in a cool, dry, dark place.
It is recommended to finish ORYZA PEPTIDE-P60 & ORYZA PEPTIDE-PC60 once open as it is highly hygroscopic. Otherwise, dessicant bag is recommended to be inserted for storage purpose.

12. Indication Examples

＜Food＞

ORYZA PROTEIN-P80
Expression：Rice Protein

ORYZA PEPTIDE-P60

Expression：Rice Peptide

＜**Cosmetic**＞

ORYZA PROTEIN-PC70

Expression：Oryza Sativa (Rice) Bran Extract

INCI name：ORYZA SATIVA (RICE) BRAN EXTACT

ORYZA PEPTIDE-PC60

Expression：Hydrolyzed Rice Bran Extract

INCI name：HYDROLYZED RICE BRAN EXTRACT

PRODUCT STANDARD

PRODUCT NAME : **ORYZA PROTEIN-P80** (FOOD)

This product is protein powder derived from rice (*Oryza sativa* Linne).

Appearance	Pale yellow to light brown powder. Light unique smell.	
Protein	Min. 80.0 %	(Kjeldahl method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2)Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 5×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)
Composition	Ingredient Content Rice Protein 100 %	

PRODUCT STANDARD

PRODUCT NAME : **ORYZA PROTEIN-PC70** (COSMETIC)

This product is protein powder derived from rice (*Oryza sativa* Linne). It guarantees minimum of 70.0 % protein.

Appearance	Light brown powder. Light unique smell.	
Protein	Min. 70.0 %	(Kjeldahl method)
Loss on Drying	Max. 10.0 %	(1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)
Composition	Ingredient / Content	
	Rice Bran Extarct	100 %

PRODUCT STANDARD

PRODUCT NAME : **ORYZA PEPTIDE-P60** (FOOD)

This product is hydrolyzed rice (*Oryza sativa* Linne) protein with an enzyme. It guarantees minimum of 60.0 % peptide. This product is water-soluble.

Appearance	Slightly yellow or light brown powder. Light unique smell.	
Peptide	Min. 60.0 %	(Kjeldahl method)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(Sodium Sulfide Colorimetric Method)
(2)Arsenic (as As_2O_3)	Max. 2 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)
Composition	Ingredient Content Rice Peptide 100 %	

PRODUCT STANDARD

PRODUCT NAME : **ORYZA PEPTIDE-PC60** (COSMETIC)

This product is hydrolyzed rice (*Oryza sativa* Linne) protein with an enzyme. It guarantees minimum of 60.0 % peptide. This product is water-soluble.

Appearance	Slightly yellow or light brown powder. Light unique smell.	
Peptide	Min. 60.0 %	(Kjeldahl method)
Loss on Drying	Max. 10.0 %	(1g, 105 ℃, 2 hr)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 20 ppm	(The Second Method of The Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 2 ppm	(The Third Method of The Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Hydrolyzed rice bran extract	100 %

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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