

ORYZA OIL & FAT CHEMICAL CO., LTD.

WALNUT POLYPHENOL

Hepatoprotective & Anti-oxidative Extract
For Metabolic Syndrome

WALNUT POLYPHENOL-P10,P30

(Powder , Food Grade)

WALNUT POLYPHENOL-WSP10

(Water-soluble Powder , Food Grade)

WALNUT POLYPHENOL-PC10,PC30

(Powder , Cosmetic Grade)

WALNUT POLYPHENOL-WSPC10

(Water-soluble Powder , Cosmetic Grade)

WALNUT POLYPHENOL-LC

(Water-soluble Liquid , Cosmetic Grade)

WALNUT SEED OIL

(Oil , Food & Cosmetic Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD

ver. 1.0 HS

WALNUT POLYPHENOL

Hepatoprotective & Anti-oxidative Extract For Metabolic Syndrome

1. Introduction

Recently, there is an increased awareness on metabolic syndrome – a condition characterized by a group of metabolic risk factors in one person. They include abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance, prothrombotic state & proinflammatory state. The dominant underlying risk factors appear to be abdominal obesity and insulin resistance. In addition, non-alcoholic fatty liver disease (NAFLD) is the most commonly associated “liver” manifestation of metabolic syndrome which can progress to advance liver disease (e.g. cirrhosis) with associated morbidity and mortality. Lifestyle therapies such as weight loss significantly improve all aspects of metabolic syndrome, as well as reducing progression of NAFLD and cardiovascular mortality.

Walnut (*Juglans regia* L. seed) is one the most popular nuts consumed in the world. It is loaded in polyunsaturated fatty acids – linoleic acid (LA), oleic acid and α -linolenic acid (ALA), an ω 3 fatty acid. It has been used since ancient times and epidemiological studies have revealed that incorporating walnuts in a healthy diet reduces the risk of cardiovascular diseases. Recent investigations reported that walnut diet improves the function of blood vessels and lower serum cholesterol. Nevertheless, walnut is rich in micronutrients (vitamins & minerals), plant sterols and polyphenols.

A joint research project was conducted between Oryza Oil & Fat Chemical Co., Ltd. and Pola Chemical Industries, Inc. to study the physiological effects of testa of walnut. Various hydrolysable polyphenolic compounds were identified which are potent antioxidants and liver protective. Correspondingly, Walnut Polyphenol is believed to be beneficial in the treatment of metabolic syndrome.



Fig. 1. Walnuts (*Juglans regia* L.)

2. WALNUT POLYPHENOLS

Most polyphenolic compounds of walnut are loaded in the testa of walnut fruit as illustrated in Fig. 2. These are hydrolysable polyphenolic compounds as shown in Fig. 3 and its principle constituents are as listed in Table 1.

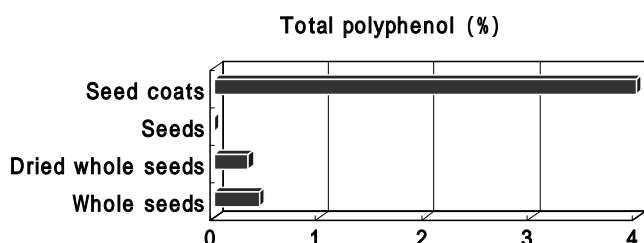


Fig. 2. The Content of Polyphenols in Walnut (%)
(Fukuda T. Polyphenols from walnuts: Structures and functions. IFT 2006 Annual Meeting, Orland, Florida, June 24-28, 2006)

Table 1. Principle Polyphenolic Constituents and Their Respective Distribution in Walnut

	(%)
Pedunculagin (1)	16.0
Ellagic acid (5)	15.8
Tellimagrandin I (6)	6.6
Casuarictin (7)	4.1
Tellimagrandin II (10)	1.2
Rugosin C (11)	1.8
Casuarinin (12)	1.0

Fukuda T. *et al.*, *BioFactors*, **21**, 251-253 (2004).

