ORYZA OIL & FAT CHEMICAL CO., LTD.

PURPLE TEA EXTRACT

- ✧ *Anti-obesity・Diet*
- ✧ *Anti-oxidant・Whitening*
- ✧ *Anti-ageing Ingredients*

FOOD・COSMETICS Ingredient

■ **Purple Tea Extract-P**
(Water Soluble Powder, FOOD Grade)

■ **Purple Tea Extract-WSP**
(Water Soluble Powder, FOOD Grade)

■ **Purple Tea Extract-PC**
(Water Soluble Powder, COSMETICS Grade)

■ **Purple Tea Extract -LC**
(Water Soluble liquid, COSMETICS Grade)

ORYZA OIL & FAT CHEMICAL CO., LTD.

Ver. 3.0 YF

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Anti-obesity • Diet
Anti-oxidant • Whitening
Anti-ageing Ingredients

1. Introduction

The history of tea (*Camellia Sinensis*) and human traces back to ancient time, the "Shen Nong" generation about 4000 years ago, who is the God of Agriculture and Medicine of ancient China. It is believed that China Sichuan is the place of origin, drinking tea has become habitual since Tang Dynasty (7~10 century), and become popular in trade. In Japan, promotional activities and knowledge of tea making and habitual consumption from China was introduced by tea envoy (Yong Zhong, Saicho, Kukai about 800 years), Eisai Zenji (1200 years) and Master Zen (1600 years). Thus tea drinking became popular in the society.

Nowadays, tea has become a healthy icon in the society due to the various healthy benefiting properties from tea, e.g. anti-oxidation, anti-mutation, anti-cancer, anti-hypertensive, lowering of elevated blood sugar level, inhibition of platelet aggregation, antibacterial and anti-viral, improve lipid metabolism and anti-allergy effects etc. [1)].

Figure 1: Purple Tea Leaves

Figure 2: Purple Tea

Purple Tea (Figure 1, 2), is a new variety tea of *Camellia Sinensis*, according to the Tea Research Foundation of Kenya (TRFK), it has been produced for 25 years with red-purple coloured leaves and rich in anthocyanins. Kenya, lies on the equator making farming resourceful, and Purple Tea Trees grow on highland of 1500-2500 meters above sea level. In view of the high exposure to UV light in the growing environment, Purple Tea is naturally abundant in polyphenols. Purple Tea is specially and carefully selected and hand-picked, only the young leaves and shoots are collected from the pesticides-free plantation.

Oryza Oil & Fat Chemical Co., Ltd., discovered a specific polyphenol compound, (1,2-di-**G**alloyl-4,6-**H**exahydroxydiphenoyl-β-D-**G**lucose) (GHG) (Figure 3) which is not found in green tea, oolong tea and black tea. GHG has been shown to demonstrate excellent anti-obesity and anti-ageing effects.

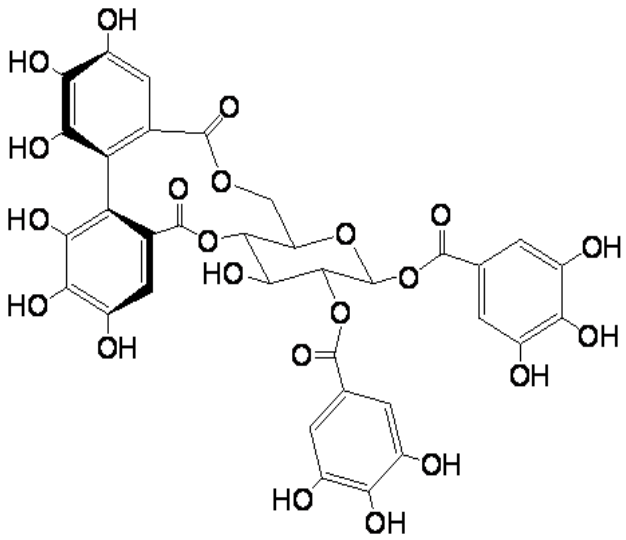

Figure 3: Functional compound specifically found in Purple Tea Extract.
(1,2-di-**G**alloyl-4,6-**H**exahydroxydiphenoyl-β-D-**G**lucose)

PURPLE TEA EXTRACT is highly recommended as **new tea-based ingredient** with excellent **health and beauty** functional activities.

1) yamamoto(maeda)marira, kagakutoseibutu, 46(3), 214-216 (2008)

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2. Functional Effect of Purple Tea Extract

Upon comparison with common teas (dry leaves) e.g. green tea, black tea and oolong tea, Purple Tea has the highest content of variety of Polyphenols antioxidants. (Figure 4)

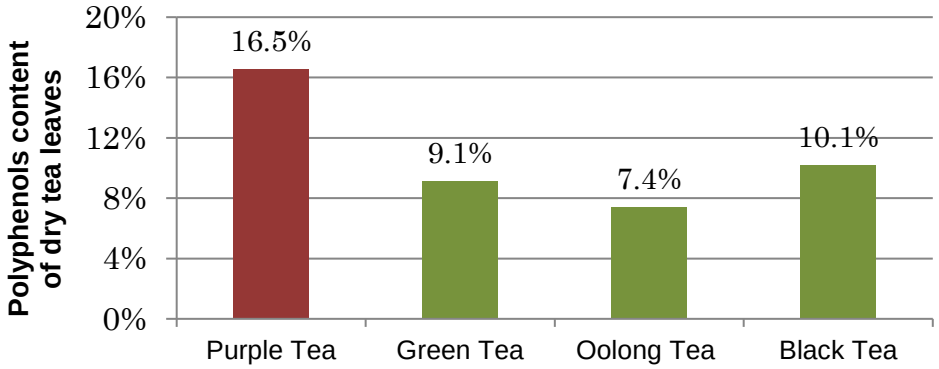
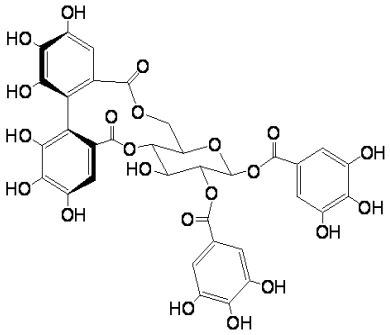
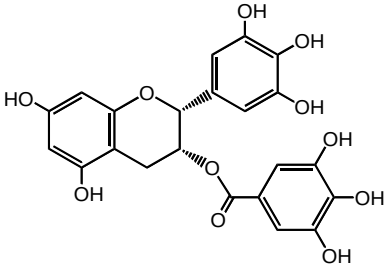


Figure 4: Total Polyphenols content of different types of dry tea leaves (by Folin-Denis method)

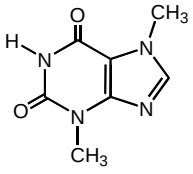
Analysis result showed that there are 5 major functional components found in Purple Tea Extract (Fig. 5, 6 and Table 1).



