Oryza

ORYZA OIL & FAT CHEMICAL CO., LTD.

TOMATO SEED EXTRACT

Extracellular matrix production cycle enhancement

Skin wasted product removal

■**TOMATO SEED EXTRACT-P**
（Water-soluble powder、Food Grade）

■**TOMATO SEED EXTRACT-PC**
（Water-soluble powder、Cosmetic Grade）

ORYZA OIL & FAT CHEMICAL CO., LTD.

ver. 1.0ST

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Extracellular matrix production cycle enhancement
Skin wasted product removal

1. Introduction

Tomato（*Lycopersicon esculentum*）is commonly known as edible plant all over the world. The species originated in Andes highlands, South America (Fig. 1). Tomato fruits contains rich functional components such as vitamin C, lycopene and GABA. Because of these knowledge, tomato is used as a raw material of functional foods and juice. In terms of the function of tomato, cholesterol lowering effect and blood pressure lowering effect have been reported. Moreover, it was reported that novel saponin compound, escleoside is identified from fully ripe tomato fruits in 2004[1,2] and one of escleoside family, escleoside A has the prevention of arteriosclerosis effect in the mice[3]. However, these are knowledge about tomato fruits and there are not any functional reports about tomato seed.

Fig. 1 Tomato and its seed

Oryza Oil & Fat Chemical Co., Ltd. has found that tomato seed contains two unique saponin compounds, lycoperoside A and H as main component and developed the powder type product which standardized these components (Tomato Seed Extract). The result of functional research about Tomato Seed Extract, we found that Tomato Seed Extract and its components have collagen and elastin production promotive effect and collagen and elastin degradation products absorption promotive effect. Tomato Seed Extract contributes to anti-wrinkle and anti-sagging as a functional ingredients of processed food and cosmetics. Furthermore, Tomato Seed Extract also available for the products in hope of synergistic effect with collagen and elastin.

1) Fujiwara S. et al., *Tetrahedron*, 60, 4915-4920 (2004).
2) Ono M. et al., *Chem. Pharm. Bull*., 54, 237-239 (2006).
3) Nohara T., *J. Trad. Med*., 27, 217-224 (2010).

2. Components of tomato seed

While there are many reports about components which are included in tomato fruits, research about components included in only tomato seed was not studied. Therefore, we researched components of tomato seed and **identified two main saponin compounds, lycoperoside A and H** (Fig.2). This research was co-worked with Kyoto Pharmaceutical University. It is novel knowledge that lycoperoside A and H are included in tomato seed as main components (Fig. 3). Moreover, These compounds do not have cell toxicity that observed in many kind of saponin compounds.

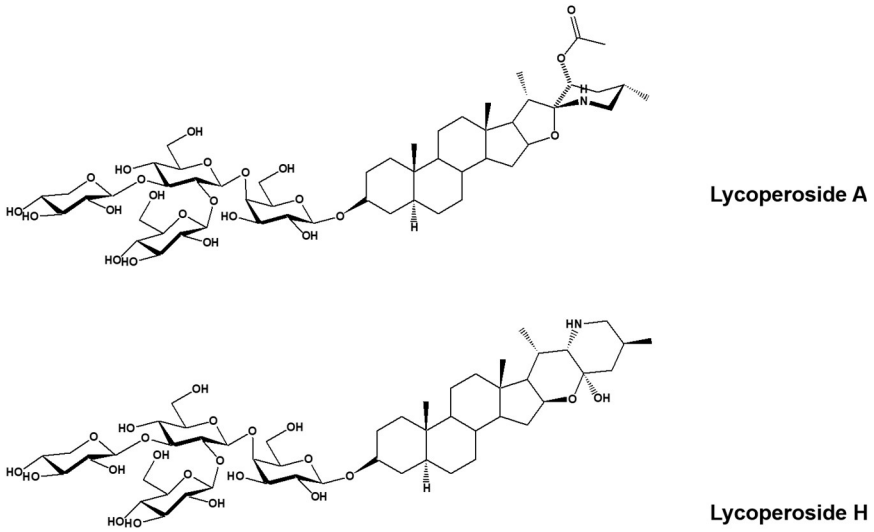

Fig. 2 Chemical structure of lycoperoside A and H

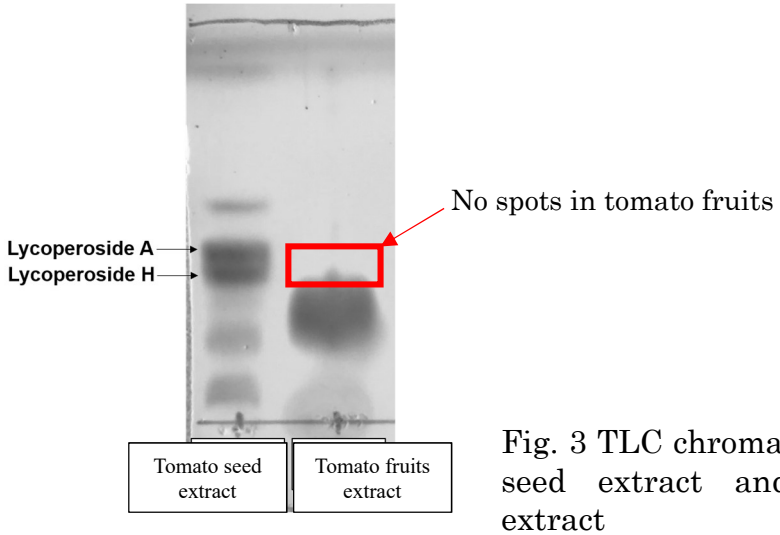

Fig. 3 TLC chromatogram of tomato seed extract and tomato fruits extract

3. Effect of Tomato Seed Extract on extracellular matrix

(1) Extracellular matrix and skin condition

Extracellular matrix (ECM) is located in dermis layer in skin and contributes to skin elasticity. ECM is consisted with collagen and elastin. Collagen and elastin are produced by fibroblast. After production, collagen and elastin are broken down and absorbed into fibroblast again (Fig. 4)[4,5]. We named this production and reduction cycle **"ECM Recycle System (ERS)"**. Skin condition is maintained freshly by ERS. Therefore, disorder of ERS causes change of cell shape and oxidative stress by accumulation of degradation products[6]. Accordingly, **it is very important maintain ERS normally to keep skin condition freshly.**