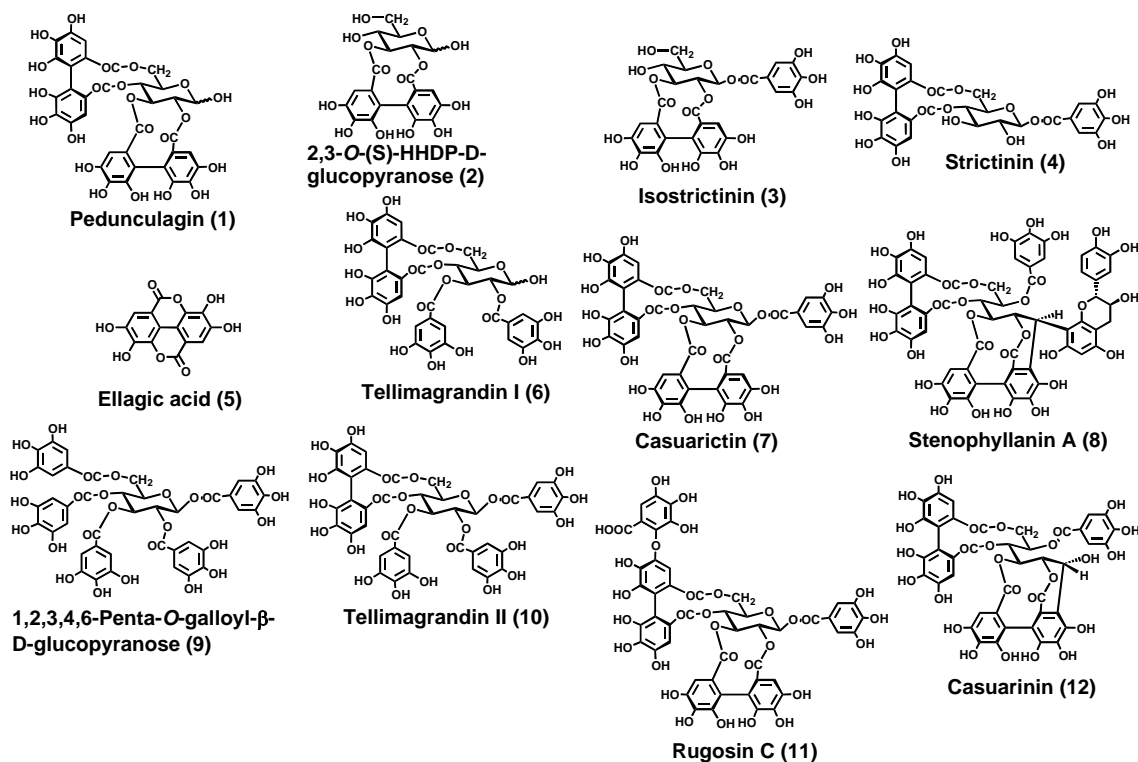


Fig. 3. Chemical Structures of Hydrolysable Polyphenolic Compounds of Walnut

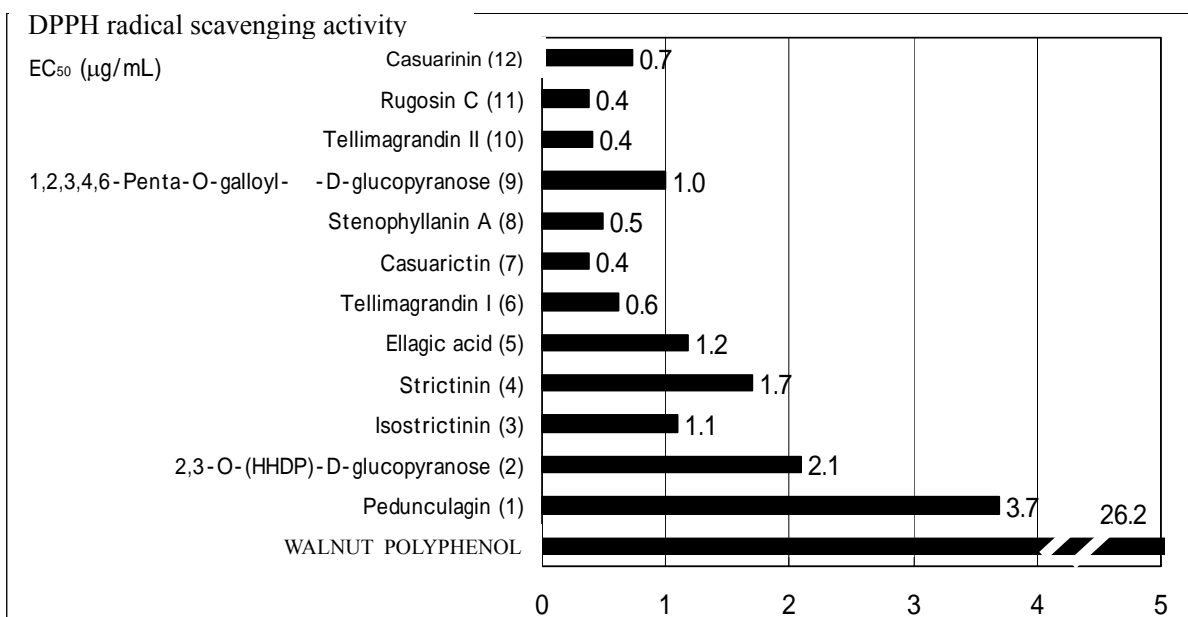
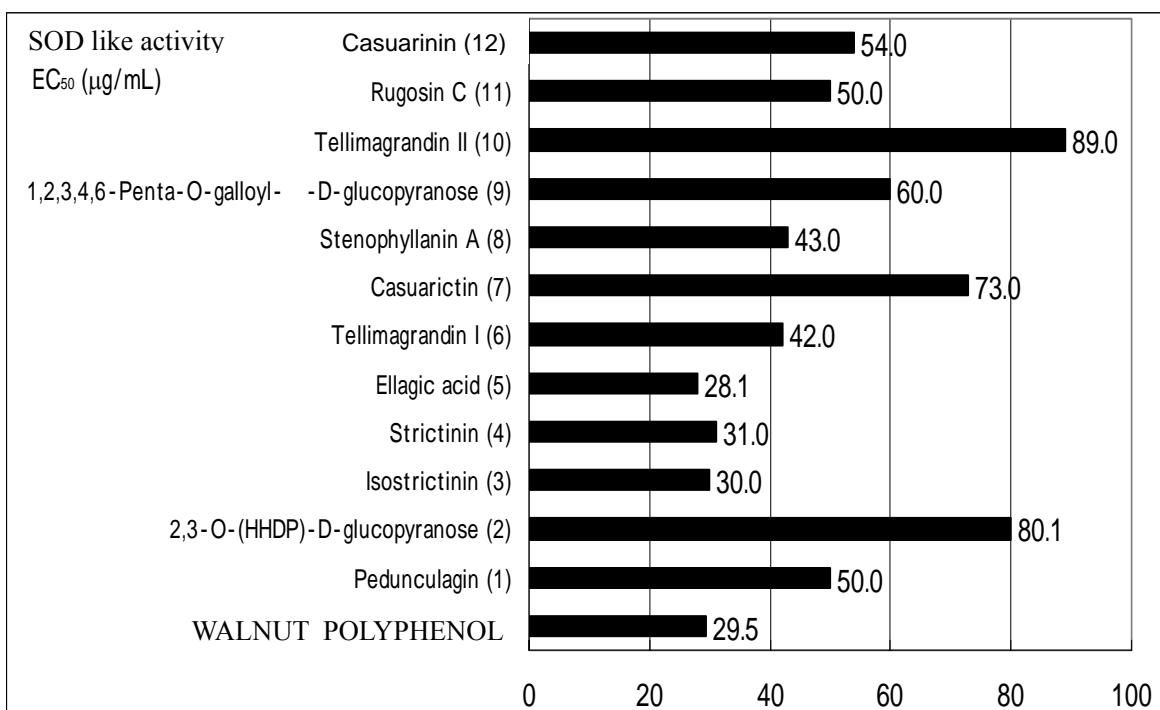


Fig. 4. (top) SOD Antioxidant Activity of Walnut Polyphenols
 (bottom) DPPH Antioxidant Activity of Walnut Polyphenols
 Fukuda T., *et al.*, Antioxidative polyphenols from walnuts (*Juglans regia* L.).
Phytochemistry, **63**, 795-801 (2003).
 EC₅₀: 50% effective concentration of sample

3. Hepatoprotective Effect of WALNUT POLYPHENOLS

(I) *In vivo*

Liver is the second largest organ in the human body. It plays a major role in metabolism of human body including glycogen storage, plasma protein synthesis and drug detoxification. It regulates a wide variety of high volume biochemical reactions and breaks down toxic substances (e.g. chemicals, viruses, alcohol etc) which may result in toxication (Fig. 5).

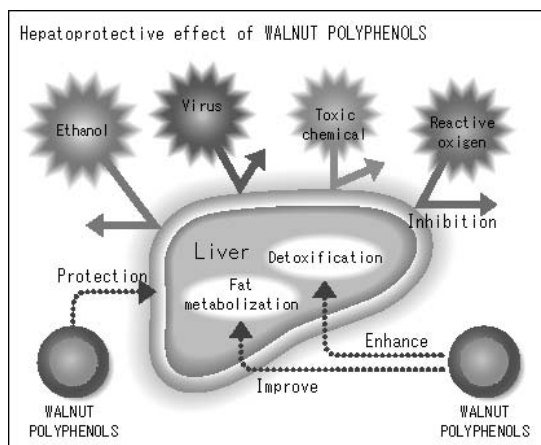


Fig. 5. Liver Functions and The Effect of Walnut Polyphenols on Liver Health

(a) The effect of Walnut Polyphenols on CCl₄-induced liver damage in mice model (detoxification)

The effect of Walnut Polyphenols on CCl₄-induced liver damage was studied. **This model is a simulated human hepatitis model as a result of oxidative stress and toxication.** Upon liver damage, the liver enzymes GOT (glutamate oxaloacetate transaminase) and GPT (glutamic pyruvic transaminase) infiltrate into the blood stream resulting in elevated serum GOT & GPT levels. Walnut Polyphenols demonstrated a dose dependent lowering effect in GOT & GPT in CCl₄-induced liver damage in mice model (Fig. 6). Meanwhile, Walnut Polyphenols is more superior than Curcumin, a commonly used hepatoprotective agent.

