Oryza

ORYZA OIL & FAT CHEMICAL CO., LTD.

YUZU SEED EXTRACT

For Whitening and beauty, Relaxation and Metabolic syndrome

- YUZU SEED EXTRACT – P
  (Powder, Food Grade)
- YUZU SEED EXTRACT – WSP
  (Water Soluble Powder, Food Grade)
- YUZU SEED EXTRACT – PC
  (Powder, Cosmetic Grade)
- YUZU SEED EXTRACT – WSPC
  (Powder, Cosmetic Grade)
- YUZU SEED EXTRACT – LC
  (Liquid, Cosmetic Grade)
- YUZU SEED OIL
  (Liquid oil, Food and Cosmetic Grade)

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Ver. 4.3 WY

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1. Introduction

Yuzu (*Citrus junos*), or Japanese grapefruit, is originated in Sichuan and Yunnan, located in the upstream region of the Yangtze River in China. It was brought to Japan during the Heian period (about a thousand years ago).

Yuzu fruits become bright yellow, as they get ripe in late November. It possesses acidic taste and citrus aroma. Its juice, peer and seeds have been utilized in various life scenes since ancient times. For example, a small slice of Yuzu is commonly used in sophisticated Japanese dishes as fine flavoring agents, characterized by its rich ingredients of limonene and pinene. Yuzu has also been used to make flavorful cakes and jam called *Yuzuneri*. Yuzu-in-the-bath is helpful in relaxation, revitalization, and relief of cold symptoms with its outstanding ingredients.

The intake of pulverized and charred seeds of Yuzu is beneficial for alleviating symptoms of body ache is a statement described in the herbal list of a pharmaceutical bibliography, edited during the Edo period (about three hundred years ago). Following this statement, Yuzu is used in the fork remedies for treatment of rheumatism, stiffness in the shoulders. The juice is beneficial for relaxation, pain relief, and cosmetics with its rich content of organic acids, such as citric acid, tartaric acid and ascorbic acid (vitamin C). Furthermore, the limonoid content of Yuzu are known to enhance blood circulation. Pectin, existing in the surface of seeds, is expected to moderate blood sugar and cholesterol levels.

Oryza Oil & Fat Chemical Co., Ltd. conducted various studies on the diverse pharmacological functions of Yuzu. It was confirmed that Yuzu Seed Extract is an excellent skin whitening and smoothing agent. Its revitalizing functionality in psychosomatic health is widely applicable to food and nutraceutical industries. The solution is here for beauty enhancement and as relaxation booster.

Yuzu Fruits

Yuzu Seeds

2. Functional Ingredients of YUZU SEED EXTRACT

(1) Recent Studies

The Designer Food Project in the United States listed citrus to be beneficial for cancer prevention. Epidemiological studies from the National Cancer Institute of United States showed an inverse relationship between the intake of citrus and the prevalence of cancer. Identified as effective cancer preventive agents are *d-limonene, limonoids* and *hesperidin*[1], all of which are in Yuzu.[2] Limonoid is a general term for triterpene derivatives found only in the *Rutaceae* and *Meliaceae* species. Thirty-six types of aglycones and glucose glycosides have been identified and isolated so far.[3] A limonoid *limonin* is a source of bitterness, and is found 1,800-fold richer in seeds than in juice (our original data). Limonoid glycosides are not bitter, and are found mainly in juice. *In vivo* studies using mice and hamsters showed that limonoids *nomilin* and *obacunon* activate glutathione-S-transferase, an enzyme that suppresses tumor formation. In addition, limonin and obacunone were reported to suppress colon cancer in rats. [4]

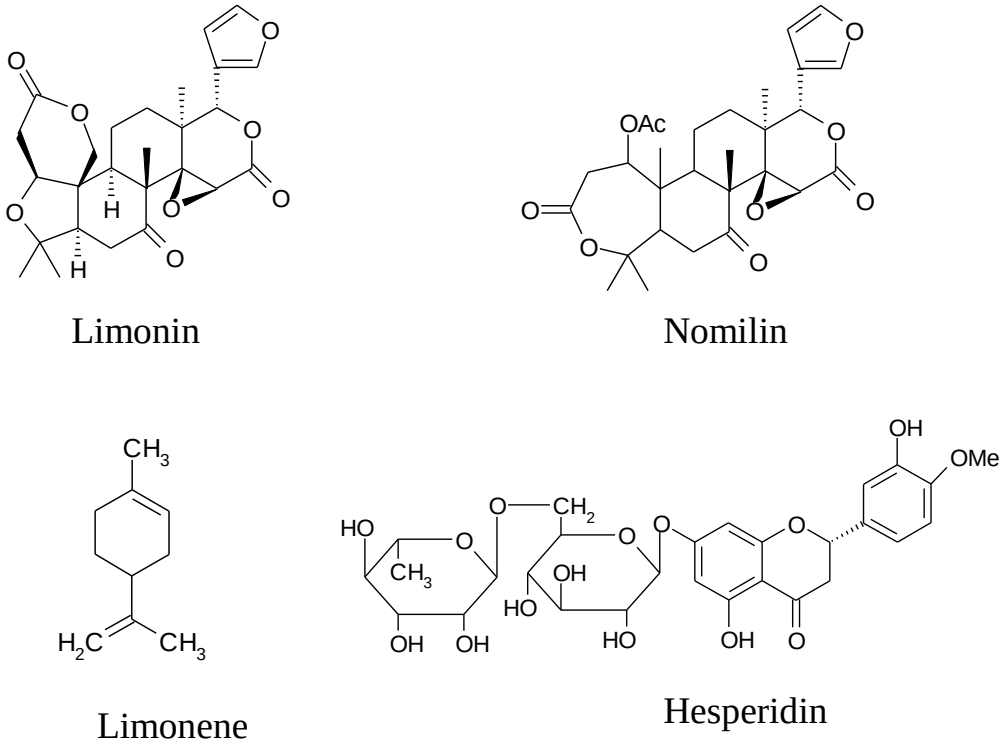


Figure 1. Chemical Structures of the Ingredients in Yuzu Seeds

1) Yano M. Cancer prevention by citrus. Nippon Shokuhin Kagaku Kogaku Kaishi, **49,** 139-144 (2002).
2) Hashinaga F., Herman Z, Hasegawa S. Limonoids in Seeds of Yuzu , Nippon Shokuhin Kogyo Gakkaishi, **37,** 380-382 (1990).
3) Hasegawa S, Ifuku Y. Biochemistry of citrus limonoids, Nippon Shokuhin Kogyo Gakkaishi, **41**, 372-380 (1990).
4) Tanaka T., Maeda M., Kohno H., Murakami M., Kagami S., Miyake M., Wada K. Inhibition of azoxymethane-induced colon carcinogenesis in male F344 rats by the citrus limonoids obacunone and limonin. *Carcinogenesis*, **22**, 193-198 (2000).

(2) Mechanism of Melanin Production and Pigmentation

What we call spots is melanin pigmentation on skin. The melanin pigmentation is caused by aging, changes in hormone balance, inflammation and exposure to ultraviolet (UV) light. Upon exposure to UV light, a signal transduction takes place from epidermis to melanocytes mediated by endotherin and phospholipase. Melanocytes are melanin-producing cells in skin, and work for defence against harmful UV light. In melanocytes, an enzyme called tyrosinase produces melanin as they receive the signal. Tyrosinase converts tyrosine, an amino acid, to dopa, then dopa quinone. Dopa quinone is further oxidized to melanin. In addition, a protein called "stem cell factor" (SCF) has been found to play an important role in skin hyperpigmentation. UV light activates SCF and endothelin to stimulate melanocytes to enhance melanin production.