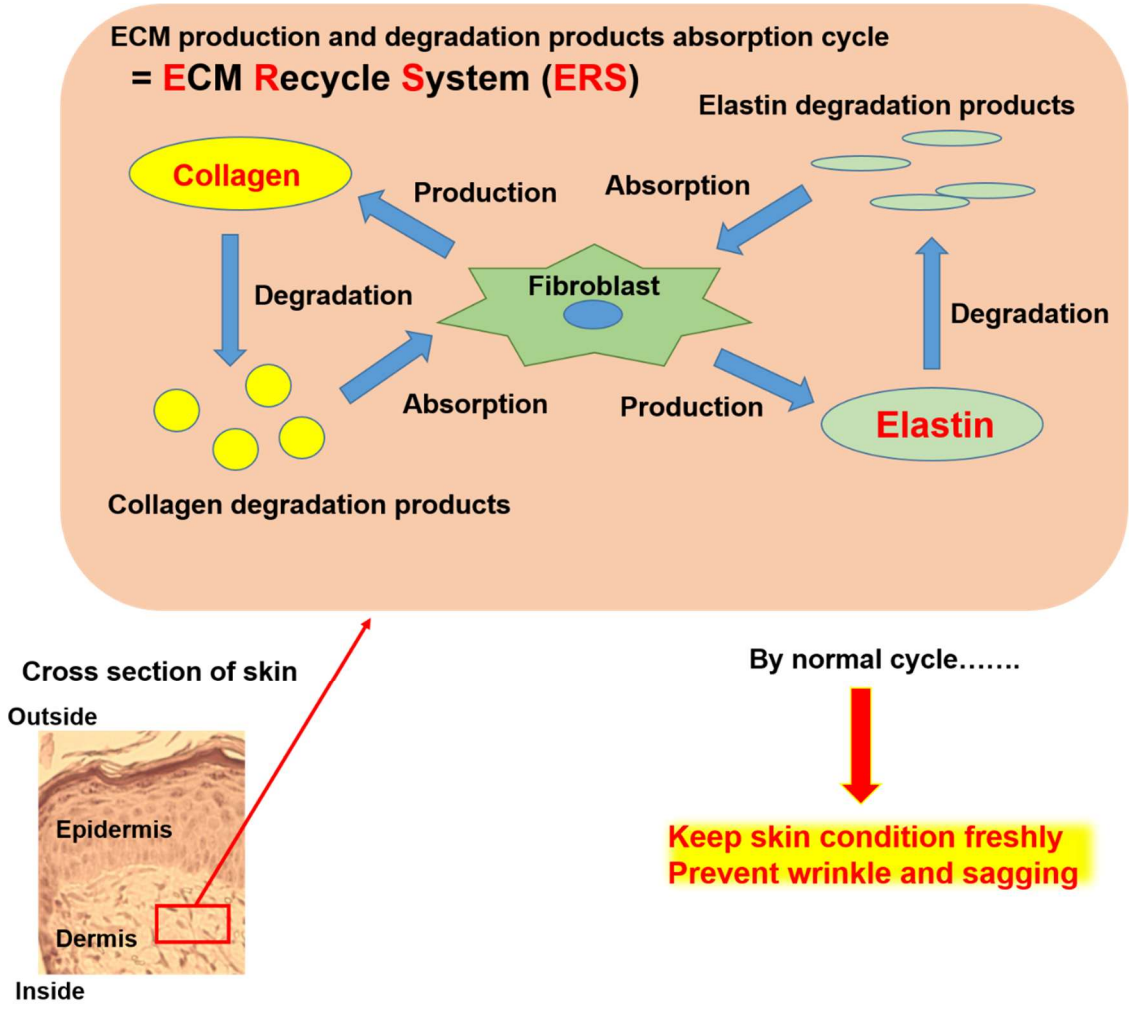

Fig. 4 ECM Recycle System (ERS) and its role in skin

4) Lars HE. Et al., *Front. Biosci*., 14, 2103-2114 (2009).
5) Amandine S. et al., *Front. Fharmacol*., 7, 1-10 (2016).
6) Fisher GJ. et al., *Am. J. Pathol.*, 174, 101-114 (2009).

(2) Effect of Tomato Seed Extract on collagen and elastin production

There are not any reports about function of lycoperoside A and H which are main components of Tomato Seed Extract. While many saponin compounds have cell toxicity, it is reported that some saponin compounds which do not have cell toxicity shows collagen production promote effect[7]. Therefore, we evaluated the effect of Tomato Seed Extract and lycoperoside on collagen production by fibroblast.

Tomato Seed Extract (1, 3, and 10 μg/mL), lycoperoside A or H (0.1, 0.3, and 1 μg/mL) were added to human skin fibroblast (TIG-103) and after cultivated 1 day, amount of collagen in cell were determined. As a result, Tomato seed Extract (3 μg/mL) and lycoperoside A (0.3 and 1 μg/mL) significantly increased amount of collagen production (Fig. 5).

From above result, we evaluated the effect on production of elastin which is main constitution factor of ECM same as collagen. Tomato Seed Extract (1, 3, and 10 μg/mL), lycoperoside A or H (1, 3, and 10 μM) were added to TIG-103 and after cultivated 1 day, amount of elastin in cell were determined. As a result, Tomato Seed Extract (10 μg/mL), lycoperoside A (3 and 10 μM) and lycoperoside H (1 μM) significantly increased amount of elastin production (Fig. 6). From these result, it is revealed that **Tomato Seed Extract and its main components, lycoperoside A increase collagen and elastin in skin fibroblast**.

7) Morikawa T. et al., *Phytochemistry,* 116, 203-212 (2015).

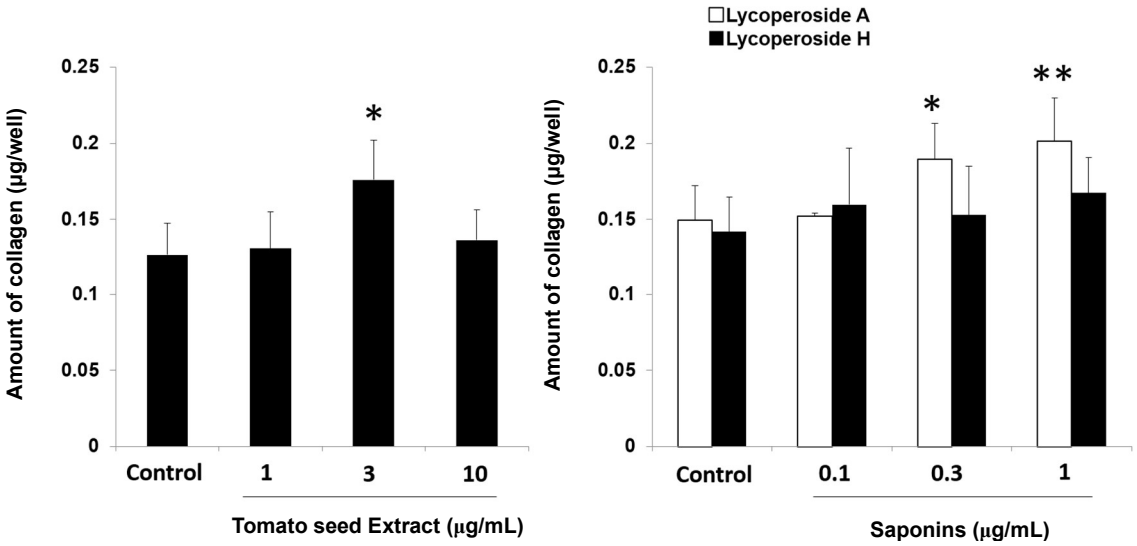

Fig. 5 Effect of Tomato Seed Extract and saponins on collagen production
n = 4, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