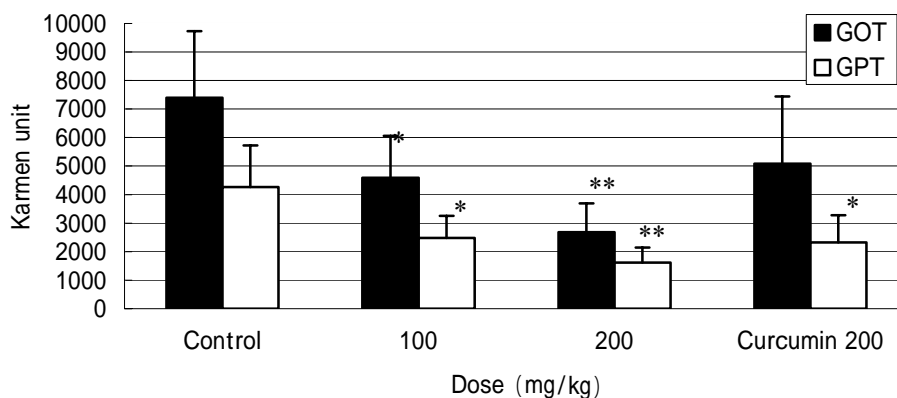


Fig. 6. The Effect of Walnut Polyphenols on CCl₄-induced liver damage in mice model (n=8, mean ± S.E., *: $p < 0.05$, **: $p < 0.01$)

【Method of Experiment】

Fasting mice (ddY, male, 5-wk old) were orally fed Walnut Polyphenols. One hour later, back of mice were subcutaneously injected with 10% CCl₄ previously diluted in olive oil (5ml/kg). Blood samples were collected 20 hours later for measurement of serum GOT and GPT.

(b) The Effect of Walnut Polyphenols on D-galactosamine–induced liver damage in mice (detoxification)

The effect of Walnut Polyphenols on D-galactosamine (D-GaIN)–induced liver damage was studied. This is a simulated human viral hepatitis model based on immune responses and histological observations. As illustrated in Fig. 7, both GOT and GPT levels are elevated in the control group 10-hour post induction of D-GaIN. Meanwhile, group treated with Walnut Polyphenols demonstrated a dose-dependent reduction on GPT level although no changes observed in the GOT level.

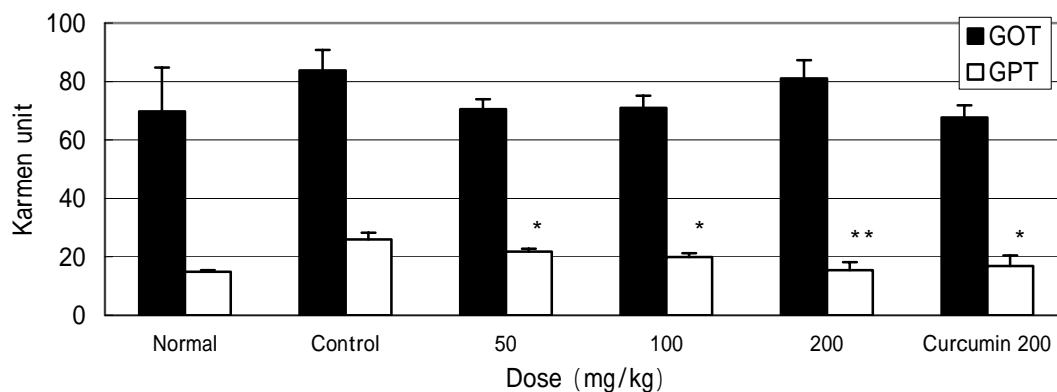


Fig. 7. The effect of Walnut Polyphenols on D-Galactosamine–induced liver damage in mice. (n=8, mean ± S.E., *: $p < 0.05$, **: $p < 0.01$)

【Method of Experiment】

Fasting mice (ddY, male, 5-wk old) were orally fed Walnut Polyphenols followed by intra-peritoneal injection of D-galactosamine (300mg/kg) 1 hour later. Blood samples were collected 20 hours later for measurement of serum GOT and GPT.

(II) *In vitro*

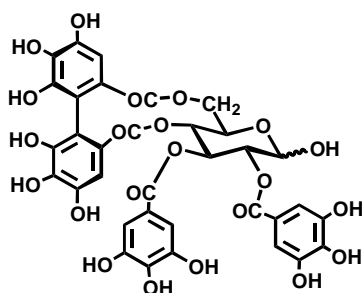
The Effect of Walnut Polyphenols on CCl₄-induced cytotoxicity in rat hepatocytes

The effect of each active component of Walnut Polyphenols on CCl₄-induced cytotoxicity in primary cultured rat hepatocytes was evaluated *in vitro*. Results showed that tellimagrandin I (6), casuarictin (7), tellimagrandin II (10), and rugosin C (11) are inhibitory on CCl₄-induced cytotoxicity in primary cultured rat hepatocytes (as tabulated in Table. 2). Meanwhile, tellimagrandin I (6) of Walnut Polyphenols is believed to be the most important active responsible for hepatoprotective effect. Nevertheless, tellimagrandin I (6) is the third largest component found within Walnut Polyphenols (Table 1).

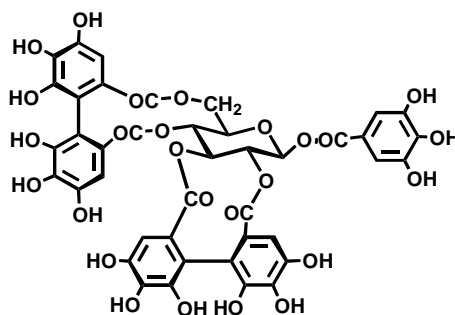
Table 2. The effect of Walnut Polyphenols and its active components on CCl₄-induced cytotoxicity in primary cultured rat hepatocytes.