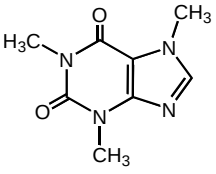
GHG (1,2-di-**G**alloyl-4,6-**H**exahydroxydiphenoyl-β-D-**G**lucose)



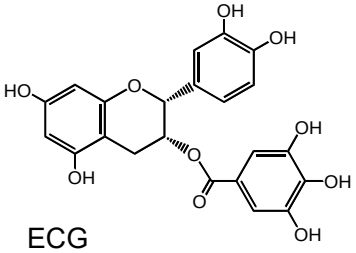
EGCG
(**E**pi**G**allo**C**atechin **G**allate)



Theobromine



Caffeine



ECG
(**E**pi**C**atechin **G**allate)

Figure 5: Functional Components of Purple Tea Extract

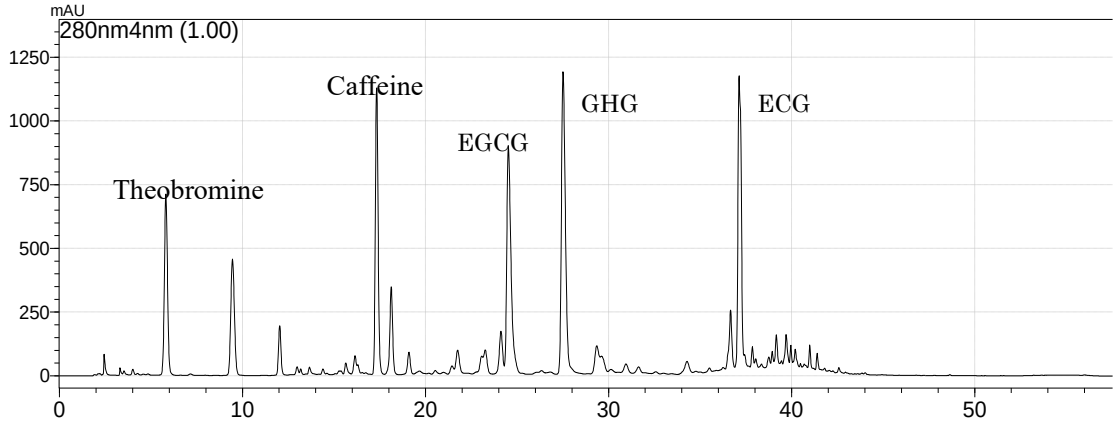


Figure 6: HPLC Chromatogram of Purple Tea Extract

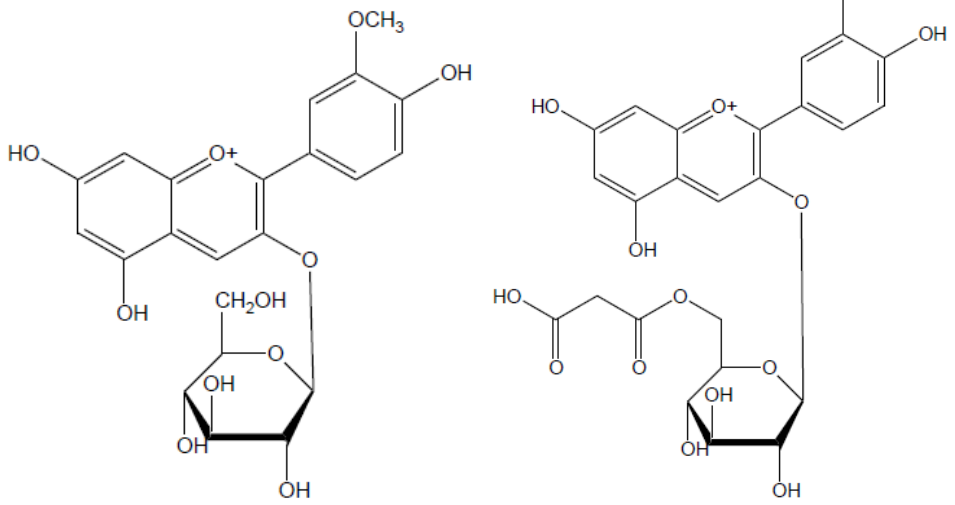
Purple Tea Extract is rich in polyphenols GHG and theobromine which is unusually found in other common teas such as green tea, black tea and oolong tea. (Table1).

Table 1: Content of functional components of Purple Tea Extract*

Functional Component	Content (%)
Total Polyphenol	50.4
Caffeine	4.5
Theobromine	1.6
GHG	7.4
EGCG	9.8
ECG	5.8
Chlorogenic Acids	0.9
Total Anthocyanin	1.5

*Extract does not contain excipients

Furthermore we analysed anthocyanins in purple tea and found that peonidin 3-*O*-(6''-malonyl glucoside and peonidin-3-*O*-glucoside were found to major anthocyanins in purple tea.



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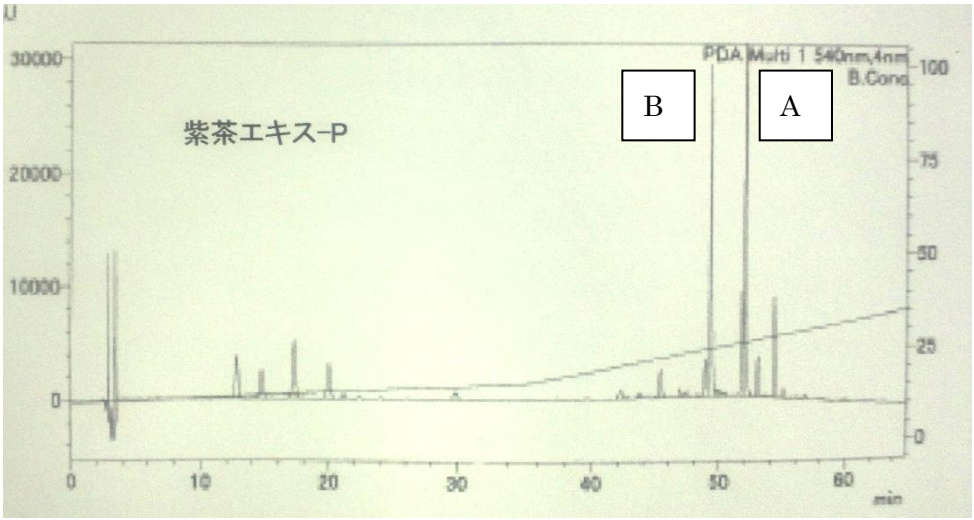

Figure 7: HPLC Chromatogram of anthocyanin fraction of Purple Tea Extract and chemical structures of major anthocyanin (right: peonidin-3-*O*-glucoside, left: peonidin 3-*O*-(6''-malonyl glucoside and)