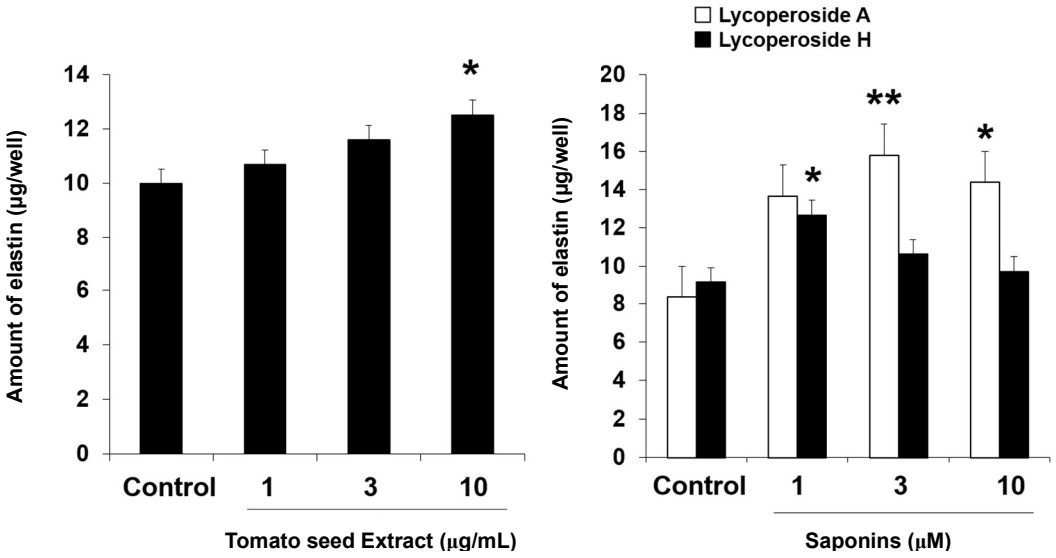

Fig. 6 Effect of Tomato Seed Extract and saponins on elastin production
n = 6, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control

（3） Effect on gene expression involved in collagen and elastin production/absorption cycle

As described above, ECM is maintained freshly by collagen and elastin production/absorption cycle. This cycle is regulated by several factors which involved in collagen and elastin production and its degradation products absorption. As shown in Fig. 7, collagen is produced by synthetic signal emitted from ① Smad and collagen degradation product is absorbed into fibroblast by ②Endo180. On the other hand, elastin is formulated by cohesion of ③ Fibulin and ④ Neuraminidase-1 is involved in absorption of elastin degradation products. Therefore, we evaluated effect of Tomato Seed Extract on gene expression of these factors.

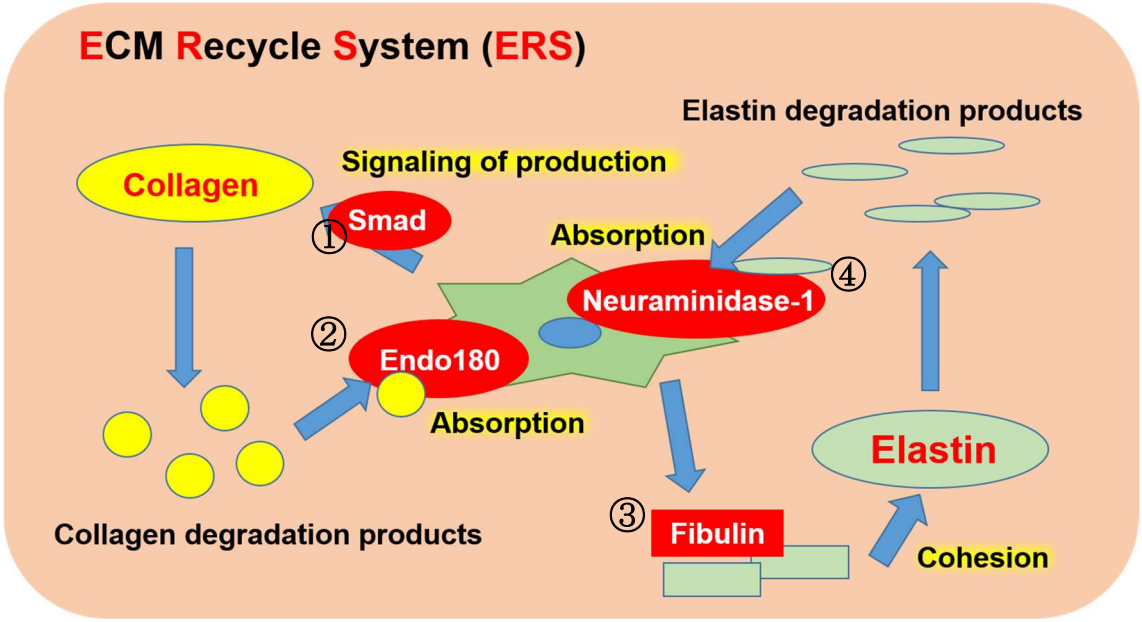

Fig. 7 Genes involved in collagen and elastin production/absorption cycle

Tomato Seed Extract (1, 3, and 10 μg/mL), lycoperoside A or H (1, 3, and 10 μM) were added to human skin fibroblast (TIG-103) and after cultivated 2 day, gene expression was determined. As a result, lycoperoside A (3 and 10 μM) significantly upregulates expression of Smad gene which involved in collagen production (Fig. 8) and Tomato Seed Extract (1 μg/mL) and lycoperoside A (3 μM) significantly upregulates expression of Endo180 gene which involved in absorption of collagen degradation products (Fig. 9).

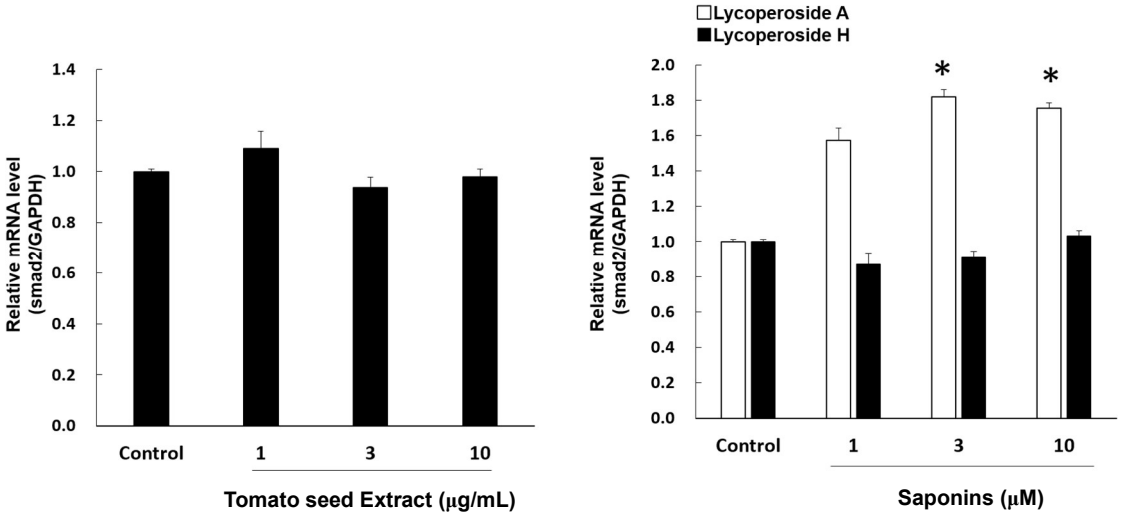

Fig. 8 Effect of Tomato Seed Extract and saponis on Smad gene expression
n = 3-6, Mean±SE, *; $P < 0.05$ vs Control