These reactions are natural defense mechanisms to protect skin from harmful UV radiation. If skin is in a good health, old cells go away with melanin, and are replaced by new melanin-less skin cells in a 28-day cycle skin turn over. However, as metabolic rates decline with ageing, the turn over does not occur regularly, resulting in accumulation of melanin pigments, that is, hyperpigmentation.

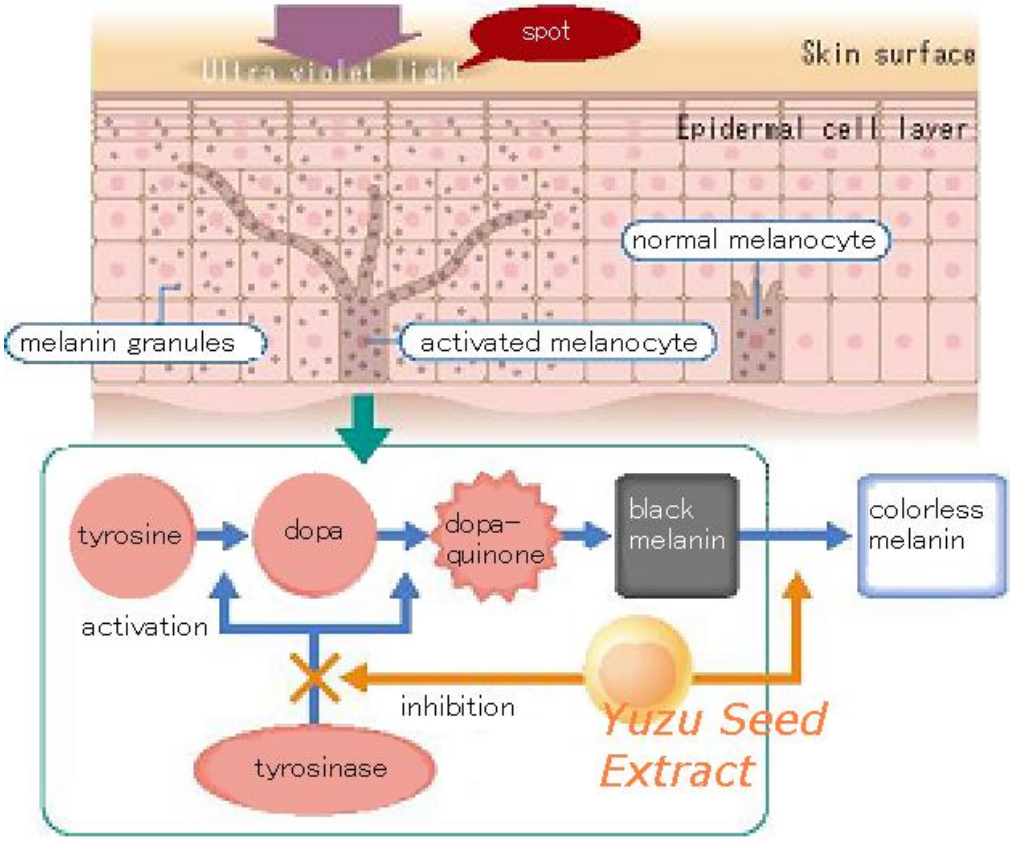


Figure 2. Mechanism of Melanin Production

3. Physiological Functions of YUZU SEED EXTRACT

(1) Skin Whitening (*In Vitro*)

B16 melanoma cells were used to examine the skin whitening effects of YUZU SEED EXTRACT, β-arbutin and vitamin C (ascorbic acid). YUZU SEED EXTRACT was found to suppress the melanin formation in a dose-dependent manner, as illustrated in Figure 3A. YUZU SEED EXTRACT showed comparative or superior inhibitory effects on melanin formation, compared to β-arbutin (Figure 3B) and vitamin C (Figure 3C), respectively.

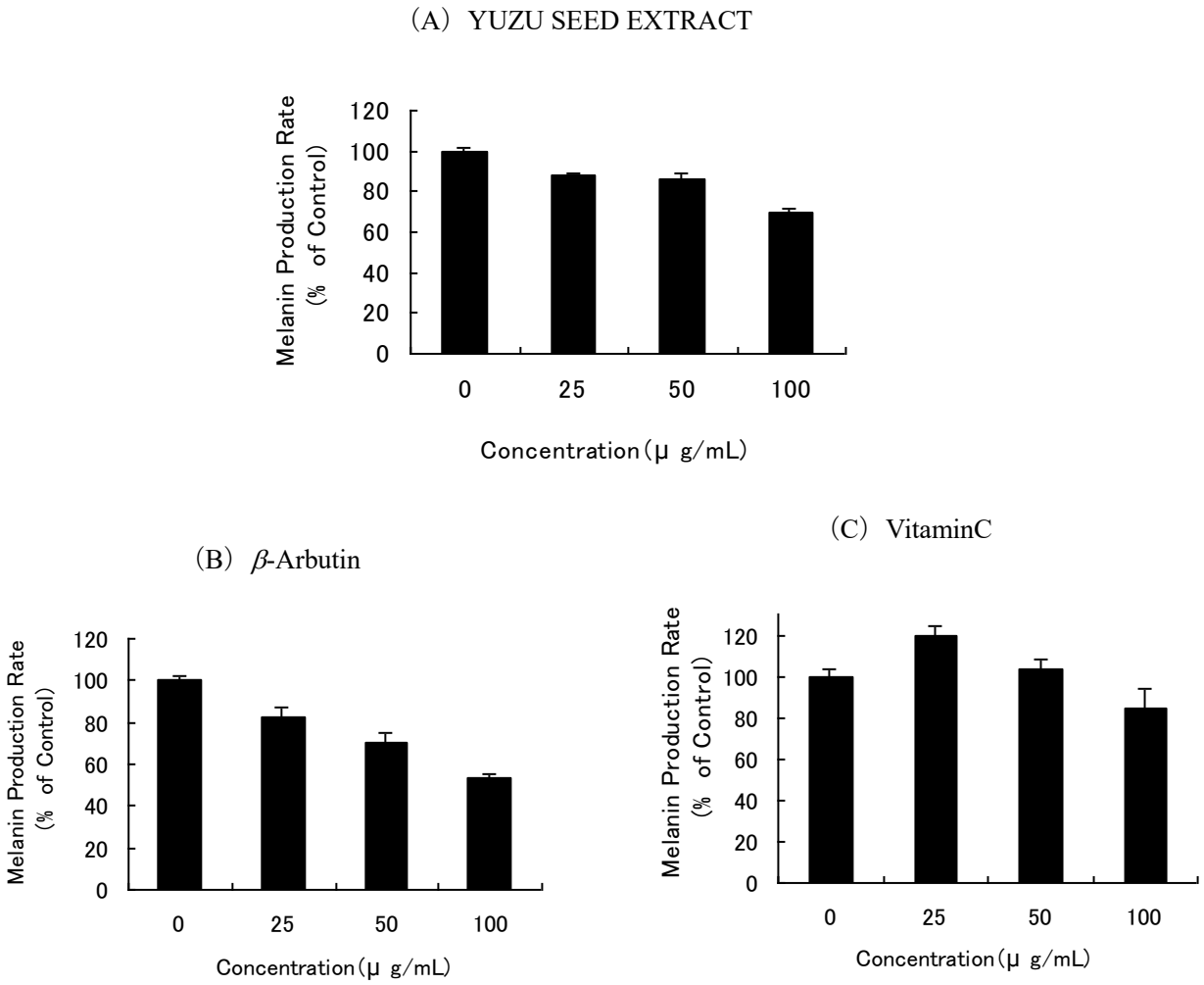


Figure 3. Effects of Plant-Origin Ingredients on Melanin Formation in B16 Melanoma Cells (mean±S.E., n=4).