3. Anti-obesity・ Diet Effect

(1) Inhibition of Lipid Absorption

In an experiment loading olive oil in mouse, Purple Tea Extract showed strong inhibitory effect on lipid absorption. In addition, at same concentration, the inhibitory effect of Purple Tea Extract was stronger than commercially available FOSHU (Food for Specific Health Use) Oolong Tea Extract. (Figure 8)

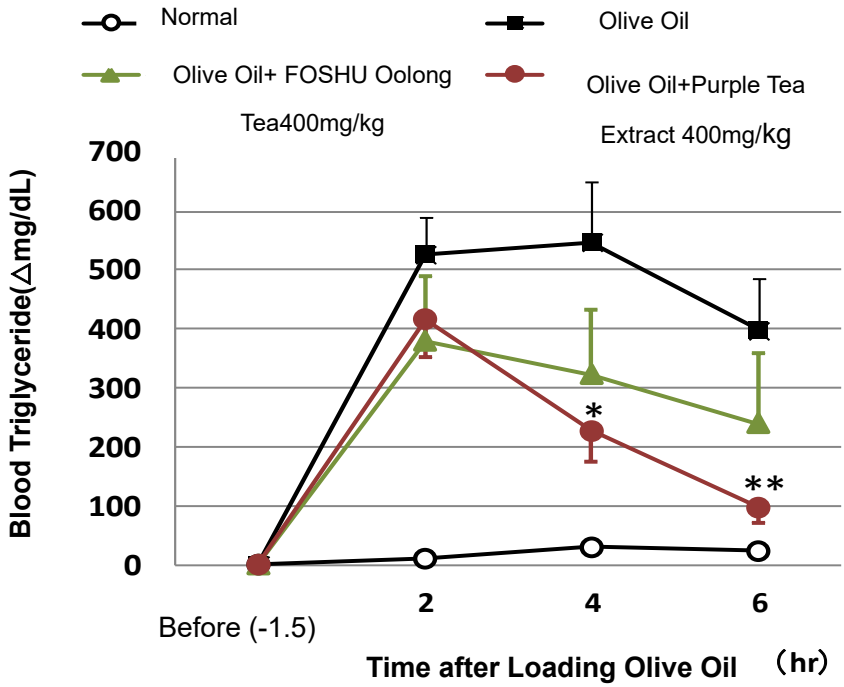

Figure 8: The effect of Purple Tea Extract on lipid absorption
(N = 5-6, mean ± S.D. * <0.05, <0.01, vs. Olive oil) treated group ** p p)

[Method of Experiment]

Male ICR mice were divided into 4 groups, each group 5-6 mice, were fasted for 15 hours. Blood sample was collected from the orbital venous sac using glass capillary. Samples containing 5% acacia gum in oral suspension (10mL/kg) was given to mice 30 minutes later. Loading of olive oil (5mL/kg) via oral route followed 1-hour later. Blood sample was collected at time 2-hour, 4-hour and 6-hour from the orbital venous sac for analysis. Blood serum was separated by centrifugation, triglyceride level was measured by enzymatic method using Triglyceride E-Test Wako, Wako Pure Chemical Industries Ltd.

(2) Inhibition of Fat Accumulation

Further experiment prompted to evaluate the effect of Purple Tea Extract on obese model. Mice were given feed with high calorie diet, high calories diet with Purple Tea Extract and FOSHU Green Tea Extract. Results showed that increased in weight gain is suppressed in mice consuming high calorie diet supplemented with Purple Tea Extract (200mg/kg), similar effect observed in mice consuming normal diet (Fig. 9). Upon comparison with FOSHU Green Tea Extract, Purple Tea Extract demonstrated a stronger effect in the prevention of weight gain caused by high calorie diet.

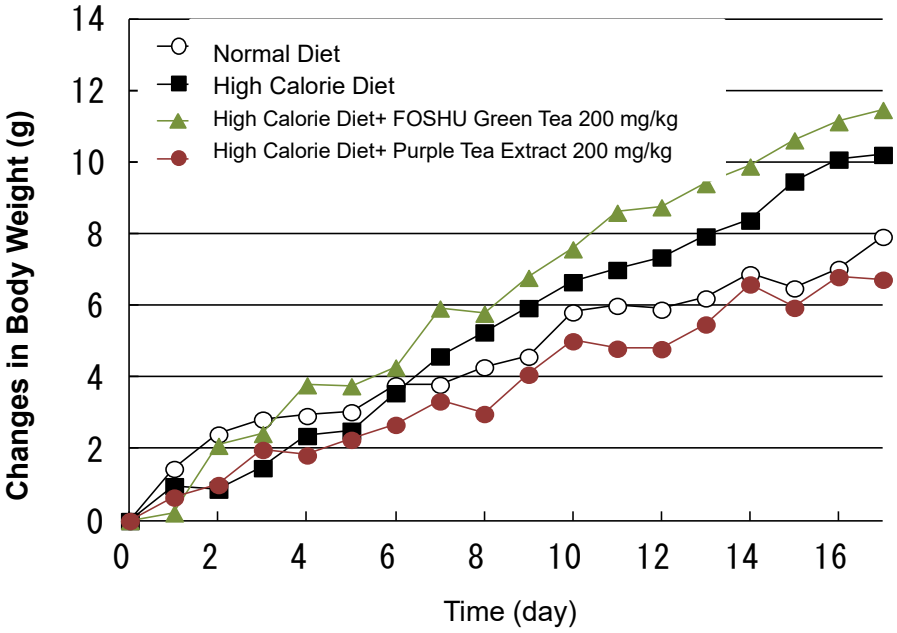

Figure 9: The effect of Purple Tea Extract on weight gain.
(N=6, mean±S.D.)

[Method of Experiment]

Male ICR mice (10-week old) were divided into 4 groups, each group 6 mice, were fed once daily (normal feed, High Fat Diet 32, High Fat Diet 32 + Purple Tea Extract 200mg/kg, High Fat Diet 32 + FOSHU Green Tea Extract). After 17-days, body weight was measured followed by fasting for 14 hours, blood sample was collected for analysis and weight of organs was measured. Blood serum and liver triglycerides was analyse by enzymatic method (Triglyceride E-Test Wako, Wako Pure Chemical Industries, Ltd.). Results were compared with mice consuming normal diet (CE-2).

As illustrated in Fig. 10, increased in weight of visceral fat (perirenal fat and epididymal fat) is suppressed in group of mice consuming high calorie diet (High Fat Diet-32) + Purple Tea Extract.