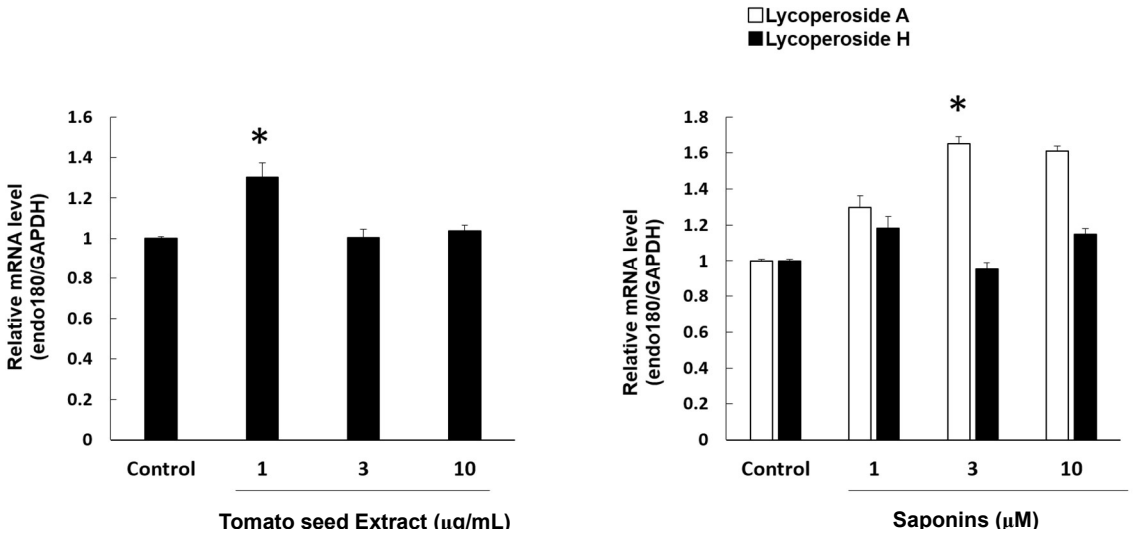

Fig. 9 Effect of Tomato Seed Extract and saponis on Endo180 gene expression
n = 4-6, Mean±SE, *; $P < 0.05$ vs Control

On the other hand, lycoperoside A (10 μM) significantly upregulates expression of Fibulin gene which involved in elastin production (Fig. 10) and lycoperoside A (3 μM) also significantly upregulates expression of Neuraminidase-1 gene which involved in absorption of elastin degradation products (Fig. 11).

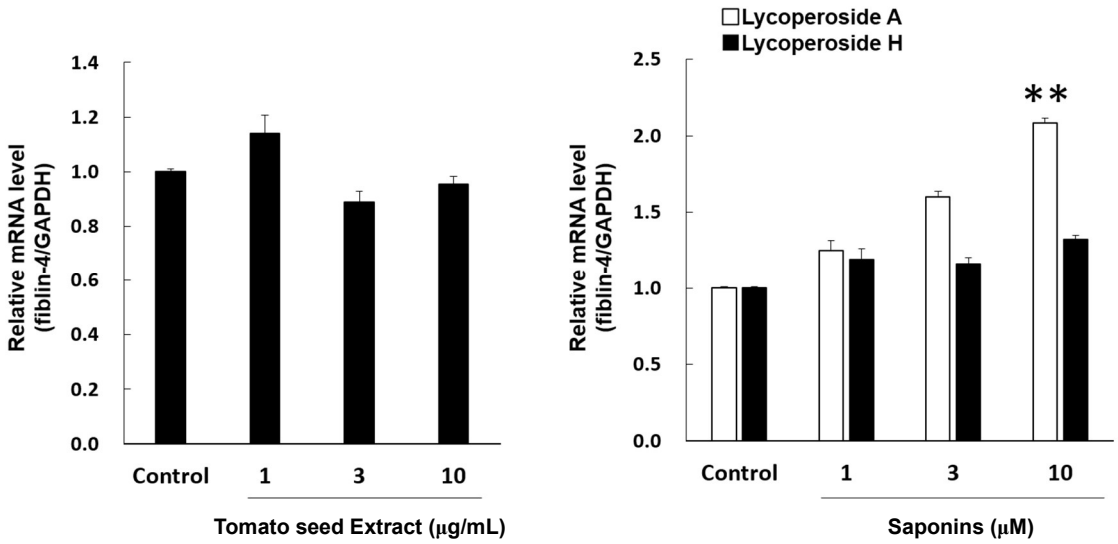

Fig. 10 Effect of Tomato Seed Extract and saponis on Fibulin gene expression
n = 5-6, Mean±SE, $^{**}P < 0.01$ vs Control

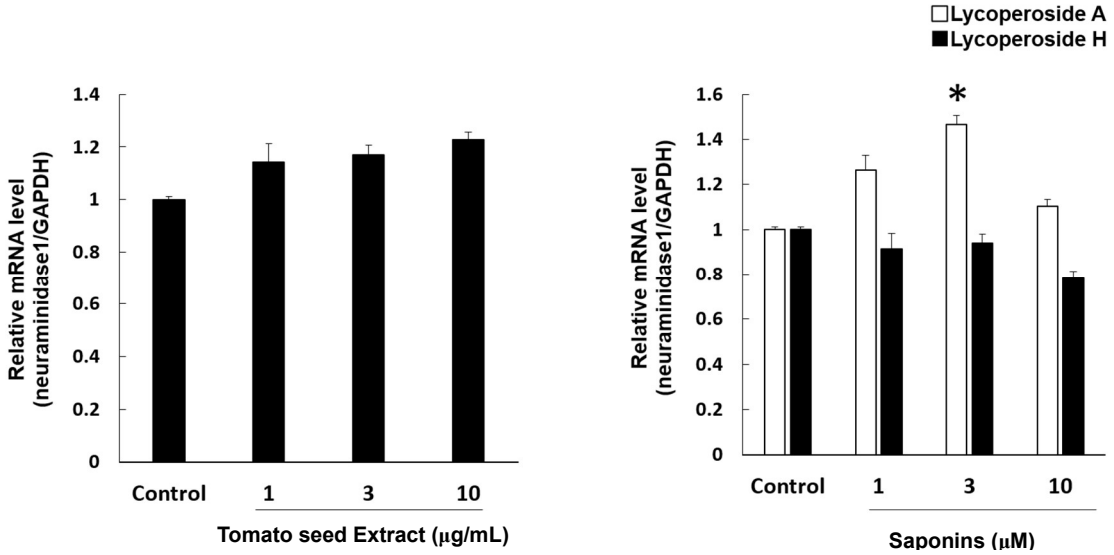

Fig. 11 Effect of Tomato Seed Extract and saponis on Neuraminidase-1 gene expression
n = 4-6, Mean±SE, $^{*}P < 0.05$ vs Control

From these results, **lycoperoside A is involved in collagen and elastin production promotive effect of Tomato Seed Extract**. Moreover, **Tomato Seed Extract significantly upregulated Endo180 gene and shows the tendency of upregulation of Neuraminidase-1 gene expression**. Lycoperoside A is also involved in this effect.

(4) Degradation products absorption enhancement effect on formulated ECM

From the results described above, it is suggested that Tomato Seed extract and its main components have the effect of ERS cycle enhancements. Accordingly, we evaluated the effect on cell sheet which formulates ECM. In usual cell test, cells do not adhere with each other tightly and do not formulate ECM. By preparing the cell sheet, cells adhere tightly and formulate ECM as a skin tissue model (Fig 12).