(Experimental Method)

B16 melanoma cells ($2.5x10^4$ cells/well) were plated in a 24-well plate in MEM with 10% fatal bovine serum, 100 units/ml of penicillin, 100 µg/ml of streptomycin and 2 mM theophylline. Solution (55µl) of YUZU SEED EXTRACT, β-arbutin and vitamin C was added to each well, followed by 3-day incubation. The culture was crushed by ultrasonication in 300 µl of PBS. The cell crush was transferred to in a 96-well assay plate and absorbance was measured at the wavelength of 415 nm using a microplate reader.

(2) Fibroblast Growth (*In Vitro*)

YUZU SEED EXTRACT is shown to stimulate fibroblast growth as illustrated in Figure 4, from the examination using human neonatal skin fibroblast, NB1RGB. YUZU SEED EXTRACT enhances growth of new skin cells, thus promotes turn over of skin.

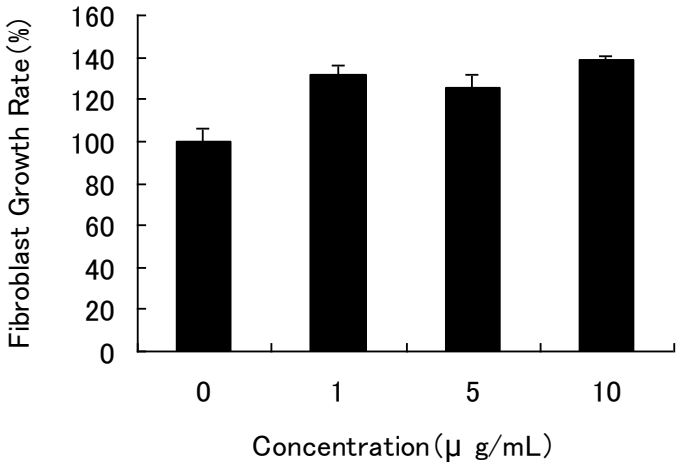


Figure 4. Effects of YUZU SEED EXTRACT on Growth of NBIRGB Fibroblast (mean ± S.E.: n=6)

(Experimental Method)

NB1RGB cells were plated ($2x10^4$ cells/well) in a 96-well plate in α-MEM with 10% fetal bovine serum, 100 units/ml of penicillin and 100µg/ml of streptomycin. YUZU SEED EXTRACT solution (10µl) was added to each well, followed by 2-day incubation. Cell growth was determined by an MTT assay.

(3) Skin Turn Over

A reconstructed artificial human skin cell model was used to examine the effect of YUZU SEED EXTRACT on skin turn over. The microscopic illustrations of skin dermal layer are shown in Figure 5.

A pronounced thickening effect on skin dermal layers (epidermis and dermis) was observed in the models treated with YUZU SEED EXTRACT in a dose-dependent manner. It was hypothesized that YUZU SEED EXTRACT promoted collagen production and fibroblast growth, resulting in thickening of the skin dermal layer.

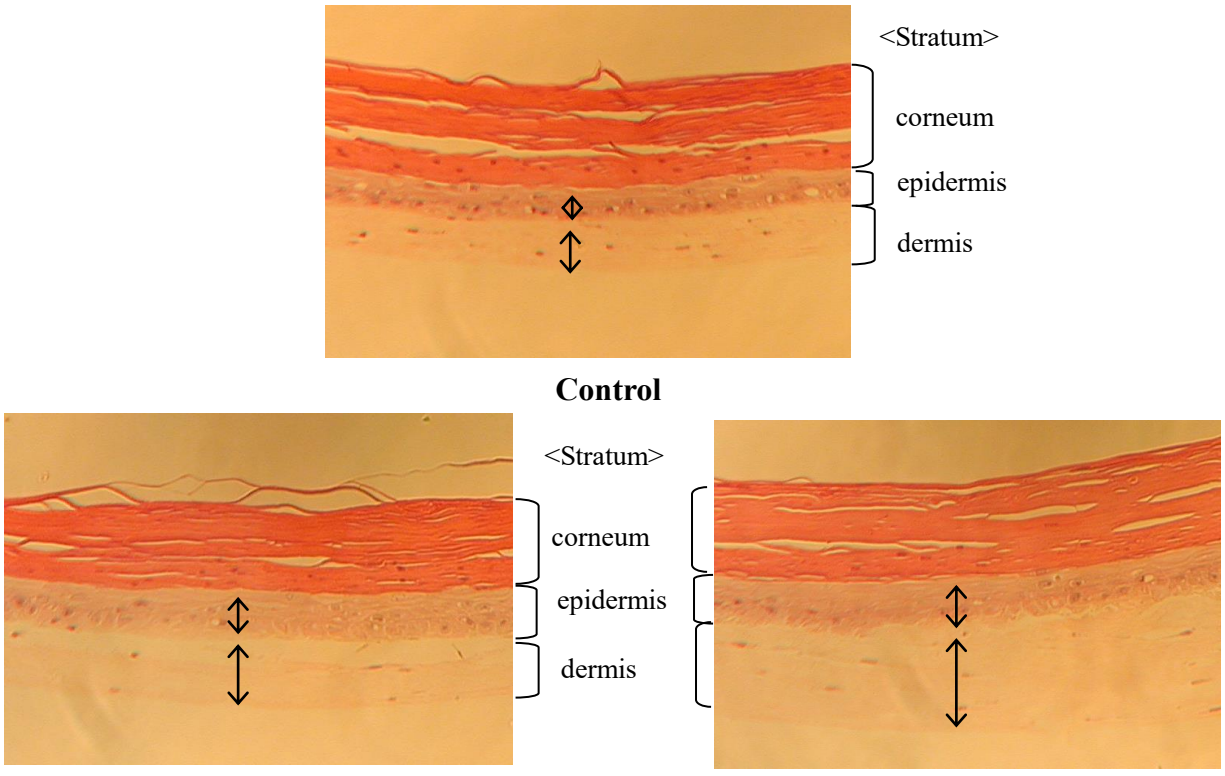


Figure 5. Microscopic Illustration of Reconstructed Skin Cell

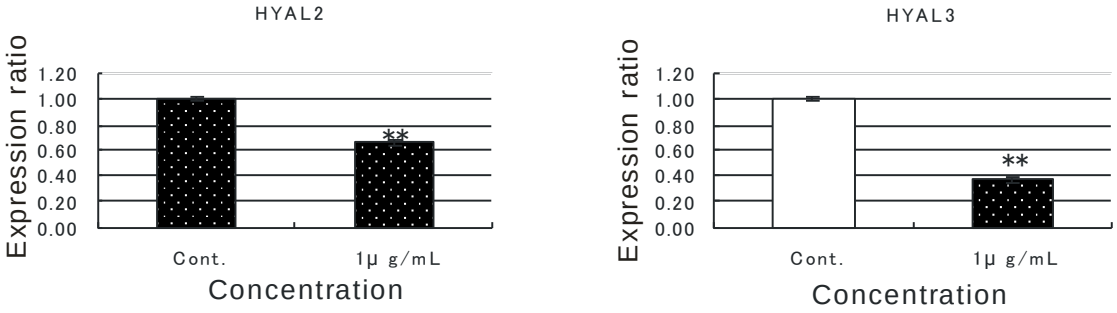
(Experimental Method)

Reconstructed Human Skin model (TESTSKIN™) was purchased from Toyobo Co., Ltd. DMSO solution of YUZU SEED EXTRACT was injected into the dermis layer of the skin model. The culture medium was replaced every 3 days while incubation continued for 6 days. The tissue specimen were stained, and then fixed in 10% formaldehyde, followed by preparation of cross-sections.