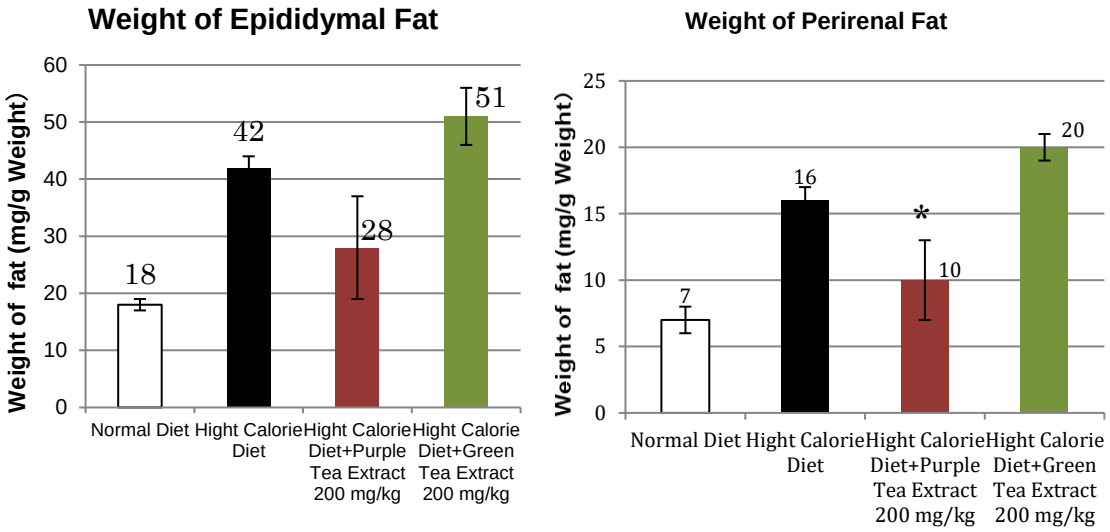

Figure 10: The effect of Purple Tea Extract on visceral fat
(N=6, mean±S.D., * p<0.05, vs High Calorie Diet group)

In addition, results also showed that liver triglyceride and blood triglyceride was reduced in group of mice consuming high calories diet (High Fat Diet-32) + Purple Tea Extract (Fig. 11).

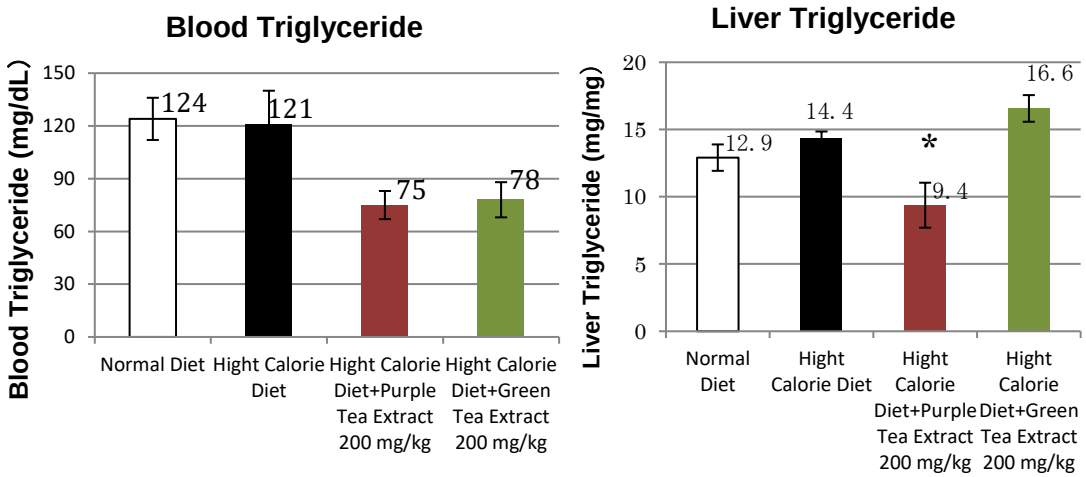

Figure 11: The effect of Purple Tea Extract on blood triglyceride
(N=6, mean±S.D., * p<0.05, vs High Calorie Diet group)

(3) Improvement on Fat Metabolism

Fatty Acids are released from adipose tissues during lipolysis and transported to the liver for metabolism. Fatty acids are transported into the mitochondria in the hepatocytes undergoing β-oxidation for energy production. Fatty acids are transported across the outer mitochondria membrane by carnitine palmitoyl transaferase (CPT-1). CPT-1 is believed to be a rate-limiting enzyme in the process of β-oxidation. Our experiment findings showed that Purple Tea Extract and its functional component, GHG, up-regulated the expression of CPT-1A in the hepatocytes (Fig. 12), therefore, Purple Tea Extract and its functional component, GHG, is believed to improve fat metabolism in the hepatocytes.

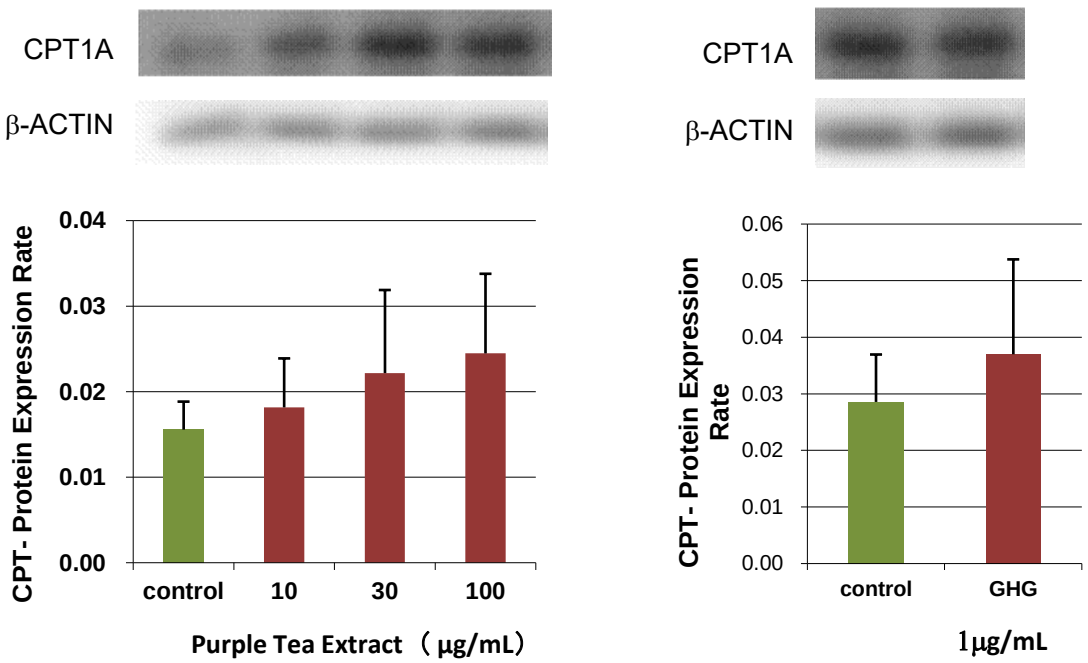

Figure 12: The effect of Purple Tea Extract & GHG on the expression of CPT in hepatocytes (N=4,mean±S.D.)