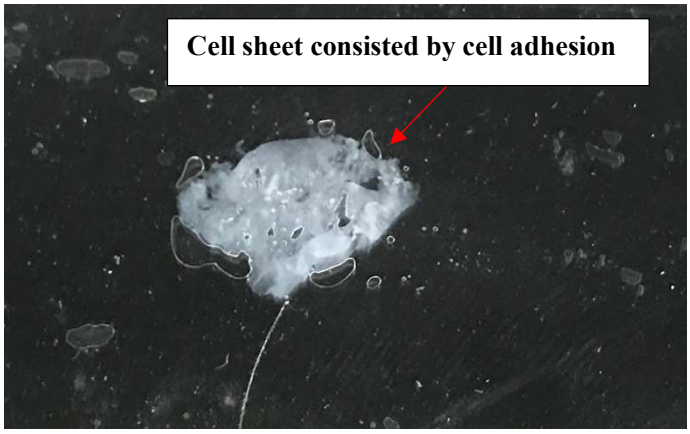

Fig. 12. Cell sheet consisted with human skin fibroblast

Firstly, H-Gly-Pro-Hyp-OH (collagen tripeptide) was added to cell sheet of human skin fibroblast (TIG-103) to duplicate accumulation of degradation products. After accumulation, Tomato Seed Extract (10 μg/mL), lycoperoside A and H (10 μM) added to cell sheet and after cultivated 1 day, degradation products were determined by immunofluorescence staining. Z stacking which is technique that series of images captured at different focus distance to create 3D image was applied to get stained degradation products in cell ECM. As a result of analysis, Amount of degradation products were removed by Tomato seed extract and saponis (Fig. 13). Namely, degradation products stained red color in Tomato Seed Extract group and lycoperoside group was decreased compere with control group. From this result, Tomato Seed Extract and lycoperosides promote absorption of degradation products in duplicate ECM.

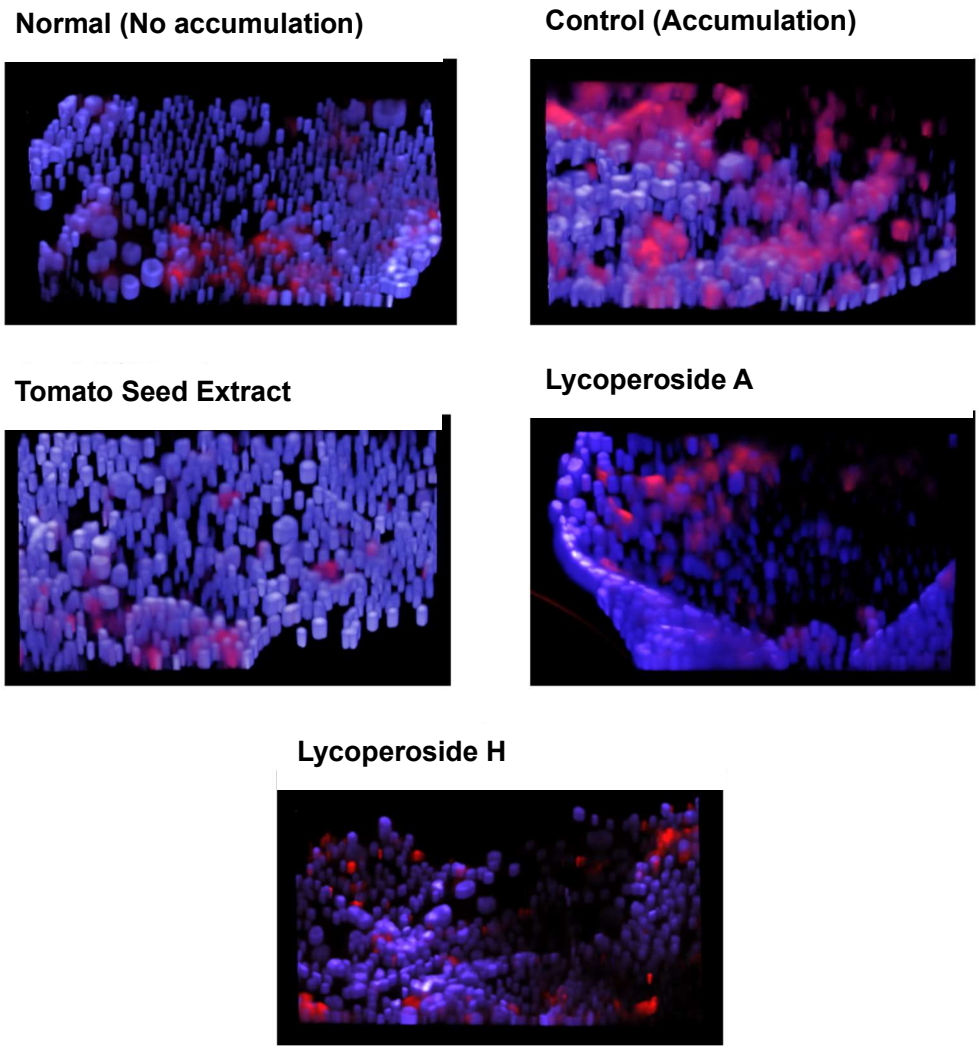

Fig. 13 Immunofluorescence staining images of ECM degradation products accumulated in cell sheet (3D images)
Red：ECM degradation products, Blue：Cell nucleus

(5) Upregulation of genes involved in absorption of degradation products on formulated ECM

In the next, we evaluated the gene expression involved in absorption of degradation products upregulation effect of Tomato Seed Extract and saponins on formulated ECM. Tomato Seed Extract (1, 3 and 10 μg/mL), lycoperoside A and H (1, 3 and 10 μM) added to cell sheet and after cultivated 1day, gene expression of Endo180 and Neuraminidase-1 were determined. As a result, gene expression of Endo180 and Neuraminidase-1 were significantly decreased by accumulation of degradation products. However, Tomato Seed Extract (10 μg/mL), lycoperoside A (1 and 3 μM) and lycoperoside H (1 μM) significantly recovered Endo180 expression (Fig. 14). Similarly, Tomato Seed Extract (10 μg/mL), lycoperoside A (1, 3 and 10 μM) and lycoperoside H (10 μM) significantly recovered

Neuraminidase-1 expression (Fig. 15). From these results, **Tomato Seed Extract and lycoperosides also upregulates gene expression involved in absorption of degradation products in formulated ECM**.

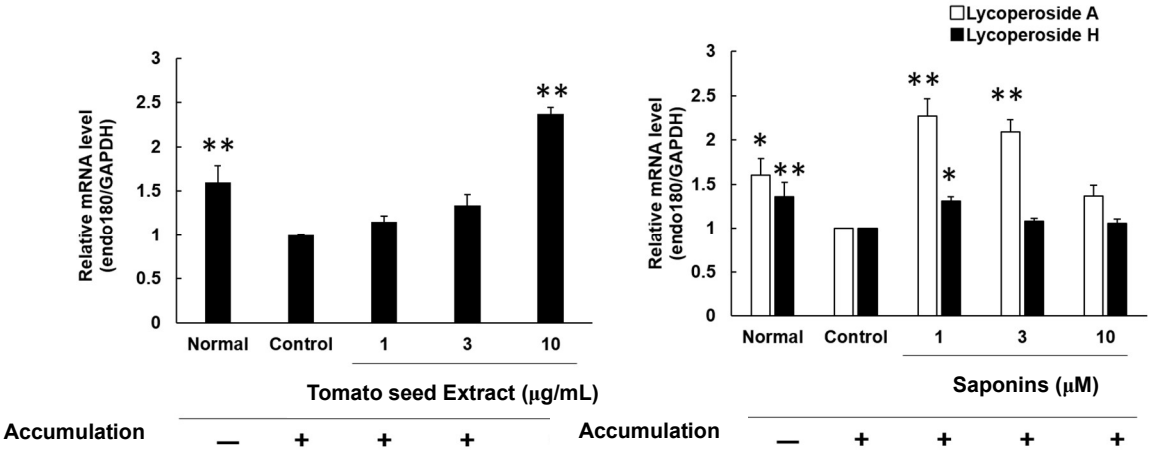

Fig. 14 Upregulation of Endo180 expression on formulated ECM
n =4-6, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control
Normal means no accumulation of ECM degradation products.
Accumulation was prepared in Control and sample group