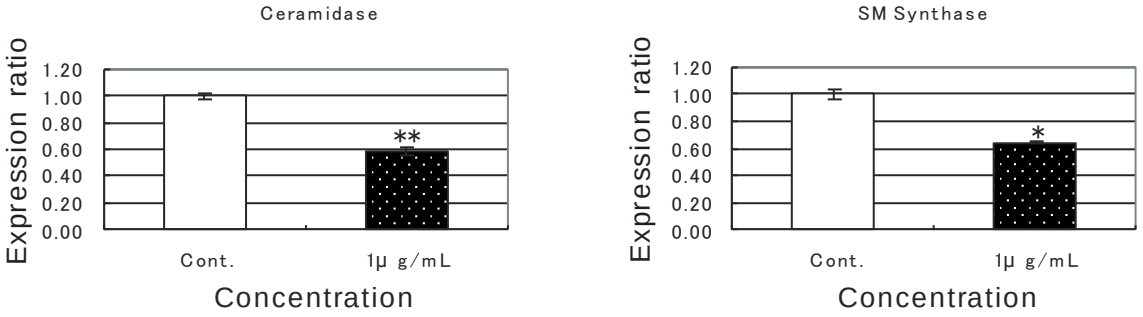
(4) mRNA expression on 3D culture of skin cells

Using 3D culture system, we evaluated the effect of YUZU SEED EXTACT on mRNA expression of several enzymes. The extract suppressed hyaluronidase 2 and 3,（HYAL2, HYAL3）, ceramidase, sphingomyelinase, and collagenase (MMP1). (Figure 6)

A：Hyaluronidase



B：Celamidase and Sphingomyelinase



C：Collagenase (MMP1)

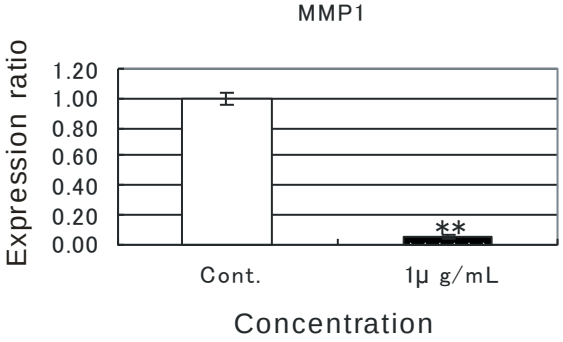


Figure 6. Effect of YUZU SEED EXTRACT on mRNA expression of lytic enzymes in 3D human skin culture system. N=4, Mean with the SE, *: $p<0.05$, **: $p<0.01$.

(Experimental method)

3D human skin cell culture system (J-TEC, Japan) was used. Cells were cultured with 1 μg/mL YUZU SEED EXTRACT for 7 days.

(5) Skin Smoothing

The effect of YUZU SEED EXTRACT on skin texture was examined using oral preparation on 8 healthy female subjects:

Number of subjects: 8

Age: 23 – 41

Dosage: 80 mg/day of YUZU SEED EXTRACT-P

Duration: 4 weeks

Parameter: Moisture of skin around region below the left eye using a moisture meter.

As illustrated in Figure 7, the skin moisture content increased from 62% to 66% with oral preparation of YUZU SEED EXTRACT. A survey was conducted on test subjects on skin texture to assess the subjective comments after the test. Survey revealed that YUZU SEED EXTRACT improved overall skin texture, as shown in Table 1. It was confirmed that skin suppleness was greatly improved with oral YUZU SEED EXTRACT at the tested dosage. Improvement on skin texture was observed and confirmed by microscopic images, as illustrated in Figure 8. YUZU SEED EXTRACT possesses excellent skin revitalizing and rejuvenating effects.

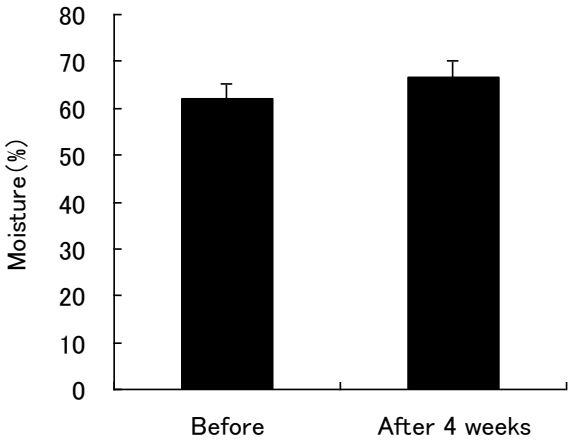
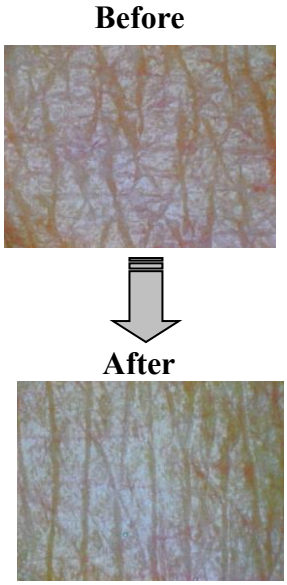


Figure 7. Changes in skin moisture with Oral Preparation of YUZU SEED EXTRACT



Subject : Female aged 24

Figure 8. Microscopic Illustration of Skin Texture after Oral Treatment of YUZU SEED EXTRACT

Table 1. Survey (Subjective) on Skin Texture after Oral Treatment of YUZU SEED EXTRACT after 4 Weeks

Item	Rate of Improvement		
Cosmetic rash	Improved : 3	No change : 5	Worsened : 0
Dryness of the face	Improved : 4	No change : 4	Worsened : 0
Flushing of the face	Improved : 2	No change : 6	Worsened : 0
Fitness in makeup	Improved : 4	No change : 4	Worsened : 0
Smoothness of the skin	Improved : 3	No change : 5	Worsened : 0
Feeling of wetness	Improved : 4	No change : 4	Worsened : 0
Skin elasticity	Improved : 2	No change : 6	Worsened : 0
Degree of dryness	Improved : 6	No change : 2	Worsened : 0
Itching	Improved : 2	No change : 6	Worsened : 0
Skin roughness	Improved : 4	No change : 4	Worsened : 0
Improvement in crease	Improved : 2	No change : 6	Worsened : 0
Dullness of the skin	Improved : 2	No change : 6	Worsened : 0