	10 (μg/mL)	Inhibition (%)	
		30	100
WALNUT POLYPHENOL	7.6 ± 0.2	13.2 ± 0.3	42.1 ± 1.8
Pedunculagin (1)	4.7 ± 0.2	3.4 ± 0.1	15.7 ± 0.7
Ellagic acid (5)	-12.6 ± 0.5	-18.5 ± -1.3	-
Tellimagrandin I (6)	66.7 ± 9.4	53.6 ± 2.8	100.0 ± 6.7
Casuarictin (7)	12.6 ± 0.2	13.9 ± 0.8	48.3 ± 1.9
Tellimagrandin II (10)	35.5 ± 3.2	20.4 ± 1.2	54.8 ± 4.6
Rugosin C (11)	15.8 ± 0.4	36.0 ± 1.4	58.8 ± 0.7
Casuarinin (12)	3.1 ± 0.1	8.9 ± 0.2	12.0 ± 0.6
Curcumin	8.5 ± 0.3	5.9 ± 0.1	-

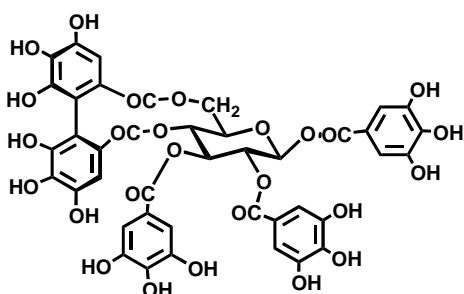
Mean ± S.E. (n=8)



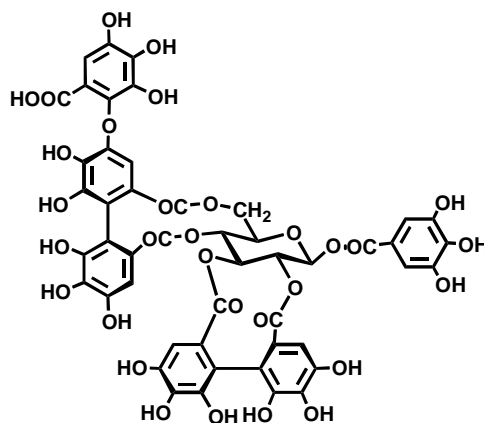
Tellimagrandin I (6)



Casuarictin (7)



Tellimagrandin II (10)



Rugosin C (11)

【Method of Experiment】

Primary cultured rat hepatocytes (4×10⁴ cells/100μL) was acquired from collagenase perfusion of fresh mouse liver. These cells were then pre-cultured for 4-hour followed by change of culture medium to 5 mM CCl₄ and test samples. After 40-hour of incubation, cell toxicity was measured using MTT Assay.

4. The Effect of Walnut on Metabolic Syndrome

(I) The Effect of Walnut on Atherosclerosis

Epidemiological studies have associated nut consumption with a reduced incidence of cardiovascular mortality. Endothelial dysfunction is associated with atherosclerosis and its risk factors, including hypercholesterolemia. Ros E. *et al.* reported that substituting walnuts for monounsaturated fat in a Mediterranean diet improves endothelium-dependent vasodilation (EDV) in hypercholesterolemic subjects. As illustrated in Fig. 9, daily intake of 8-13 walnuts for 4 weeks significantly improve the EDV of 21 hypercholesterolemic males and females.

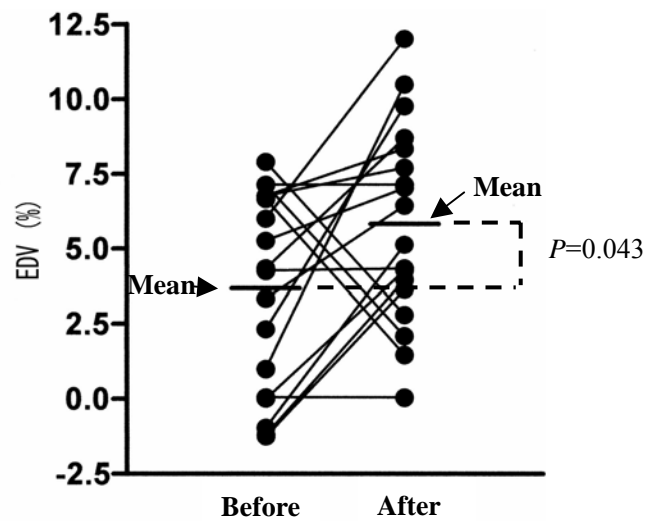


Fig. 9. The effect of Walnut enriched diet on endothelium-dependent vasodilation (EDV) of hypercholesterolemic patients.

Ros E. *et al.*, A walnut diet improves endothelial functions in hypercholesterolemic subject. *Circulation*, **109**, 1609-1614 (2004).

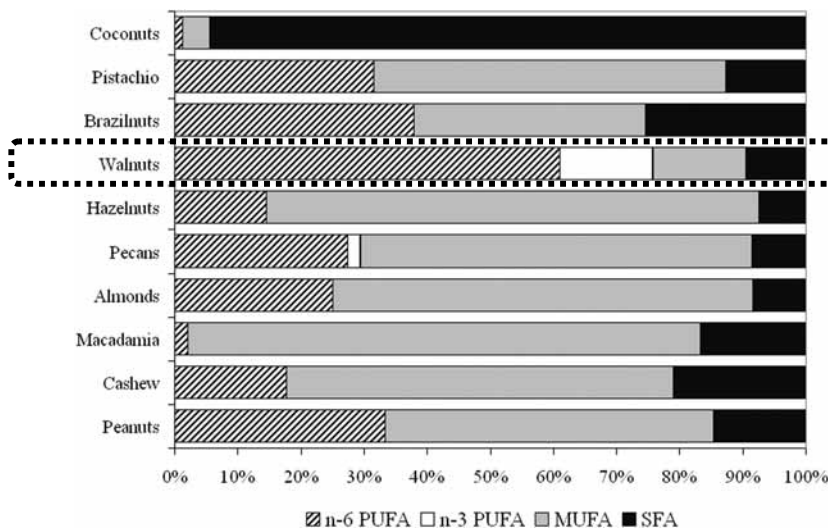


Fig. 10. The Fatty Acids Composition of various nuts (PUFA: polyunsaturated fatty acid, MUFA: monounsaturated fatty acid, SFA: saturated fatty acid)

Mukuddem-Petersen J., *et al.*, A systematic review of the effects of nuts on blood lipid profiles in humans. *J. Nutr.* **135**, 2082-2089 (2005).

Fig. 10 illustrated the fatty acids composition of various nuts commonly available in our diet. Walnuts, differ from other nuts, are loaded with α -linolenic acid, a plant ω -3 fatty acids, which may have protective effect on cardiovascular health by reducing blood cholesterol and triglyceride levels.

(II) The Effect of Walnut on Cholesterol

There are several reports documented the beneficial effect of walnuts on hyperlipidemia. [Mukuddem-Petersen J., *et al.*, *J. Nutr.* **135**, 2082-2089 (2005)] In one study conducted by Iwamoto M *et al.* on Japanese subjects are particularly interesting. Iwamoto M *et al.*, reported that incorporating moderate quantities of walnuts into the average Japanese diet while maintaining the intake of total dietary fat and energy decreases serum total cholesterol concentration and favorably modifies the lipoprotein profile in Japanese, particularly in women. In this study, daily intake of 43 to 57g of walnuts was incorporated into Japanese diet for 4 weeks to 40 healthy Japanese men and women. As illustrated in Fig. 11, blood cholesterol of test subjects was lowered, particularly in women.