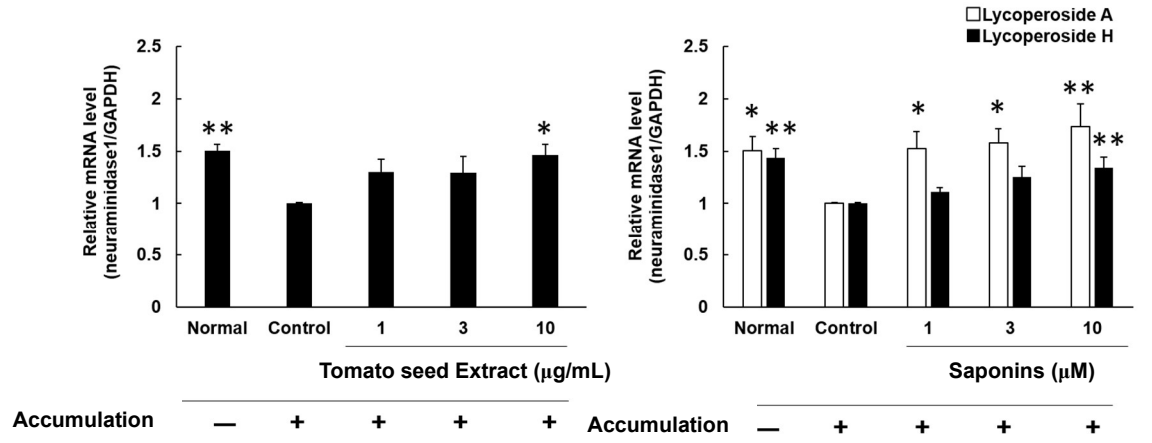

Fig. 15 Upregulation of Neuraminidase-1 expression on formulated ECM
n =4-6, Mean±SE, *; $P < 0.05$, **$P < 0.01$ vs Control
Normal means no accumulation of ECM degradation products.
Accumulation was prepared in Control and sample group

4. Effect on amount of intercellular lysosome (Wasted products removal)

As described above, it is suggested that Tomato Seed Extract and its main components, lycoperosides have collagen and elastin production/absorption cycle enhancement effect. Therefore, we evaluated the effect on amount of LAMP-1 (Lysosomal-associated membrane protein-1) which is lysosome involved in removal of wasted products not only collagen and elastin degradation products but also other degradation products in ECM. Similarly with collagen and elastin degradation products, it has been reported that accumulation of wasted products in ECM causes skin aging and deteriorating of skin condition[6].

Tomato Seed Extract (3 μg/mL), lycoperoside A and H (10 μM) added to human skin fibroblast (TIG-103) and after cultivated 2 days, LAMP-1 were determined by immunofluorescence staining. As a result, compare with control, amount of LAMP-1 was increased in fibroblast by Tomato Seed Extract and lycoperosides (Fig. 16). In addition, the effect of lycoperoside A was especially remarkable. From this result, **Tomato Seed Extract and lycoperosides have the removal of wasted products effect in ECM by increasing amount of lysosome involved in absorption and degradation of wasted products.**

6) Tashiro K. et al., *BBRC.*, 443, 167-172 (2014).

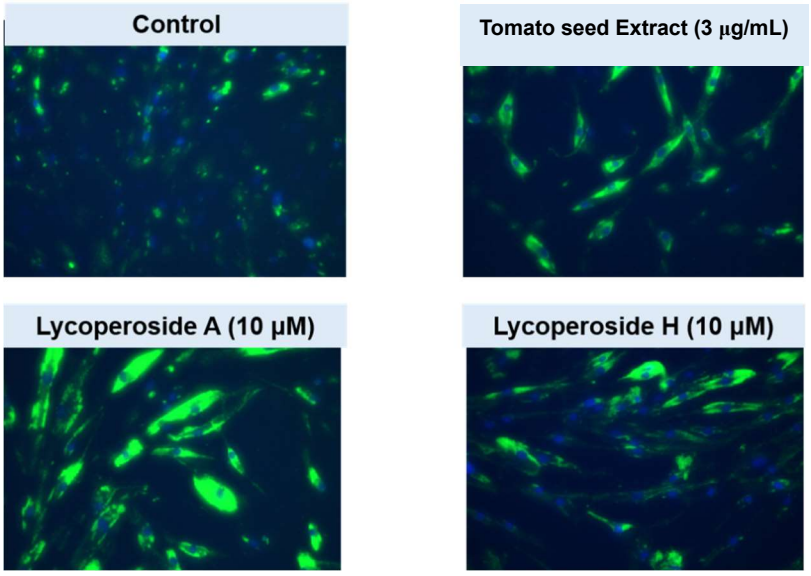

Fig. 16 Effect of Tomato Seed Extract and lycoperosides on amount of LAMP-1
Green：LAMP-1, Blue：Cell nucleus

5. Human clinical test

We conducted the human clinical test (open trial) to evaluate the effect of Tomato Seed Extract-P on skin condition. Subjects were 18 healthy adult (Age: 36.8±12.2). Female were 12 (Age: 36.7±13.6) and male were 6 (Age: 37.0±10.0) among subjects. **All subjects ingested Tomato Seed Extract –P by 200 mg/day for 4 weeks**. Collagen score and thickness of dermis in cheek and inside of upper arm were measured to evaluate the skin condition. In addition, questionnaire about skin condition was also performed. As a result, **collagen score in cheek and inside of upper arm were significantly increased** after ingestion and **thickness of dermis in inside of upper arm was also significantly increased.** Moreover, questionnaire items **"skin moisture", "Improvement of sagging", "Improvement of wrinkles" and "Elasticity" were significantly improved** after ingestion. From these results, Ingestion of Tomato Seed Extract-P contributes improvement of wrinkle and sagging by increasing amount of collagen and thickness of dermis.

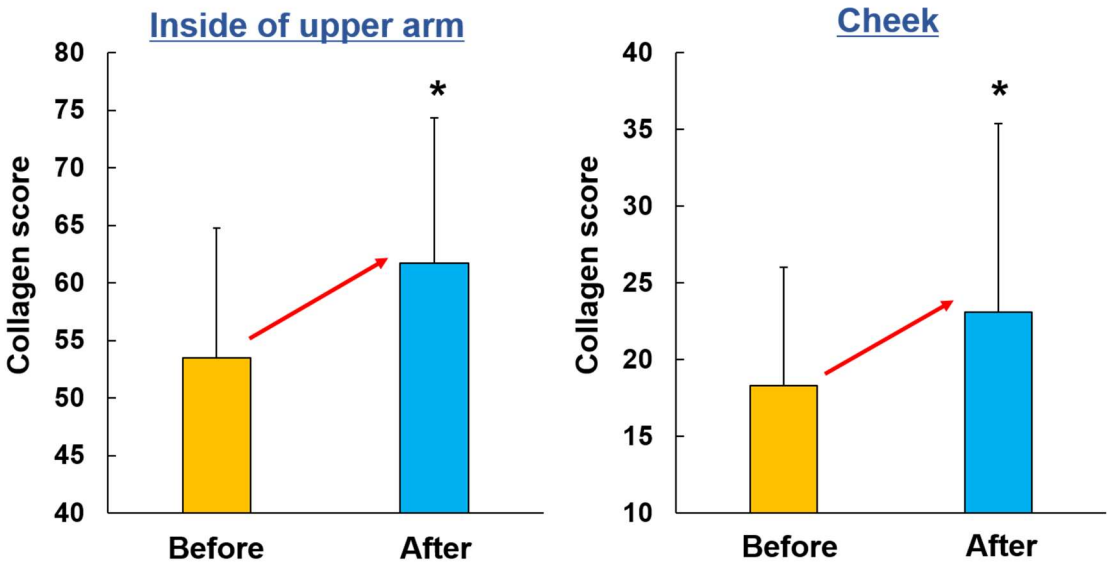

Fig. 17 Change in collagen score before and after ingestion
n = 18, Mean±SD, *; $P < 0.05$ vs before ingestion