(6) Relaxation

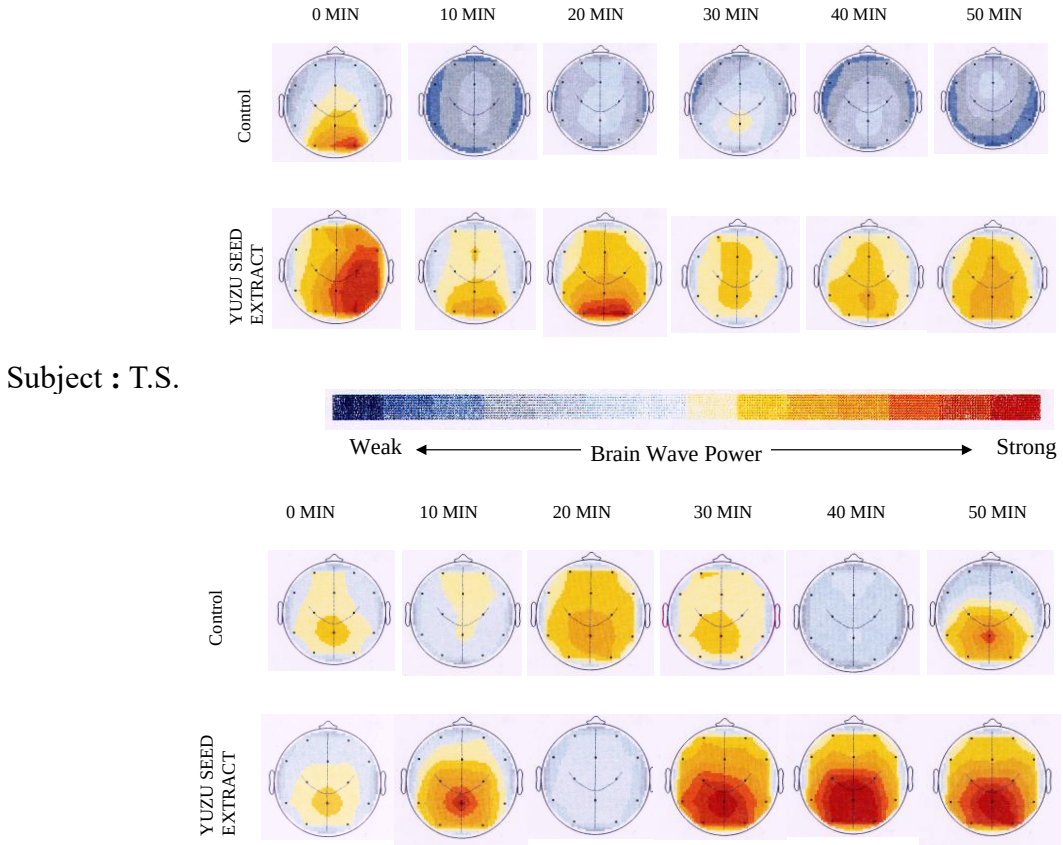
A. α-wave Propagation

Yuzu traditionally has been used for relaxation. Relaxation effects of YUZU SEED EXTRACT were assessed by observation on brain α-wave (a relaxation index) of subjects using topography.

Subjects were acclimatized in room temperature of 27℃, relative humidity of 55% for 1 hour. Tow hundred mg of YUZU SEED EXTRACT wrapped in oblate was given with 100 ml of water. Only water was given for control. After 20 minutes, stress (mental arithmetic) was loaded for 5 minutes and resting brain waves were then measured for 50 minutes.

In subject T.S., strong α-wave was observed within 50 minutes after stress loading when YUZU SEED EXTRACT was given. Similarly, marked α-wave was observed in subject M.A. from 30 to 50 minutes after stress loading when YUZU SEED EXTRACT was given. On the other hand, no α-wave was detected in the control test.

Appearance of α-wave strongly suggests that YUZU SEED EXTRACT possesses anti-stress and relaxation effects.



Subject : T.S.

Subject : M.A.

Figure 9. Brain α-Wave Topography

B. Suppression of Chromogranin A Secretion in Saliva

ChromograninA (CgA) is known to increase with stress loading, so its secretion is authorized as an index which represents mental stress. In this examination, stress (mental arithmetic) was loaded to subjects for 5 minutes, and then CgA in saliva was measured. The time-course was illustrated in Figure 10. In subject T.S., increasing amount of CgA was observed 0 to 50 minutes after stress loading. In subject M.A., sheering amount of CgA was observed 20 to 50 minutes after stress loading. On the other hand, when YUZU SEED EXTRACT was given to subjects, no increase in the amount of CgA was observed, making drastic contrast with and without YUZU SEED EXTRACT. This examination demonstrated that YUZU SEED EXTRACT has an anti -stress functionality.

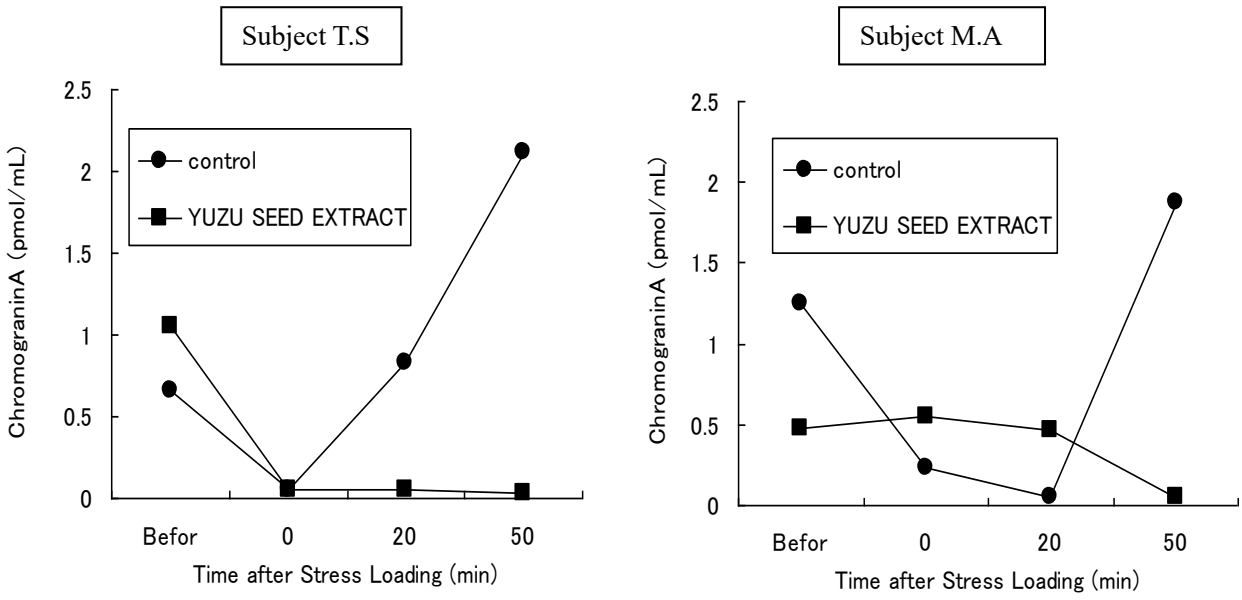


Figure 10. Suppression of ChromograninA Secretion in Saliva

(7) Improvement of Blood Circulation

The effect of YUZU SEED EXTRACT on blood circulation was examined in a single-dose oral administration clinical trial using thermograph.

Subjects were acclimatized to room temperature of 25ºC, relative humidity of 50%. Subjects were given 100 ml of water, 2 to 3 hours after breakfast. The upper and lower limbs of subjects were immersed into cold water of 14ºC for 1 minute. Changes in skin temperature were determined using thermographic illustration for 30 minutes immediately after immersion.

Two hours after lunch, 200 mg of YUZU SEED EXTRACT wrapped in oblate was given to subjects with 100 ml of water and their limbs were immersed into cold water of 14ºC for 1 minute. Changes in skin temperature were determined using thermographic illustration for 30 minutes immediately after immersion.

The thermographs clearly showed the improvement of blood circulation of subjects, as illustrated in Figure 11. Temperature of limbs recovered faster when YUZU SEED EXTRACT was given.