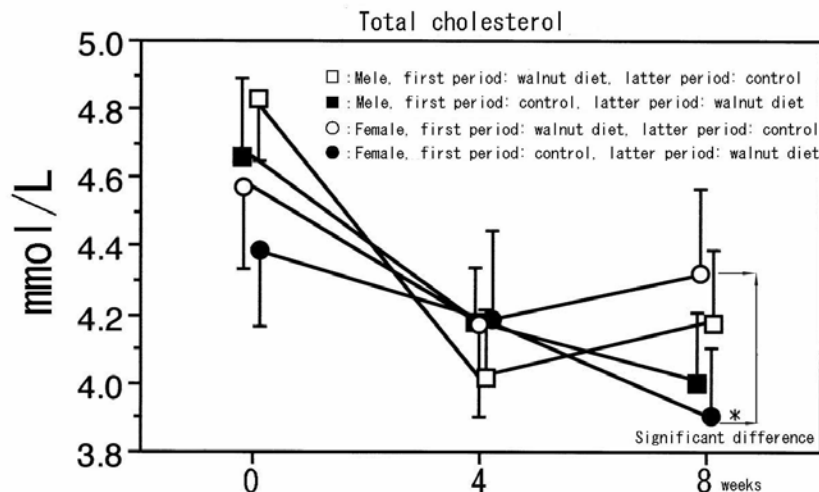
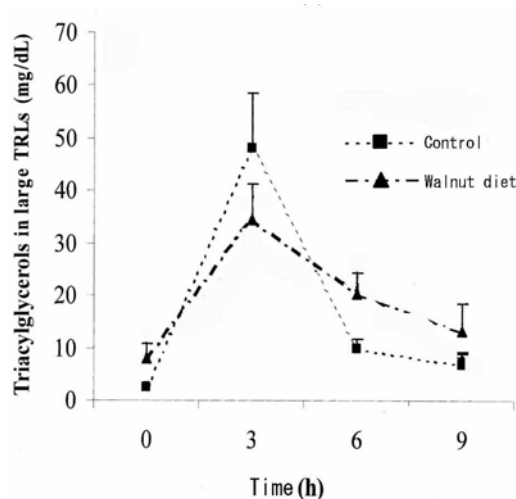


Fig. 11. The effect of Walnuts on Total Cholesterol (*: $p < 0.05$)

Iwamoto M. *et al.*, Walnuts lower serum cholesterol in Japanese men and women. *J. Nutr.* **130**, 171-176 (2000).



On the other hand, Bellido C.E. *et al.*, reported that walnut-enriched meals effectively prevented post prandial lipidemia where triacylglycerol in large triacylglycerol is significantly reduced.

Fig. 12. The Effect of Walnuts on postprandial blood triacylglycerol.

Bellido C. E. *et al.*, Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor κ B in peripheral blood mononuclear cells from healthy men. *Am. J. Clin. Nutr.*, **80**, 1487-1491 (2004).

(III) The effect of Walnut Polyphenols on Diabetes Mellitus

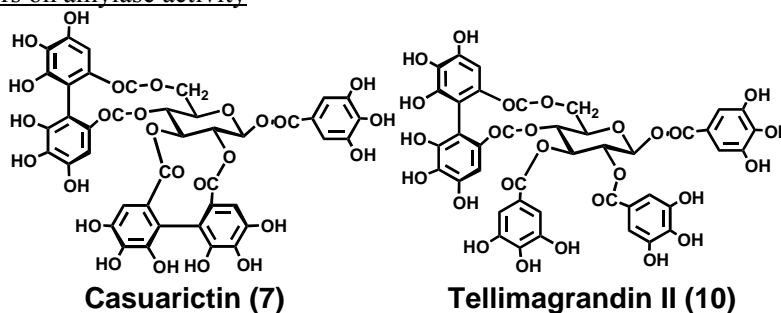
Dr. Fukuda of Pola Chemical Industries, Inc. has conducted extensive studies on the effect of Walnut Polyphenols on diabetes mellitus especially on the enzyme glycosidases. The IC₅₀ of each active component of Walnut Polyphenols on glycosidases is tabulated in Table 3. Walnut extract and its polyphenolic components, especially casuarictin (7) and tellimagradin II (10) demonstrated strong inhibitory activity against amylase. Meanwhile, tellimagradin I (6), tellimagradin II (10) & casuarictin (7) are three most potent walnut polyphenolic components against maltase activity. Walnut Extract and its polyphenolic components showed similar inhibition against the enzyme sucrose.

Table 3. IC₅₀ of Walnut Polyphenols on glycosidases

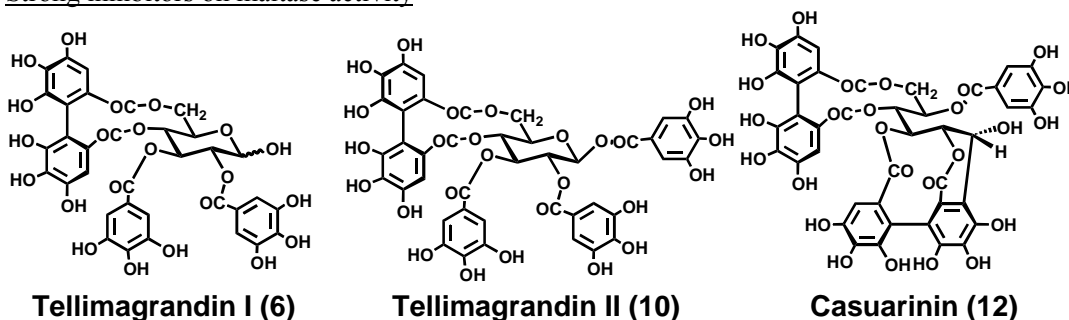
	Inhibitory activity : IC ₅₀ (mg/mL)		
	Sucrose	maltase	Amylase
Pedunculadin (1)	0.50	0.70	0.11
2,3-HHDP-Glc. (2)	0.67	0.83	0.13
Isostrictinin (3)	0.41	0.31	0.062
Strictinin (4)	0.26	0.20	0.053
Tellimagradin I (6)	0.33	0.041	0.013
Casuarictin (7)	0.30	0.18	0.0033
Stenophyllanin A (8)	0.92	0.31	Not examined
Tellimagradin II (10)	0.43	0.025	0.0019
Rugosin C (11)	0.60	0.32	0.017
Casuarinin (12)	0.40	0.046	0.018
Walnut extract	>1	0.40	0.070
non resin-attached fraction	>1	>1	>1
resin-attached fraction	0.61	0.11	0.011

Fukuda, *et al.*, the 50th Meeting of the Japanese Society of Pharmacognosy, Sep. 12-13, 2003 (Tokyo), the 51st Meeting of the Japanese Society of Pharmacognosy, Sep. 9-10, 2004 (Kobe).

Strong inhibitors on amylase activity



Strong inhibitors on maltase activity



In a research conducted by Fukuda *et al.*, reported that walnut polyphenol-rich fraction (WPF) lowered elevated blood glucose level post-loading of starch and sucrose in mice model (Fig. 13).