[Method of Experiment]

HepG2 hepatocytes was cultured in medium containing test sample for 24-hour. Cells were collected and the protein expression of CPT was analyzed by Western Blot method.

(4) Inhibition of Pancreatic Lipase

Further in-vitro experiment was conducted to evaluate the effect of Purple Tea Extract and its functional component, GHG, on the effect of pancreatic lipase. Pancreatic lipase is the enzyme that involved in the degradation and absorption of fat at the intestine. Results showed that Purple Tea Extract and GHG inhibited pancreatic lipase with increasing concentration (Fig. 13).

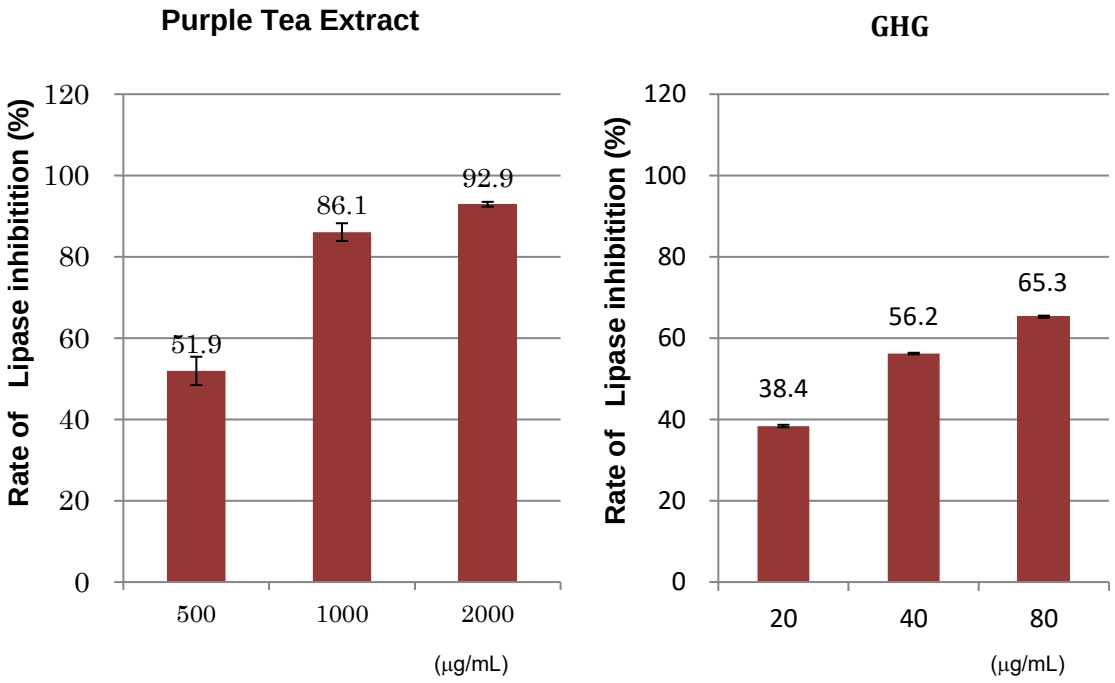

Figure 13: The effect of Purple Tea Extract and GHG on Pancreatic lipase.
(N=3, mean±S.D.)

[Method of Experiment]

Porcine derived pancreatic lipase (SIGMA Co.) was used and analyzed by Lipase Kit-S (Dainippon Pharmaceutical).

(5) Effects of Purple Tea Extract and GHG on Clock Gene Expression in Muscle Cells

Recently several clock genes have been reported to affect to mental and metabolic conditions. Thus we evaluated the effect of purple tea extract and GHG on a clock gene expression in muscle cells.

[Method of Experiment]

Mouse muscle cells (C2C12) were treated with purple tea extract（0.1-10 μg/mL）or GHG（0.1-3 μg/mL）for a week. Then RNA was extracted and evaluated mRNA expression of Bmal1 which highly expresses in night and regulates mRNA suppressing fat accumulation.

[Results]

As shown in Fig. 14, purple tea extract and GHG enhanced Bmal1 expression. Chatterjee *et al.* reported that Bmal1 is an important clock gene for fat acculumation and muscle fibers. Therefore increase in Bmal1 is expected to fat reduction, muscle increase and thicker muscle fiber. Through this event, Purple Tea Extract can be expected to reduce fat accumulation with increase in muscle fibers.

Reference) Chatterjee et al., *Jounal of Cell Science* 126, 2213-2224 (2013)

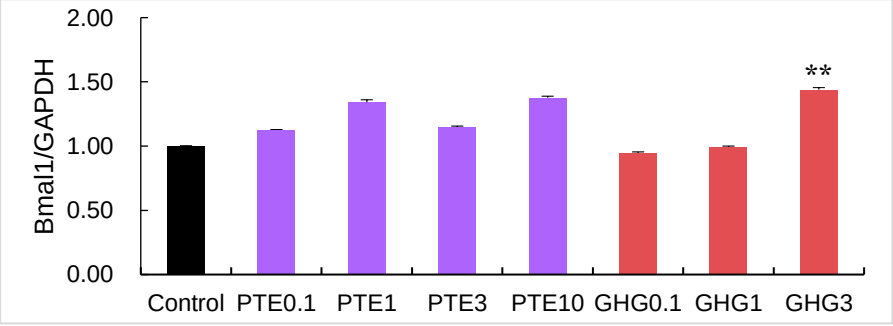

Figure 14: The effect of Purple Tea Extract (PTE) and GHG on Bmal1 expression. (mean±S.D., **: p<0.01)

(6) Human Monitor Test

Part 1.

Human Monitor Test was conducted on healthy volunteers (Male: 11, Female:7) for 4-week continuous oral consumption of Purple Tea Extract to verify its functional effect on beauty and diet on human being.

[Test sample]

Purple tea Extract in hard capsules (equiv. to Purple Tea Extract-P 100mg).

[Test subjects]

Male volunteers: 11, aged 23-48 year-old, (average age: 32.9 years old)
Female volunteers: 7, aged 27-60 year-old, (average age: 41.0 years old)

[Test method]

Test subjects were fasted the day prior to the start of the test, blood sample was collected, and measurements of:
subcutaneous fat, thickness of the upper arm, body weight, body fat percentage, parameter of the hip and waist, parameter of the abdomen, water content and sebum level of the cheek (measured by CORNEOMETER SM825) were taken prior to the test while collagen density (collagen score) of skin dermis was determined by DermaLab Ultrasound Imaging System.
Test subjects were required to take the sample (containing Purple Tea Extract-P 100mg) once daily after breakfast for 4 weeks. Upon completion of the test, above test parameters measurement was taken again for comparison. Significant differences correspond to t-test between the 2 groups.