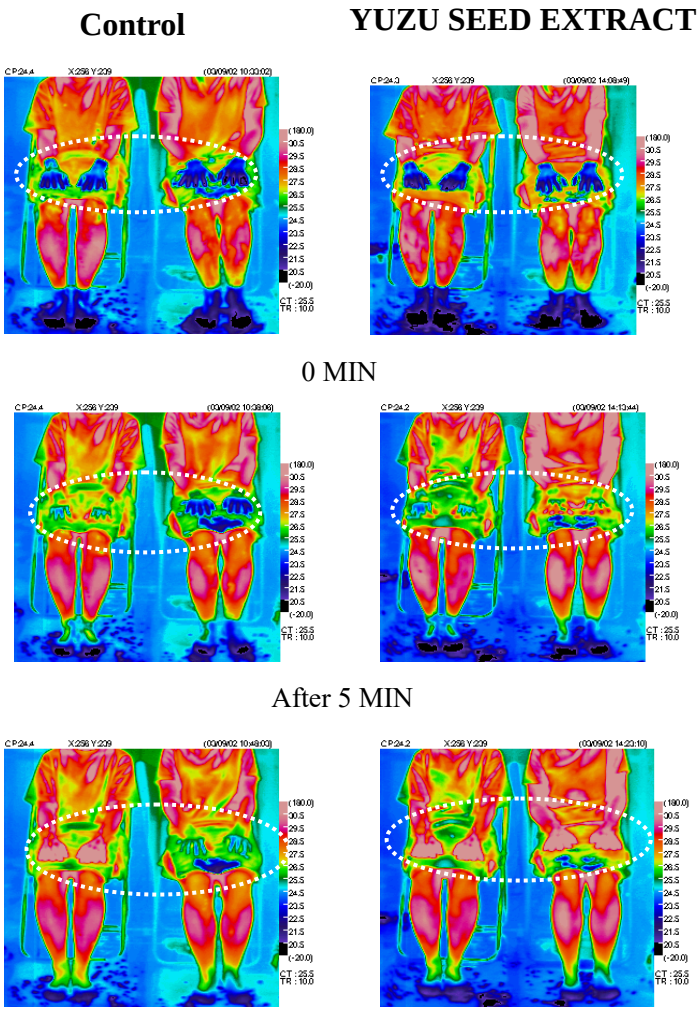


Figure 11. Thermographs Taken after Low-Temperature Loading

(8) Effect on Lipid Parameters on High Fat Diet-fed Rats (Joint Study with Nagasaki Prefectural University of Siebold)

Rats were fed high fat and cholesterol diet and lipid parameters were determined. As a result, YUZU SEED EXTRACT suppressed body weight gain and fat amount. (Figures 12, Table 2).

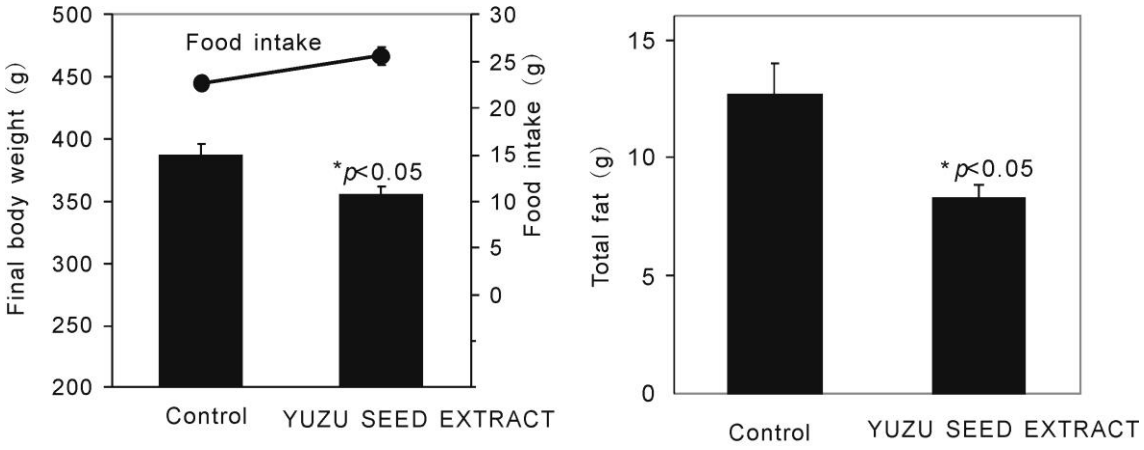


Figure 12. Effect of YUZU SEED EXTRACT on body weight and total fat in rats.
(n=6, mean with the SE)

YUZU SEED EXTRACT enhanced serum HDL-cholesterol and suppressed serum and hepatic triglyceride (Fig. 13, Tables 3, 4).

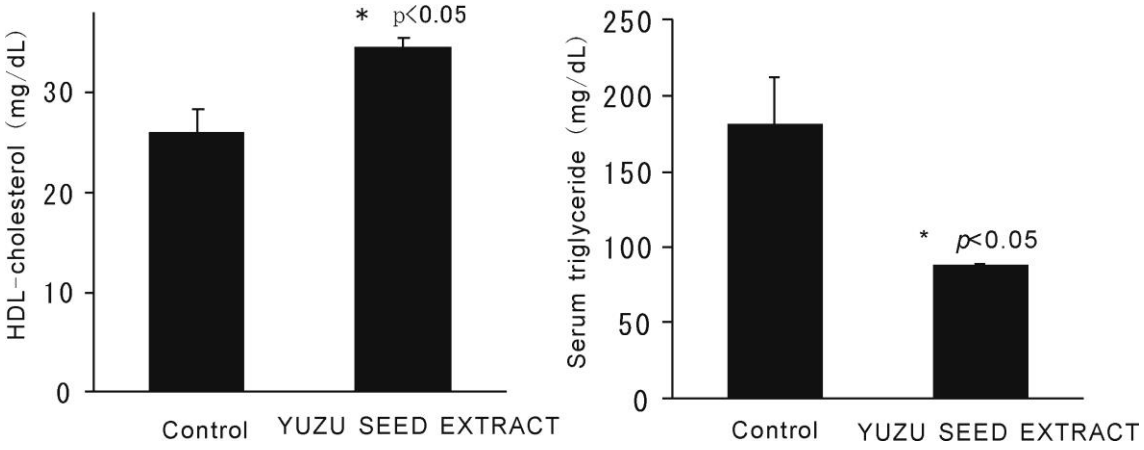


Figure 13. Effect of YUZU SEED EXTRACT on sereum HDL-cholesterol and triglyceride in rats.
(n=6, mean with the SE)

In addition, activity of hepatic metabolizing enzymes were evaluated. YUZU seed extract suppressed the activity of fatty acid synthase and activated malate dehydrogenalse and carnitine palmitoyltransferase (Figure 14, Table 5).

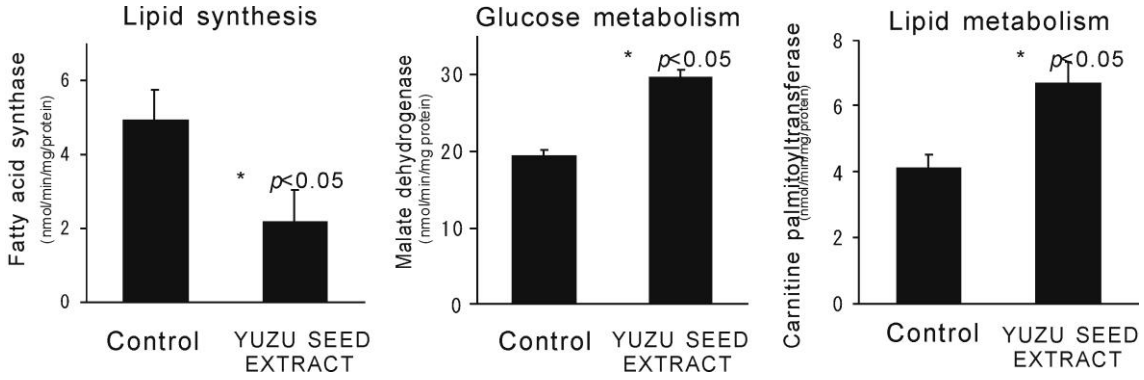


Figure 14. Effect of YUZU SEED EXTRACT on hepatic enzyme activities in rats. (n=6, mean with the SE)

Table 2. Parameters

	Control	YUZU SEED EXTRAC
Initial body weight (g)	143±2	142±2
Final body weight (g)	386±10	355±7*
Increase in body weight (g)	244±9	213±7*
Food intake (g)	22.7±0.5	25.6±1.0*
Food efficacy	0.40±0.01	0.31±0.02*
Liver (g)	24.5±1.0	23.8±0.4
(g/100 g body weight)	6.33±0.13	6.71±0.16
Total fat(g)	12.65±1.31	8.27±0.55*
(g/100 g body weight)	3.23±0.26	2.37±0.16*

Mean±S.E. (n=6), * $p < 0.05$.