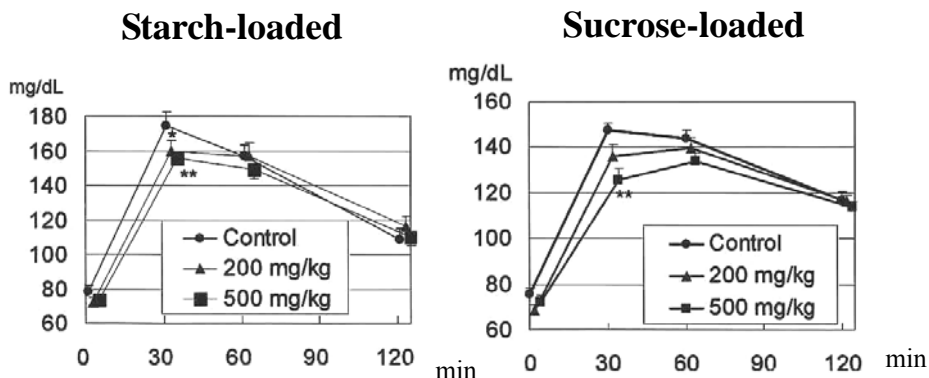


Fig. 13. The effect of Walnut Polyphenol-rich Fraction (WPF) on Blood Glucose Level Post-Loading of Starch and Sucrose in Mice (mean \pm S.E., n=23, *: $p < 0.05$, **: $p < 0.01$)

Fukuda *et al.*, The 50th Meeting of the Japanese Society of Pharmacognosy, Sep 12-13, 2003 (Tokyo), The 51st Meeting of the Japanese Society of Pharmacognosy, Sep 9-10, 2004 (Kobe).

[Method of Experiment]

Blood sample was collected from fasting mice (ddY, male, 10-week old) for measurement of initial blood glucose level prior to loading of starch and sucrose. Test sample A (containing Walnut polyphenol-rich fraction [WPF] and soluble starch 2g/kg) and test sample B (containing Walnut polyphenol-rich fraction [WPF] and sucrose 2g/kg) were orally given to mice 20 minutes later. Blood samples were collected at 30, 60, and 120 minutes for measurement of blood glucose level.

In addition to the above findings, research also noticed that Walnut polyphenol-rich fraction (WPF) has triglyceride lowering effect and urine peroxide lowering effect in genetically inherited Type II diabetes mellitus (*db/db*) mice as shown in Table 4.

Table 4. The effects of walnut polyphenol-rich fraction (WPF) on genetically inherited type II diabetes mellitus mice.

	Genotype	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Urine 8-OHdG/creatinine (ng/mg creatinine)
Normal	<i>db/+m</i>	25.6 \pm 0.8**	63.5 \pm 3.2**	69.3 \pm 9.3**	84.0 \pm 12.4**
Control	<i>db/db</i>	37.4 \pm 2.4	103.8 \pm 19.1	177.0 \pm 59.1	122.5 \pm 25.5
WPF	<i>db/db</i>	36.5 \pm 2.3	106.7 \pm 15.0	121.6 \pm 37.0**	94.8 \pm 24.9*

N=6-8, mean \pm S.D., *: $p < 0.05$, **: $p < 0.01$.

[Method of Experiment]

Mice (C57BL/KsJ-*db/db*, male, 9-week old) were fed walnut polyphenol-rich fraction 200mg/kg/day for 4-week. After 4-week, mice were placed in individual metabolic cages for urine collection for 8 hours and blood sample was collected after overnight starvation. Urine 8-OHdG, blood glucose and triglyceride levels were measured respectively.

Fukuda T., *et al.*, Effect of the walnut polyphenol fraction on oxidative stress in type 2 diabetes mice. *BioFactors* **21**, 251-253 (2004).

5. Other Functional Activities

Skin-lightening activity (Inhibition of hyperpigmentation)

The effect of Walnut Polyphenols on skin hyperpigmentation was examined using B16 melanoma cells *in vitro*. Pre-cultured B16 melanoma cells were incubated in medium containing Walnut Polyphenols and melanin cells formation was determined. As illustrated below, Walnut Polyphenols inhibited melanin formation at concentration 1 to 30 μ g/mL. Apparently, Walnut Polyphenols is more superior than the popular skin-lightening agent, ascorbic acid and arbutin upon data comparison (Fig. 14)

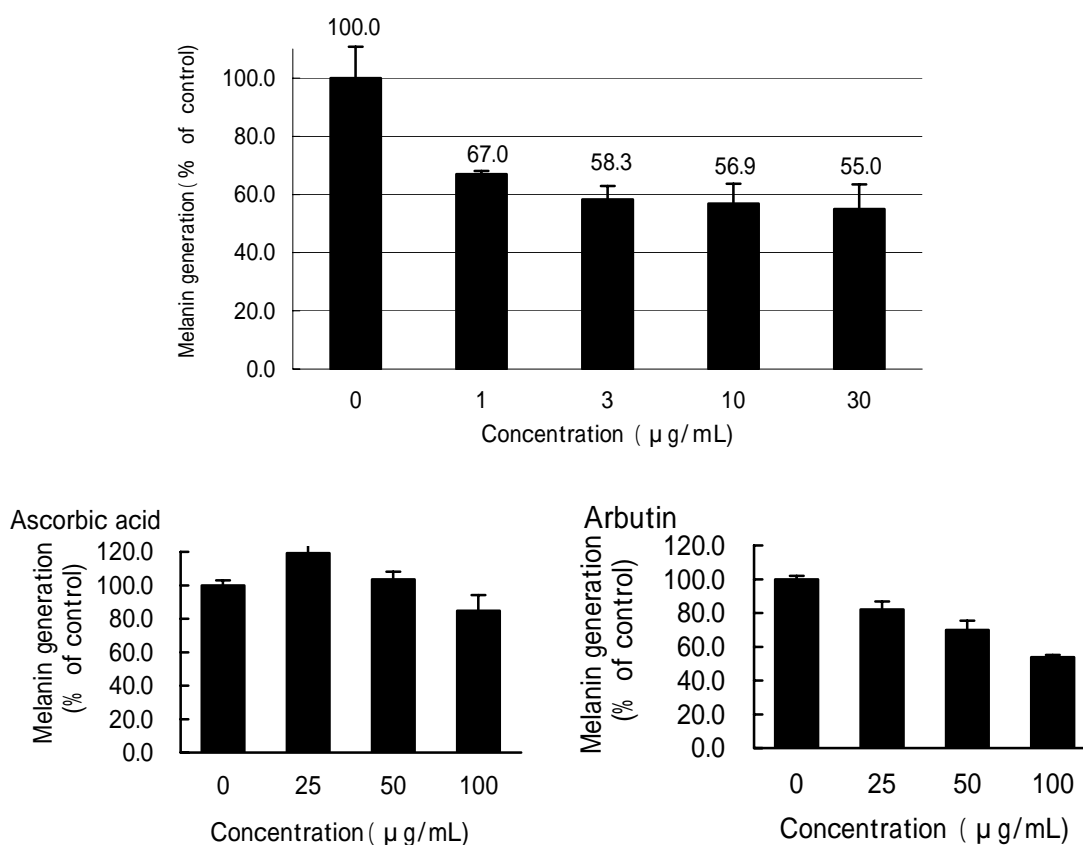


Fig. 14. The Effect of Walnut Polyphenols, Ascorbic acid and Arbutin on B16 Melanoma cells. (mean \pm S.D., n=5)

Data of The Effect of Ascorbic Acid & Arbutin on B16 Melanoma cells were cited from: Aitani M. and Shimoda H. *Japan Food Science*, **44**, 58-63 (2005).