[Results and Discussion]

As showed in Figure 15 and Table 2, thickness of subcutaneous fat of upper arm and abdomen was reduced after 4-week of daily oral intake of Purple Tea Extract capsule (containing Purple Tea Extract-P 100mg). The percentage of body fat reduced significantly among the female test subjects. No significant changes observed in the blood profile analysis, however, there was a reduction in blood glucose level and LDL-cholesterol level among the female test subjects.
Analysis results of DermaLab Ultrasound Imaging System showed that moisture level

and collagen score of the cheek increased while the sebum level decreased after the 4-week intake of Purple Tea Extract capsules.

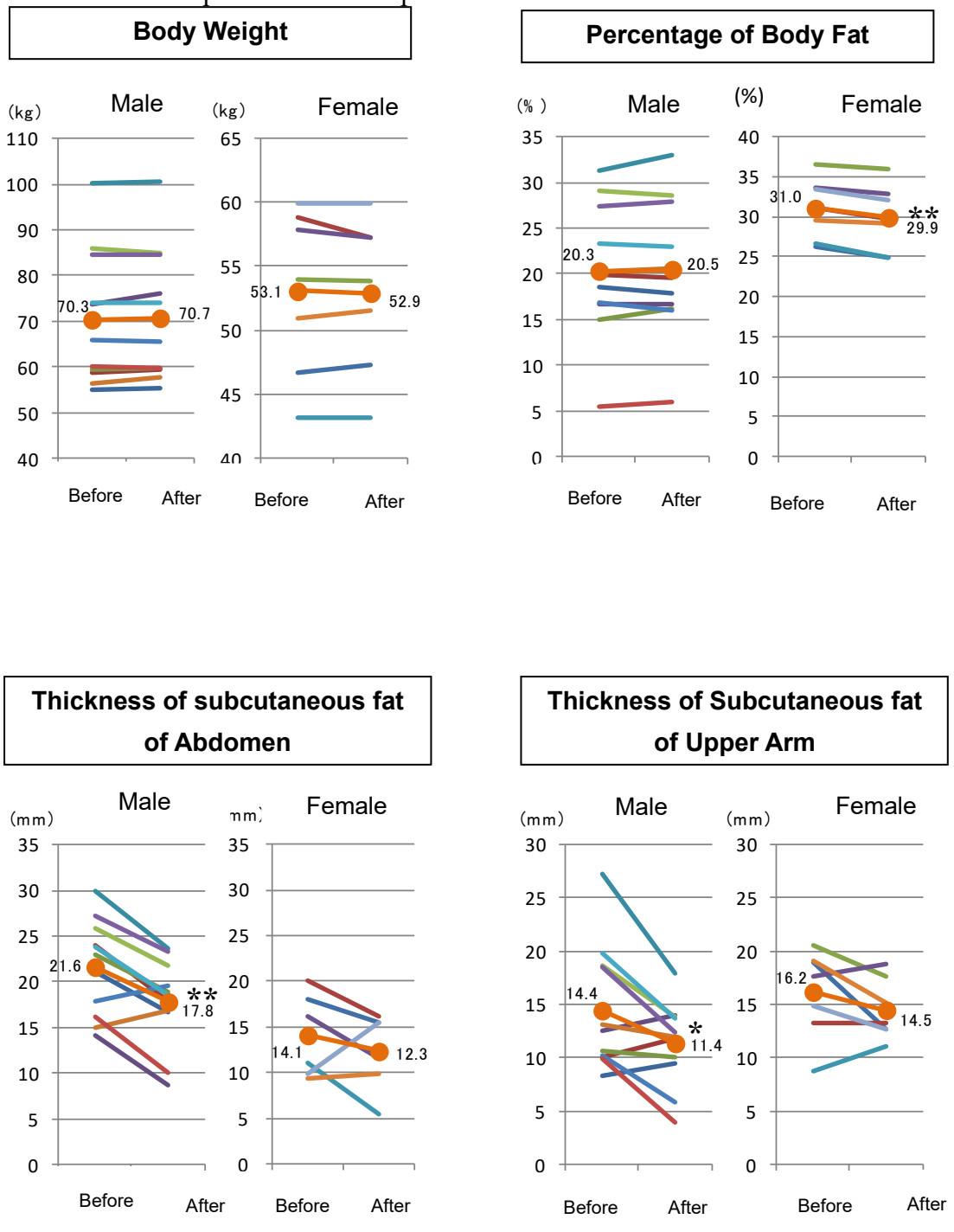

Figure 15A: The effect of Purple Tea Extract on human via oral route (* ⬬ * mean, , * $p < 0.05$, ** $p < 0.01$, vs. before intake)