Table 3. Lipid parameters

	Control	YUZU SEED EXTRAC
Total cholesterol (mg/dL)	168±14	152±15
HDL-cholesterol (mg/dL)	25.8±2.5	34.4±1.0*
HDL/Total cholesterol (%)	15.9±2.1	23.9±2.5*
Triglyceride (mg/dL)	180±32	87.0±2.3*
Phospholipid (mg/dL)	184±13	155±4*
Lipoxygenase (nmol/mL)	15.6±1.0	11.2±0.7*
SOD (%)	16.5±0.9	15.4±0.6

Mean±S.E. (n=6), * $p < 0.05$.

Table 4. Hepatic lipid (mg/g)

	Control	YUZU SEED EXTRAC
Cholesterol	69.1±2.4	73.4±1.5
Triglyceride	75.6±6.7	50.4±5.1*
Phosphlipid	27.7±0.5	36.1±1.3*

Mean±S.E. (n=6), * $p < 0.05$.

Table 5. Activity of metabolizing enzymes (nmol/min/mg protein)

	Control	YUZU SEED EXTRAC
Fatty acid synthase	4.88±0.84	2.14±0.90*
Glucose 6-phosphate dehydrogenase	17.2±2.2	18.5±1.3
Malate dehydrogenase	19.3±0.84	29.7±0.93*
PAP	4.46±0.19	5.13±0.18
Carnitine palmitoyldehydrogenase (CPT)	4.12±0.37	6.68±0.62*

Mean±S.E. (n=6), * $p < 0.05$.

(Experimental method)
AIN-93 diet containing 5% YUZUSEED EXTRACT, 0.5% cholesterol and 0.125% sodium cholate was fed tl SD rats for 4 weeks.

4. Stability

(1) Thermostability

The thermostability of YUZU SEED EXTRACT was examined. The limonin content of YUZU SEED EXTRACT remain stable after heating at either 100 and 120℃ for 1 hour. YUZU SEED EXTRACT is highly stable at food processing temperatures.

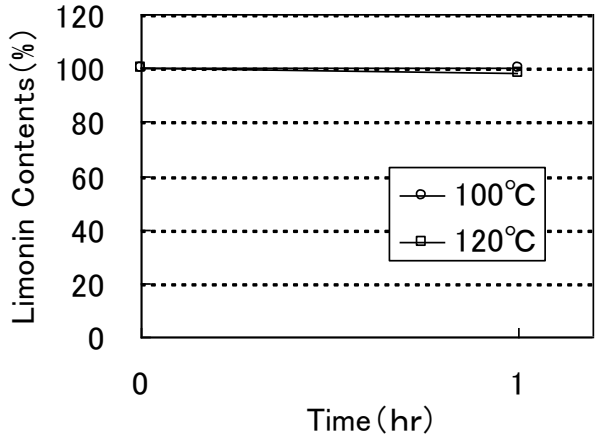


Figure15. Thermal Resistance of YUZU SEED EXTRACT (100% of The Initial Value)

(2) pH Stability

The effect of pH on aqueous solutions of YUZU SEED EXTRACT was examined at room temperature for 1 week. The content of limonin remained totally. YUZU SEED EXTRACT is highly stable at pH of 3 - 10.

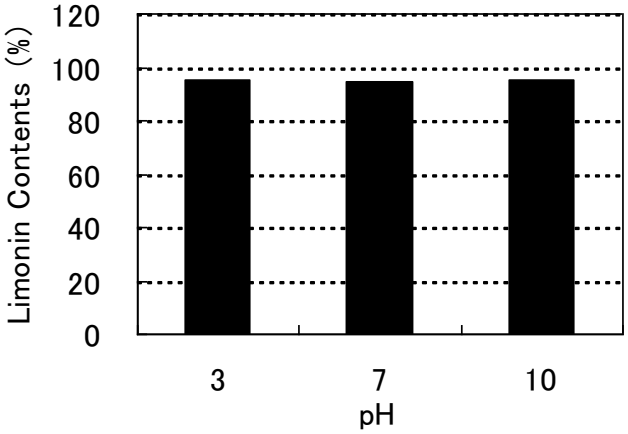


Figure16. pH Stability (100% of The Initial Value)

(3) Stability of Liquid Formulation

Water-soluble YUZU SEED EXTRACT (product name: YUZU SEED EXTRACT-WSP), was prepared in 0.7% aqueous solution (pH 3.5), and stored either at room temperature, 40°C or 5°C for 4 weeks under light protection. Neither sedimentation nor colour changes were observed visually. YUZU SEED EXTRACT -WSP is highly stable in acidic conditions.

5. Nutritional Information

	-P	-WSP	Method
Water	0.52 g	0.13 g	heat drying method under ordinal pressure
Protein*1	3.76 g	0.94 g	kieldahl method
Fat	1.64 g	0.41 g	acid fat dissolution method
Ash	2.28 g	0.57 g	direct ashing method
Carbohydrate*2	91.8 g	97.5 g	
Energy*3	397 kcal	398 kcal	
Dietary Fiber	0.1 g	0.0 g	prosky method
Sodium	5.2 mg	1.3 mg	atomic absorption spectrophotometory

*1） N=6.25
*2） 100 – (moisture + protein + fat + ash)
*3） Factors for calculating the energy value：protein, 4; fat, 9; carbohydrate, 4; dietary fiber, 2
Test trustee：SRL, Inc.
Date of issue of the test result report：September 2, 2004
Research result issue number：No. 200408200016

6. Safety Profile (in non-excipient form)

(1) Residual Agricultural Chemicals

Assayed Items	Results	Detection Limits	Assay Method
Ditianon	Not Detected	0.5 ppm	gas chromatography

Test trustee：Kyusai analysis institute Co., LTD.
Date of issue of the test result report：April 17, 2004
Research result issue number：No. 20040902-32

(2) Acute toxicity（LD_{50}）

According to the Guidelines for Single-Dose Toxicity Tests of Pharmaceutical Products, YUZU SEED EXTRACT (in non-excipient form) was orally administered to

male and female mice of the ICR strain (5 weeks old) at a dose of 5,000 mg/kg under a fasting condition, and then they were kept and observed for 14 days. Neither fatality, abnormalities in body weight gain compared to control group, nor macroscopic examinations of the organs in autopsy was observed. Thus, the LD_{50} value (p.o.) of YUZU SEED EXTRACT was estimated to be over 5,000 mg/kg in both male and female mice.

(3) Eye irrelative test

By using animal alternative method, BCOP method, we examined eye irrelativeness of YUZU SEED EXTRACT-PC. The extract was found to be mild eye irritant.

(4) Repeated insult patch test (RIPT)

RIPY of YUZU SEED EXTRACT-PC in 30 healthy mail and female subjects. No toxicity, irritation and allergic reactions were observed. The decision was “very good”.

(5) Mutagenicity (Ames test)

By using Salmonellas (TA1535, TA1537, TA98, TA100) and *E. Coli*（WP2uvrA), Ames study of YUZU SEED EXTRACT-PC was performed. No mutagenicity was observed at 33 to 5,000 μg/plate.