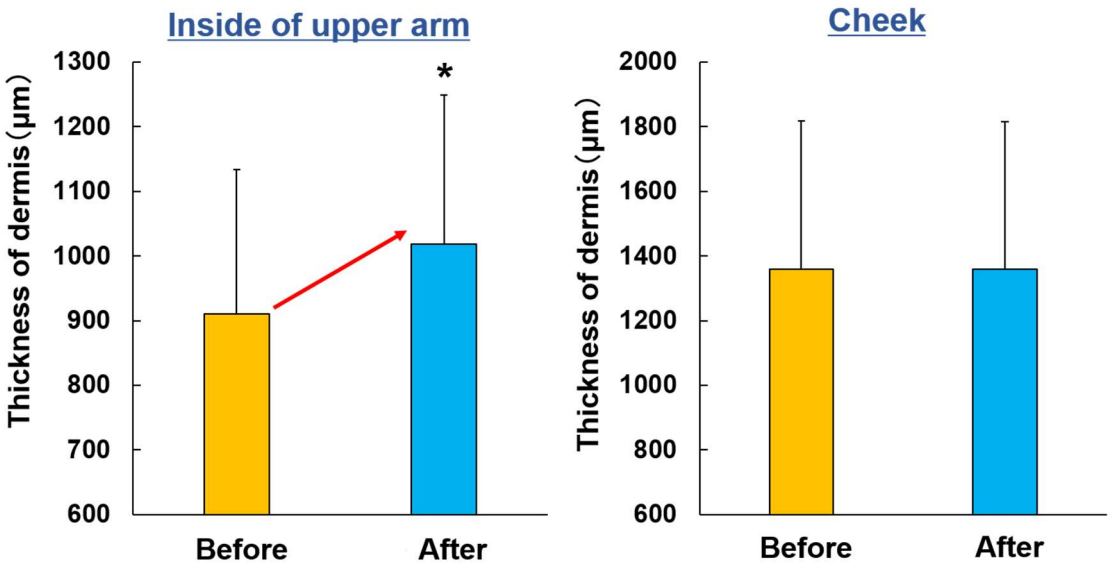

Fig. 18 Change in thickness of dermis before and after ingestion
n = 18, Mean±SD, *; $P < 0.05$ vs before ingestion

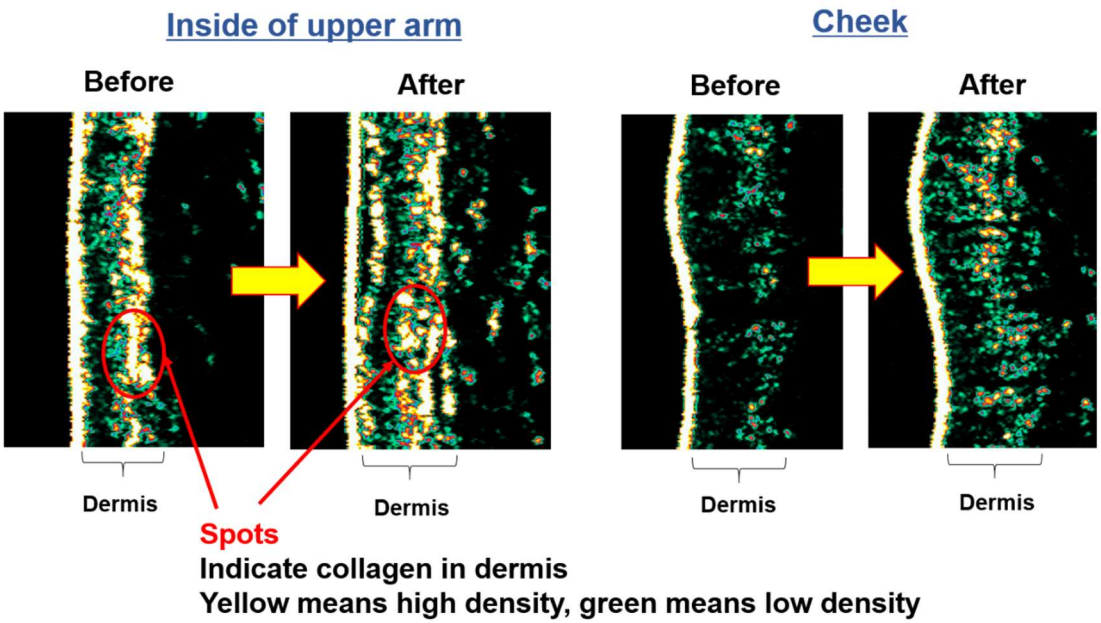

Fig. 19 Example of improvement

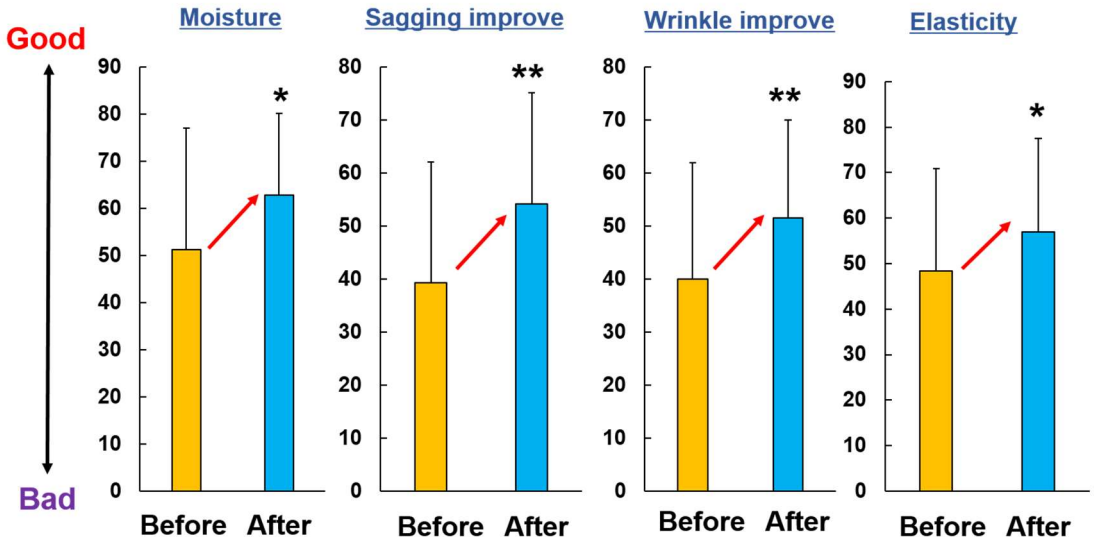

Fig. 20 Change in questionnaire before and after ingestion
n = 18, Mean±SD, *; $P < 0.05$, **; $P < 0.01$ vs before ingestion

6. Product stability of Tomato Seed Extract

(1) Heat stability

Heat stability of Tomato Seed Extract-P was examined by heating at 100℃ and 120℃ continuously for 1hour. As shown in Fig. 21, total lycoperosides contents were not decreased after heating. Therefore, Tomato Seed Extract-P is highly stable upon heating at normal food processing temperature.