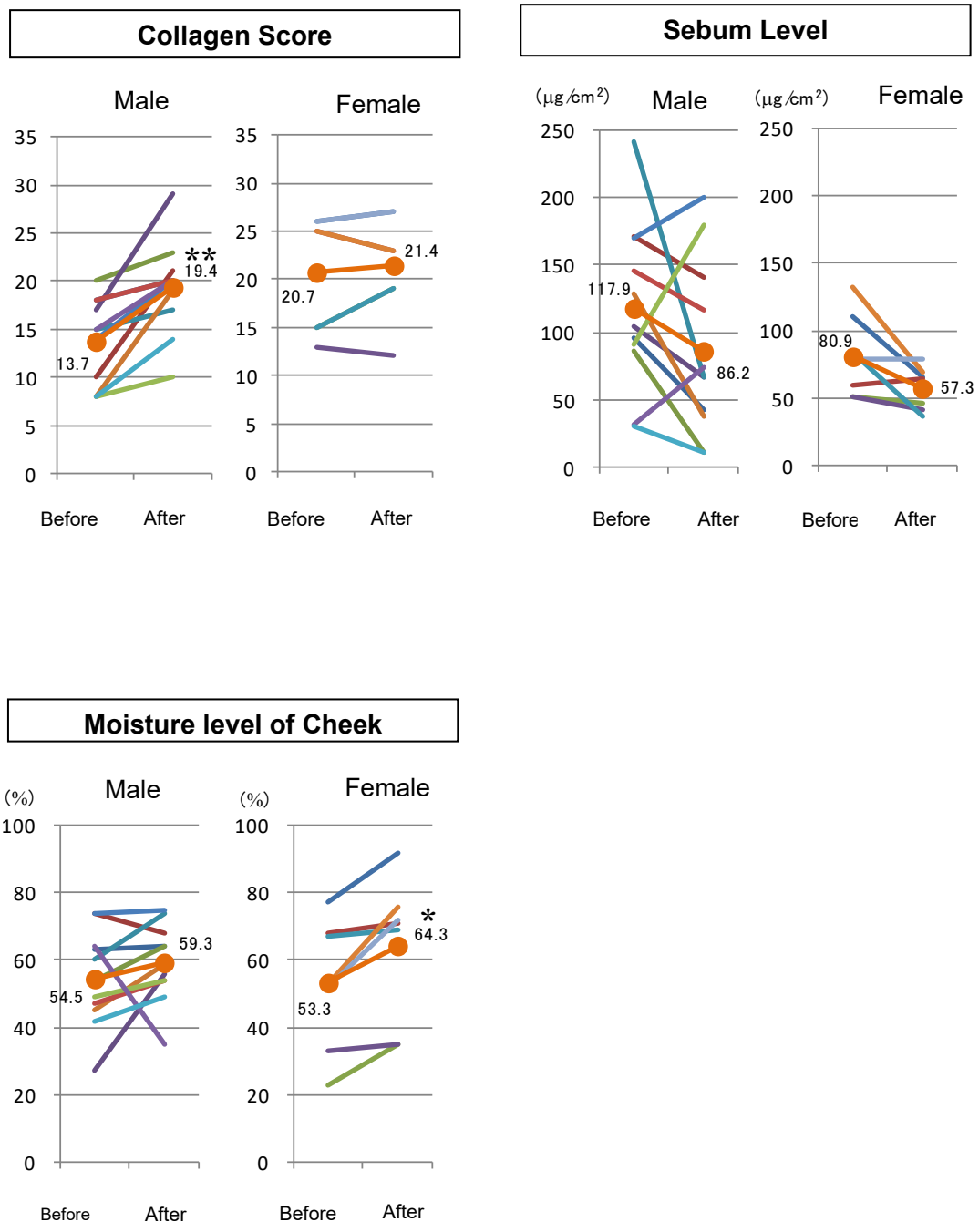

Figure 15B: The effect of Purple Tea Extract on human via oral route (* * mean, , * $p <$ 0.05, ** $p < 0.01$, vs. before intake)

Table 2: The effect of Purple Tea Extract on physical parameter of human via oral route (mean, , * $p < 0.05$, ** $p < 0.01$, vs. before intake)

① **Physical Parameters**

	Male		Female	
	Before	After	Before	After
BMI (kg/m^2)	23.5±4.3	23.5±4.2	20.7±1.5	20.5±1.3
Weight (kg)	70.3±14.8	70.7±14.5	53.1±6.4	52.9±6.0
Body Fat (%)	20.3±7.3	20.5±7.4	31.0±3.8	29.9±±4.1**
Muscle mass (%)	33.4±3.0	33.3±3.0	27.4±1.7	27.7±1.9
Waist (cm)	82.6±10.9	82.8±12.4	70.8±4.8	71.8±5.1
Hip (cm)	97.3±7.7	97.3±7.6	92.2±3.0	90.3±1.9**
Ratio of waist/hip	0.85±0.05	0.85±0.07	0.77±0.05	0.80±0.05
Abdominal fat (mm)	21.6±5.3	17.8±4.8**	14.1±4.5	12.3±4.2
Fat of upper arm (mm)	14.4±5.8	11.4±3.9*	16.2±0.4	14.5±0.3

② **Blood Profile Analysis**

	Male		Female	
	Before	After	Before	After
TG (mg/dL)	93.0±48.7	99.2±39.3	52.8±10.5	58.0±14.4
Free Fatty Acid (mEq/L)	0.6±0.2	0.5±0.2	0.5±0.2	0.7±0.4
Phospholipids (mg/dL)	214.2±31.5	216.5±21.6	239.8±28.8	231.5±34.2
Total Cholesterol (mg/dL)	188.3±33.3	194.9±29.5	224.2±39.4	222.5±38.5
LDL-C (mg/dL)	114.5±35.6	116.0±33.6	141.7±42.8	135.0±40.5
HDL-C (mg/dL)	59.1±9.8	54.5±19.8	75.0±15.5	75.7±15.3
Blood glucose (mg/dL)	91.9±11.5	91.5±11.7	88.5±9.5	83.0±6.9
Total Protein (g/dL)	7.4±0.3	7.4±0.3	7.3±0.4	7.5±0.6
Albumin (g/dL)	4.8±0.2	4.8±0.2	4.4±0.4	4.6±0.3
Urea Nitrogen (mg/dL)	14.9±3.6	13.0±2.3	14.3±3.8	12.2±3.1
Uric Acid (mg/dL)	6.1±1.6	6.0±1.5	4.7±0.7	4.4±0.6
Total Bilirubin(mg/dL)	0.6±0.2	0.6±0.2	0.6±0.3	0.8±0.2
Creatinine (mg/dL)	0.8±0.1	0.8±0.1	0.6±0.1	0.6±0.1

Figure 16 showed the analysis results of Dermlab Ultrasound Imaging System where collagen score of the cheek increased among female subjects and among male subjects with significance ($p<0.01$) after 4-week of oral intake of Purple Tea Extract.