7. Recommended Daily Dosage

Recommend daily dosage of YUZU SEED EXTRACT is 200 – 300 mg. The Ministry of Health, Labour and Welfare approved the products of YUZU SEED EXTRACT as foodstuffs.

8. Applications

Applications	Examples
Foods	drinks (beverage, juice, *etc*.), soft gel capsule, hard capsule, tablet, candy, chewing gum, cookies, chocolate, jelly, *etc*.
Cosmetics	base cosmetics (lotion, milk, cream, *etc*.) body cosmetics (body lotion, body cream, *etc*.) cleansing cosmetics (soap, *etc*.) makeup cosmetics (lipstick, foundation, *etc*.) *etc*.

YUZU SEED EXTRACT-WSP is highly soluble in water, so it is suitable for wide range of water-soluble preparation in food and cosmetic industries, for example, beverages or conditioners and lotions.

9. Packaging

YUZU SEED EXTRACT – P (Powder, for food)

YUZU SEED EXTRACT – WSP（Powder, water-soluble, for food）
5kg Interior packaging：aluminum-coated plastic bag
Exterior packaging：cardboard box
YUZU SEED EXTRACT – PC（Powder, for Cosmetic）
YUZU SEED EXTRACT – WSPC（Powder, for Cosmetic）
5kg Interior packaging：aluminum-coated plastic bag
Exterior packaging：cardboard box
YUZU SEED EXTRACT – LC（liquid, for Cosmetic）
5kg Interior packaging：cubic polyethylene container
Exterior packaging：cardboard box

10. Storage

Store in cool and dry place. Avoid direct sunlight and humidity.

11. Expression

YUZU SEED EXTRACT-P
YUZU SEED EXTRACT -WSP
Expression: YUZU SEED EXTRACT
YUZU EXTRACT
YUZU SEED OIL
Expression: Yuzu Seed Oil

YUZU SEED EXTRACT-PC
YUZU SEED EXTRACT -WSPC
INCI name: Dextrin
Citrus Junos Seed Extract
YUZU SEED EXTRACT-LC
INCI name: Butylene Glycol
Water
Citrus Junos Seed Extract
YUZU SEED OIL
INCI name: *Citrus Junos Seed Oil*

PRODUCT STANDARD

PRODUCT NAME

YUZU SEED EXTRACT-P

(FOOD)

This product is extracted with aqueous ethanol from Yuzu seed, the seeds of *Citrus junos* (Rutaceae). It gcontains minimum 0.5% limonin.

Appearance	Slightly yellow-brown powder with slight unique smell	
Limonin	Min. 0.5%	(HPLC)

Certificaion Test

Nomilin: After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to nomilin, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed according to the following conditions for 5 μl of test solution and standard solution. The peak of nomilin is found in the HPLC chromatogram of test solution.

Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists,1g,105℃,2h)

Purity Test

(1)Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)
(2)Arsenic (as AS_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1 $\times 10^3$ cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Yuzu Seed Extract	40 %
Maltodextrin	60 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

YUZU SEED EXTRACT-WSP

(FOOD)

This product is extracted with aqueous ethanol from Yuzu seed, the seed of *Citrus junos,* (Rutaceae). This product is water-soluble.

Appearance	White or slightly yellow-brown powder with slight unique smell

Certificaion Test

Limonin: After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to limonin, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed accor-ding to the following conditions for 5 μl of test solution and standard solution. The peak of limonin is found in the HPLC chro-matogram of test solution.

Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists,1g,105℃,2h)

Purity Test

(1)Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Dextrin	90 %
Yuzu Seed Extract	10 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

YUZU SEED EXTRACT-PC

(COSMETIC)

This product is extracted with aqueous ethanol from Yuzu seed, the seeds of *Citrus junos* (Rutaceae). It contains minimum 0.5% limonin.

Appearance	Slightly yellow-brown powder with slight unique smell	
Limonin	Min. 0.5%	(HPLC)
Certificaion Test		
Nomilin	After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to limonin, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed accor-ding to the following conditions for 5 μl of test solution and standard solution. The peak of limonin is found in the HPLC chromatogram of test solution.	
Loss on Drying	Max. 10.0 %	(1g,105℃,2h)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max.1ppm	(The Third Method Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

9.Composition

Ingredients	Contents
Dextrin	60 %
Citrus Junos Seed Extract	40 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

YUZU SEED EXTRACT-WSPC

(COSMETIC)

This product is extracted with aqueous ethanol from Yuzu seed, the seeds of *Citrus junos* (Rutaceae). This product is water-soluble.

Appearance: White or slightly yellow-brown powder with slight unique smell

Certificaion Test

Limonin: After methanol (HPLC grade) is added to 0.1 g of this product in a 10 ml volume flask, and the flask is treated with ultrasonic wave for 1 minutes. The solution is filtered through a 0.45 μm PTFE filter after addition of methanol (HPLC grade) to adequate volume. For preparation of standard solution, methanol (HPLC grade) is added to limonin, and the concentration is prepared 0.2 mg/ml (standard solution). HPLC analysis is performed accor-ding to the following conditions for 5 μl of test solution and standard solution. The peak of limonin is found in the HPLC chro-matogram of test solution.

Loss on Drying	Max. 10.0 %	(1g,105℃,2h)
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max.1ppm	(The Third Method Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

9.Composition

Ingredients	Contents
Dextrin	90 %
Citrus Junos Seed Extract	10 %
Total	100 %

PRODUCT STANDARD

PRODUCT NAME

YUZU SEED EXTRACT-LC

(COSMETIC)

This product is extracted with aqueous 1,3-butylene glycol from Yuzu seeds, the seeds of *Citrus junos* (Rutaceae).

Appearance	Slightly yellow color liquid with slight unique or no smell	
Certification Test Polyphenols	Mix this product (0.5 ml) with water (2.0 ml), and Folin-Denis reagent (0.2 ml) and saturated Na_2CO_3 solution (0.4 ml) are added. The solution reveals blue color.	
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second Method of Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(The Third Method of Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1 $\times 10^2$ cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredients	Contents
Butylene Glycol	69 %
Water	30 %
Citrus Junos Seed Extract	1 %
Total	100 %

PRODUCT STANDARD
PRODUCT NAME

YUZU SEED OIL

FOOD

This product is oil extracted and refined from the seed of *Citrus junos* (Rutaceae).

Appearance	Slight yellowish clear liquid oil with slight unique aroma.	
Acid Value	Max. 0.50	
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(Sodium Sulfide Colorimetric Method)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation, The Third Method, Apparatus B)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition	Ingredient	Content
	Yuzu Seed Oil	99.90 %
	Mix Tocopherol	0.05 %
	L-Ascorbic Acid Palmitate	0.05 %

Ref: Japan Oil Chemists` Society.

PRODUCT STANDARD
PRODUCT NAME

YUZU SEED OIL

COSMETIC

This product is oil extracted and refined from the seed of *Citrus junos* (Rutaceae).

Appearance	Slight yellowish clear liquid oil with slight unique aroma.	
Acid Value	Max. 0.50	
Purity Test		
(1)Heavy Metals (as Pb)	Max. 10 ppm	(The Second method of Japanese Standards of Quasi-Drug Ingredients)
(2)Arsenic (as As_2O_3)	Max. 1 ppm	(The Third method of Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Negative	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Citrus junos seed Oil	99.90 %
Mix Tocopherol	0.05 %
L-Ascorbic Acid Palmitate	0.05 %

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:+81-3-5209-9150　FAX:+81-3-5209-9151
E-mail: tokyo@oryza.co.jp

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