6. Stability of Walnut Polyphenols

(I) Thermostability

Fig. 15 illustrated data of Thermostability of Walnut Polyphenols (without binder). The polyphenols content remained stable at temperature 100°C and 120°C for 1 hour. Walnut Polyphenols is highly stable.

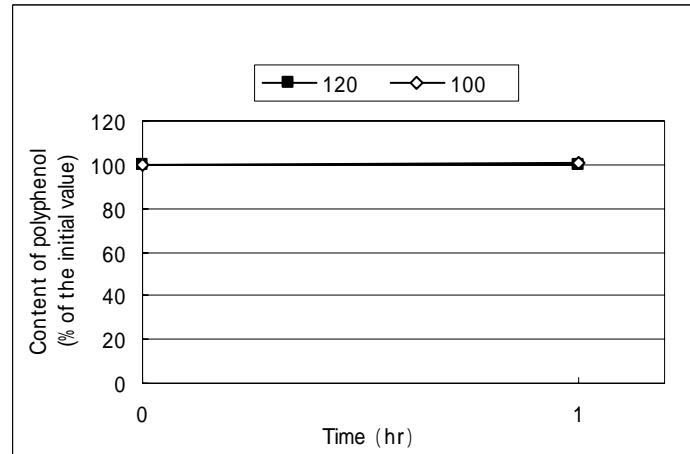


Fig. 15 . Thermostability of Walnut Polyphenols

(II) pH Stability

Fig. 16 illustrated data of pH Stability of Walnut Polyphenols on Day 1 and on Day 7. Walnut Polyphenols solution (0.5%) was prepared and stored under different pH at room temperature for 1-day and 1-week. Results showed that Walnut Polyphenols is highly stable at acidic and neutral conditions but degraded at alkaline condition.

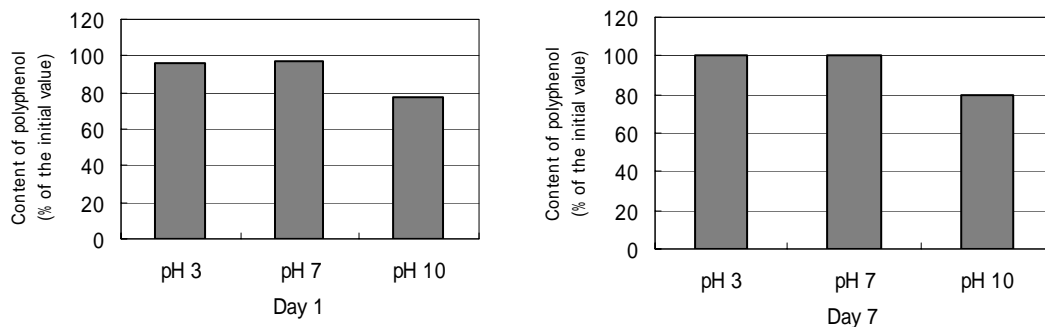


Fig. 16 . pH stability of Walnut Polyphenols

7. Nutrition Information (Walnut Polyphenols)

	-P10, -WSP10	-P30	Note	Method
Water	1.9 g/100g	1.9 g/100g		Vacuum superheating drying method
Protein	1.5 g/100g	4.1 g/100g	1	Kjeldahl method
Fat	14.0 g/100g	37.8 g/100g		Acid decomposition
Ash	1.7 g/100g	4.5 g/100g		Direct incineration
Carbohydrate	82.5 g/100g	51.7 g/100g	2	
Energy	464 kcal/100g	570 kcal/100g	3	Modified Atwater method
Dietary fiber	1.2> g/100g	3.3> g/100g		Prosky method
Sodium	203 mg/100g	549 mg/100g		Atomic absorption spectrophotometry

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

Test trustee : SRL

Data : September 15, 2006

Report No. : 2006090400032

8. Safety Profile of Walnut Polyphenols

(I) Residual Agricultural Chemical

Walnut Polyphenols (without binder) is conformed to regulation stipulated for 447 residual agricultural chemical compounds. No residual agricultural chemicals detected as confirm by test trustee.

Test trustee : Masis Co. Ltd.

Data : September 4, 2006

Report No. : 7035

(II) Acute Toxicity (LD₅₀)

Fasting male and female mice (ddY, 5-week old) were given orally 2,000mg/kg Walnut Polyphenols (no binder) in accordance to Single Dose Toxicity Test Guideline for Pharmaceuticals. Mice were maintained for observation for 14 days. No fatal event occur nor abnormal changes observed upon comparison with control group. No evident abnormalities detected in organs upon autopsy. Oral LD₅₀ of Walnut Polyphenols is deduced to be >2,000mg/kg for both male and female mice.

(III) Human Consumption Test

4 male volunteers were given oral Walnut Polyphenols (without binder) 50mg/day for 4 weeks. Blood profile screening was carried out for analysis prior to and after the test. No abnormal reading detected in blood profile screening.

Blood profile screening: Total bilirubin, Total protein, Albumin, AST, ALT, LDH, LAP, γ -GTP, cholinesterase, amylase, lipase, L-CAT, LDL-cholesterol, total cholesterol, triglyceride, phospholipid, FFA, HDL-cholesterol, Na, K, serum Fe, TIBC, UIBC, urea nitrogen, uric acid, glucose, hemocytes.

9. Dosage Recommendation

The recommended daily dosage of Walnut Polyphenol-P10 & Walnut Polyphenol-WSP10 is 50 to 150 mg

10. Crude Material Equivalent

1g of Walnut Polyphenols is equivalent to 200 edible portion of walnuts. Recommended daily consumption of walnut is 10 – 30 walnuts.