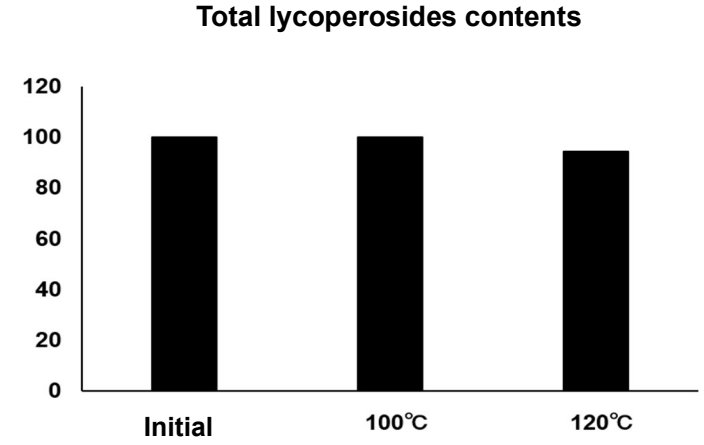

Fig. 21. Heat stability of Tomato Seed Extract-P
Heating for 1 hours at each temperature

(2) pH stability

The pH stability of Tomato Seed Extract-P was examined by stored at different pH value at room temperature and for 1 week. No alteration was observed neither in the color of extract nor in the total lycoperosides content.

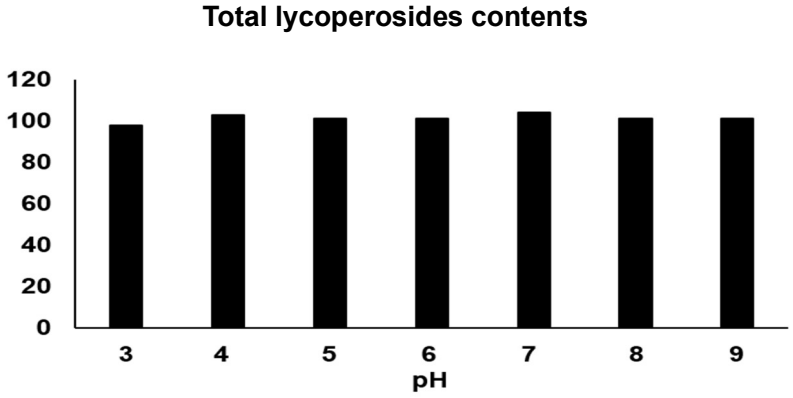

Fig. 22. pH stability of Tomato Seed Extract-P

7. Nutrition profiles

Analyzed item (/100g)	Tomato Seed Extract-P, PC	Method
Water (g)	3.2	Heating drying method under normal pressure
Protein (g)	0.8	Kjeldahl method, nitrogen protein conversion factor: 6.25
Fat (g)	0.5	Acid decomposition method
Ash (g)	0.3	Direct incineration method
Carbohydrate (g) －Sugar (g) －Fiber (g)	95.2 95.0 0.2	Refer note 1 Prosky's method
Energy (kcal)	388	Refer note 2
Sodium (mg)	22.7	Atomic absorption spectrophotometory
Sodium chloride equivalent (g)	0.06	Refer note 3

The nutritional information of Tomato Seed Extract was analyzed according to the standard in nutrition labeling (March 30, 2015; No 139 Eishin)

Note 1: Calculation: 100-(water + protein + fat + ash)

Note 2: Energy conversion factor: Protein 4, fat 9, sugar 4, dietary fiber 2

Note 3: In terms of sodium

Test trustee: SUNATECH/ Dte of analysis: April 18, 2019

Test No: 160804527-001-01

8. Safety profiles

（1） Residual agricultural chemicals

Tomato Seed Extract was screened and analyzed for residual agricultural chemicals (535 items) stipulated under the Food Sanitation Act and Pesticides Control Act, presence of the test items was lower than the allowed limits.
Test trustee: Masis Co., Ltd.; Center for Food Safety Evaluation and Analysis
Date: November 28, 2018
Report No: 123136

（2） Acute toxicity（LD_{50}）

Acute Toxicity test was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products. Tomato Seed Extract 2000 mg/kg was orally given to fasted ICR mice (6 weeks old). After 14 days, no abnormalities and fatal event were observed at 2000 mg/kg. No abnormalities were observed under macroscopic examination upon autopsy. Thus, LD_{50} of Tomato Seed Extract is deduced to be > 2000 mg/kg.

（3） Mutagenicity（Ames test）

Ames test was conducted to evaluate the mutagenicity of Tomato Seed Extract using *Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *E. coil* WP2 at concentration 19.5-5,000 μg/plate. No mutagenicity was observed.

9. Recommended dosage

We recommend 200 mg/day of Tomato Seed Extract-P based on the result of human clinical trial.

10. Application

	Applications	Indication	Examples
Food	Nutritional supplement, Beauty food	-Extracellular matrix production cycle enhancement -Skin wasted product removal	Beverages, Hard and soft capsules, tablets, Candies, Chewing gums, Gummies, Cookies, Chocolates, Wafers, Jellies etc.
Cosmetic	Beauty		Toners, Lotions, Packs, Body gels etc.

11. Packing

Tomato Seed Extract-P（PC）
1 kg, 5kg Interior packing: Aluminium bag
Exterior packing: Cardboard box

12. Storage

Store in a dry, ventilated location. Keep away from high temperature and sun light, store in the closed containers.

13. Expression

<Food>

Tomato Seed Extract-P

Maltodextrin, Tomato Seed Extract

<Cosmetic>

Tomato Seed Extract -PC

Maltodextrin, Tomato Extract

INCI name: MALTODEXTRIN (AND) SOLANUM LYCOPERSICUM (TOMATO) EXTRACT

PRODUCT STANDARD

PRODUCT NAME : **TOMATO SEED EXTRACT-P**

(FOOD)

This product is extracted with aqueous ethanol from the seed of tomato (*Lycopersicon esculentum*).
It contains a minimum of 0.5% total lycoperosides.

Appearance	Pale white to pale yellowish white powder with slightly characteristic odor.	
Total lycoperosides	Min. 0.5%	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 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. 1×10^3 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
Maltodextrin	95%
Tomato Seed Extract	5%
Total	100%

Expiry date	2 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high temperature and sun light.

PRODUCT STANDARD

PRODUCT NAME : **TOMATO SEED EXTRACT-PC**

(COSMETIC)

This product is extracted with aqueous ethanol from the seed of tomato (*Lycopersicon esculentum*). It contains a minimum of 0.5% total lycoperosides.

Appearance	Pale white to pale yellowish white powder with slightly characteristic odor.	
Total lycoperosides	Min. 0.5%	(HPLC)
Loss on Drying	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105ºC, 2 hr)
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 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	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	95%
Tomato Seed Extract	5%
Total	100%

Expiry date	2 years from date of manufacturing.
Storage	Store in a dry, ventilated location. Keep away from high temperature and sun light.

This standards and test methods are referred to General Notices and General Tests, Processes and Apparatus of The Japanese Standards of Quasi-drug Ingredients, unless otherwise specified.

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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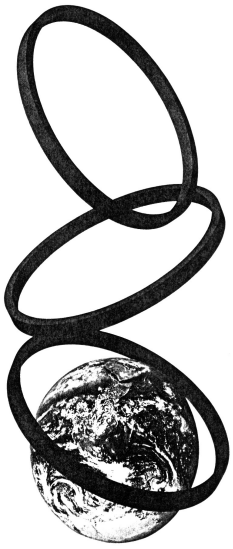

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