11. Commercial Application

	Application	Claim	Example
Foods	Hepatoprotection, prevention of metabolic syndrome, anti-oxidation, beautifying	Hepatoprotection, prevention of metabolic syndrome, diabetes, hyperlipidemia, hypertension, anti-oxidation, whitening	Beverages, hard & soft capsules, tablets, candies, chewing gum, chocolates, wafers, jellies, etc
Cosmetics	Whitening		Body lotion, body gel, etc

12. Packaging

WALNUT POLYPHENOL-P10, -P30 (powder , food grade) , -WSP10(water-soluble powder , food grade)

5kg Interior package : aluminium bag
Exterior package : cardboard box

WALNUT POLYPHENOL-PC10, -PC30 (powder , cosmetics grade) , -WSPC10 (water-soluble powder , cosmetics grade)

5kg Interior package : aluminium bag
Exterior package : cardboard box

WALNUT POLYPHENOL-LC (water-soluble liquid , cosmetics grade)

5kg Interior package : cubic polyethylene container
Exterior package : cardboard box

WALNUT SEED OIL (oil , food and cosmetics grade)

16kg Interior package : tinplate can
Exterior package : cardboard box

13. Storage

Store in cool, dry dark place

14. Expression

<Food>

WALNUT POLYPHENOL -P10 , -P30 , -WSP10

Example : walnut extract , walnut polyphenol

WALNUT SEED OIL

Example : walnut oil , walnut seed oil

<Cosmetics>

WALNUT SEED OIL

Example : walnut seed oil

PRODUCT STANDARD
PRODUCT NAME

WALNUT POLYPHENOL-P10

Food

This product is extracted with aqueous ethanol from seed coats of walnut (*Juglans regia* L.) or its congeners. It guarantees minimum 10.0 % polyphenols and 0.1% tellimagrandin I.

<u>Appearance</u> smell	Light brown to dark brown powder with slightly unique	
<u>Polyphenols</u>	Min. 10 %	(Folin-Denis method)
<u>Tellimagrandin I</u>	Min. 0.1 %	(HPLC)
<u>Loss on drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 °C, 2 hr)
<u>Purity test</u> (1) Heavy metals	Max. 20 ppm	(The Japanese Standards for Food Additives)
(2) Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
<u>Standard Plate Counts</u>	Max. 3×10^3 cfu / g (Analysis for Hygienic Chemists)	
<u>Moulds and Yeasts</u>	Max. 1×10^3 cfu/ g (Analysis for Hygienic Chemists)	
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	Ingredients	Contents
	Walnuts extract	33 %
	Dextrin	67 %
	Total	100 %

PRODUCT STANDARD

PRODUCT NAME

WALNUT POLYPHENOL-P30

Food

This product is extracted with aqueous ethanol from seed coats of walnut (*Juglans regia* L.) or its congeners. It guarantees minimum 30.0 % polyphenols and 0.3% tellimagrandin I.

<u>Appearance</u>	Brown to dark brown powder with slightly unique smell	
<u>Polyphenols</u>	Min. 30 %	(Folin-Denis method)
<u>Tellimagrandin I</u>	Min. 0.3 %	(HPLC)
<u>Loss on drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 °C, 2 hr)
<u>Purity test</u>		
(1) Heavy metals	Max. 20 ppm	(The Japanese Standards for Food Additives)
(2) Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
<u>Standard Plate Counts</u>	Max. 3×10^3 cfu / g (Analysis for Hygienic Chemists)	
<u>Moulds and Yeasts</u>	Max. 1×10^3 cfu/ g (Analysis for Hygienic Chemists)	
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	Ingredients	Contents
	Walnuts extract	90 %
	Dextrin	10 %
	Total	100 %

PRODUCT STANDARD
PRODUCT NAME

WALNUT POLYPHENOL-WSP10

Food

This water-soluble product is extracted with aqueous ethanol from seed coats of walnut (*Juglans regia* L.) or its congeners. It guarantees minimum 10.0 % polyphenols and 0.1% tellimagrandin I.

<u>Appearance</u> smell	Light brown to dark brown powder with slightly unique	
<u>Polyphenols</u>	Min. 10 %	(Folin-Denis method)
<u>Tellimagrandin I</u>	Min. 0.1 %	(HPLC)
<u>Loss on drying</u>	Max. 10.0 %	(Analysis for Hygienic Chemists, 1 g, 105 °C, 2 hr)
<u>Purity test</u> (1) Heavy metals	Max. 20 ppm	(The Japanese Standards for Food Additives)
(2) Arsenic	Max. 1 ppm	(Standard Methods of Analysis in Food Safety Regulation)
<u>Standard Plate Counts</u>	Max. 3×10^3 cfu / g (Analysis for Hygienic Chemists)	
<u>Moulds and Yeasts</u>	Max. 1×10^3 cfu/ g (Analysis for Hygienic Chemists)	
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	Ingredients	Contents
	Walnuts extract	33 %
	Dextrin	67 %
	Total	100 %

PRODUCT STANDARD

PRODUCT NAME

WALNUT POLYPHENOL-LC

Cosmetics

This water-soluble product is extracted with aqueous BG from seed coats of walnut (*Juglans regia* L.) or its congeners.

<u>Appearance</u>	Brown liquid with slightly unique smell	
<u>Certification test</u> (Polyphenols)	Dissolve 30 μL of this product in 3.5 mL water. Add 0.2 mL Folin-Denis reagent into the solution followed by 0.4 mL saturated Na ₂ CO ₃ . The solution changes to blue.	
<u>Purity test</u>		
(1) Heavy metals	Max. 10 ppm	(The second method)
(2) Arsenic	Max. 1 ppm	(The third method)
<u>Standard Plate Counts</u>	Max. 1 × 10 ² cfu / g (Analysis for Hygienic Chemists)	
<u>Moulds and Yeasts</u>	Max. 1 × 10 ² cfu/ g (Analysis for Hygienic Chemists)	
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)

<u>Composition</u>	Ingredients	Contents
	Water	50 %
	BG	49 %
	Walnuts extract	1 %
	Total	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD

PRODUCT NAME

WALNUT SEED OIL

Food

This product is oil extracted from seeds of walnuts (*Juglans regia* L.) or its congeners.

<u>Appearance</u>	Clear oil of light yellowish color with slightly unique smell.	
<u>Acid value</u>	Max. 0.5	
<u>Iodine value</u>	140 ~ 183	
<u>Saponification value</u>	188 ~ 196	
<u>Color value</u>	Max. 3	(Gardonar method)
<u>Purity test</u>		
(1) Heavy metals	Max. 10 ppm	(The second method)
(2) Arsenic	Max. 1 ppm	(The third method)
<u>Standard Plate Counts</u>	Max. 1×10^2 cfu / g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Negative	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>	Walnut seed oil	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

PRODUCT STANDARD

PRODUCT NAME

WALNUT SEED OIL

Cosmetics

This product is oil extracted from seeds of walnuts (*Juglans regia* L.) or its congeners.

<u>Appearance</u>	Clear oil of light yellowish color with slightly unique smell	
<u>Acid value</u>	Max. 0.5	(The first method, 10 g)
<u>Iodine value</u>	140 ~ 183	
<u>Saponification value</u>	188 ~ 196	
<u>Color value</u>	Max. 3	(Gardonar method)
<u>Purity test</u>		
(1) Heavy metals	Max. 10 ppm	(The second method)
(2) Arsenic	Max. 1 ppm	(The third method)
<u>Standard Plate Counts</u>	Max. 1×10^2 cfu / g	(Analysis for Hygienic Chemists)
<u>Moulds and Yeasts</u>	Negative	(Analysis for Hygienic Chemists)
<u>Coliforms</u>	Negative	(Analysis for Hygienic Chemists)
<u>Composition</u>	Walnut seed oil	100 %

Ref: The Japanese Standards of Quasi-Drug Ingredients.

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

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