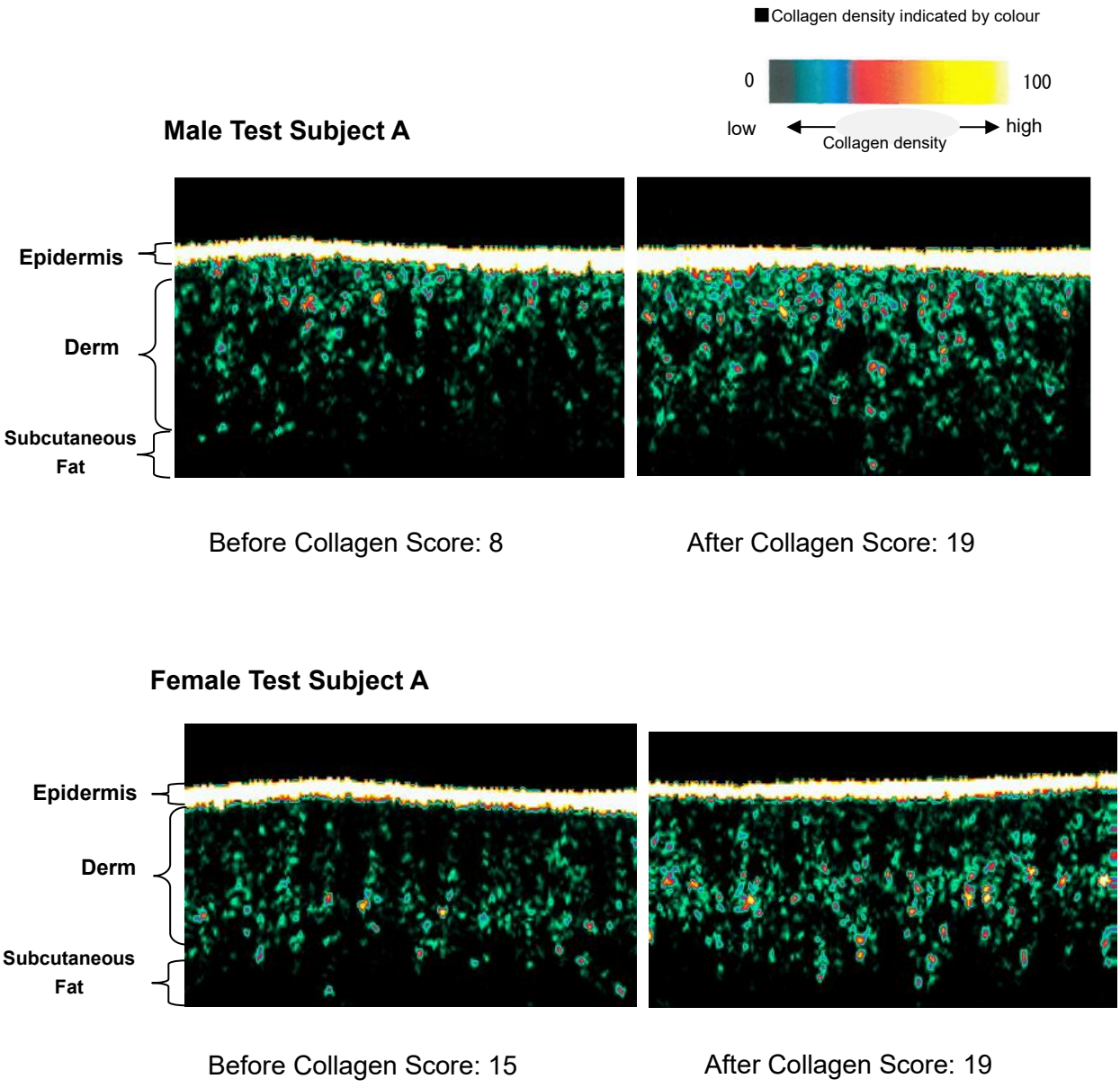

Figure 16: The effect of Purple Tea Extract on the ultrasound images of cheek

Part 2.

Human Monitor Test of purple tea leaves was also conducted on healthy male volunteers for 4-week continuous oral consumption.

Reference) Shimoda H., Hitoe S., Nakamura S., Matsuda H. Purple tea and its extract suppress diet-induced fat accumulation in mice and human subjects by inhibiting fat absorption and enhancing hepatic carnitine palmitoyltransferase expression. *Int. J. Biomed. Sci.* 11, 67-75 (2015).

[Test sample]

Purple tea leaves (1.5 g/portion).

[Test subjects]

Male volunteers with mild obesity: 10, aged 32-69 year-old, (average age: 47.1 years old)

[Test method]

The experiment was performed in accordance with the 6th revision of the Declaration of Helsinki of 2008. On the first day of the test period, blood samples were collected from fasted test subjects. Then BMI, bodyweight, body fat mass, abdominal fat, muscle amount, body fat ratio, muscle ratio, basal metabolism, moisture, waist, hip, waist: hip ratio, abdominal subcutaneous fat thickness, right upper arm subcutaneous fat thickness were measured. After the measurement, dried purple tea leaves (1.5 g/portion) were given to each subject. The subjects ingested the tea extracted from the purple tea portion with hot water (100-200 mL) twice a day for 4 weeks. On the last day of the test period, measurements of obesity parameters were carried out again to compare them with the values before ingestion.

[Results and Discussion]

No adverse effects were observed during the test period. As shown in Table 3, after a 4-week ingestion of purple tea, BMI, bodyweight, body fat mas, abdominal fat, body fat ratio, waist size, hip size, and abdominal and right upper arm fat thickness were significantly reduced comparing to the values before ingestion. On the other hand, muscle ratio significantly increased after the ingestion of purple tea. There were no significant changes in blood parameters, however, HDL-cholesterol and HbA1c levels tended to be lower than those of before ingestion. (Table 4).

Table 3. Comparison of Obesity Parameters Before and After Four-Week Ingestion of Purple Tea

	Before Ingestion	After Ingestion
BMI (kg/m^2)	27.0±0.6	26.8±0.6*
Body weight (kg)	80.8±3.2	79.9±3.1*
Body fat mass (kg)	21.8±1.5	21.0±1.4**
Abdominal fat (arbitral unit)	135.0±8.5	123.5±8.5**
Muscle amount (%)	24.9±1.0	25.0±1.0
Body fat ratio (%)	26.8±1.2	26.1±1.2*
Muscle ratio (%)	30.9±0.5	31.4±0.5**
Basal metabolism (kcal)	1789±73	1768±70*
Moisture (%)	43.2±1.6	39.4±4.3
Waist (cm)	97.6±1.6	94.2±1.7**
Hip (cm)	106.0±1.5	102.8±1.6*
Waist/hip ratio	0.92±0.01	0.92±0.01
Abdominal subcutaneous fat thickness (mm)	28.5±1.4	24.4±1.8*
Right upper arm subcutaneous fat thickness (mm)	28.5±2.6	21.5±1.6**

Values are indicated in average value and standard error (n=10). Paired *t*-test was used for evaluation of significance. Significant differences are indicated as *: $p < 0.05$, **: $p < 0.01$, vs. before ingestion.

Table 4. Comparison of Blood Parameters Before and After Four-Week Ingestion of Purple Tea Extract

	Before Ingestion	After Ingestion
Triglyceride (mg/dL)	143.7±19.4	125.9±23.2
Free fatty acid (mEq/L)	0.63±0.05	0.55±0.04
Total cholesterol (mg/dL)	195.8±8.6	183.2±6.6
LDL-cholesterol (mg/dL)	120.8±8.9	110.4±6.8
HDL-cholesterol (mg/dL)	53.6±4.7	51.9±4.3 p=0.09
Blood glucose (mg/dL)	102.7±5.6	99.2±4.5
HbA1c (%)	5.8±0.2	5.6±0.2 p=0.07

Values are indicated in average value and standard error (n=9). Paired *t*-test among was used for evaluation of significance. No significance was observed.

Shimoda H., Hitoe S., Nakamura S., Matsuda H. Purple tea and its extract suppress diet-induced fat accumulation in mice and human subjects by inhibiting fat absorption and enhancing hepatic carnitine palmitoyltransferase expression. *Int. J. Biomed. Sci.* 11, 67-75 (2015).

4. Anti-ageing of Skin

Inhibition of Lipid Peroxidation

Lipid peroxidation occurs when lipids in the cell membrane undergone oxidative degradation. Exposure of cells to ultraviolet light is an example. Chain reactions from lipid peroxidation may result in increased accumulation of lipid peroxide that is responsible for ageing skin, cell damage and inflammation (Figure 17).

In an experiment using normal human epidermal keratinocytes, Purple Tea Extract has been shown to inhibit lipid peroxidation induced cytotoxicity (Figure 18). This inhibitory effect on lipid peroxidation-induced cytotoxicity is specifically to Purple Tea Extract and not commonly found in other tea such as oolong tea, green tea and tea. In addition, upon comparison with generally popular tea polyphenols such as ECG and EGCG, GHG which is loaded in Purple Tea Extract has been shown to have the most potent effect on lipid peroxidation-induced cytotoxicity (Figure 19). Therefore, it is suggestive that GHG is the active functional component of Purple Tea.

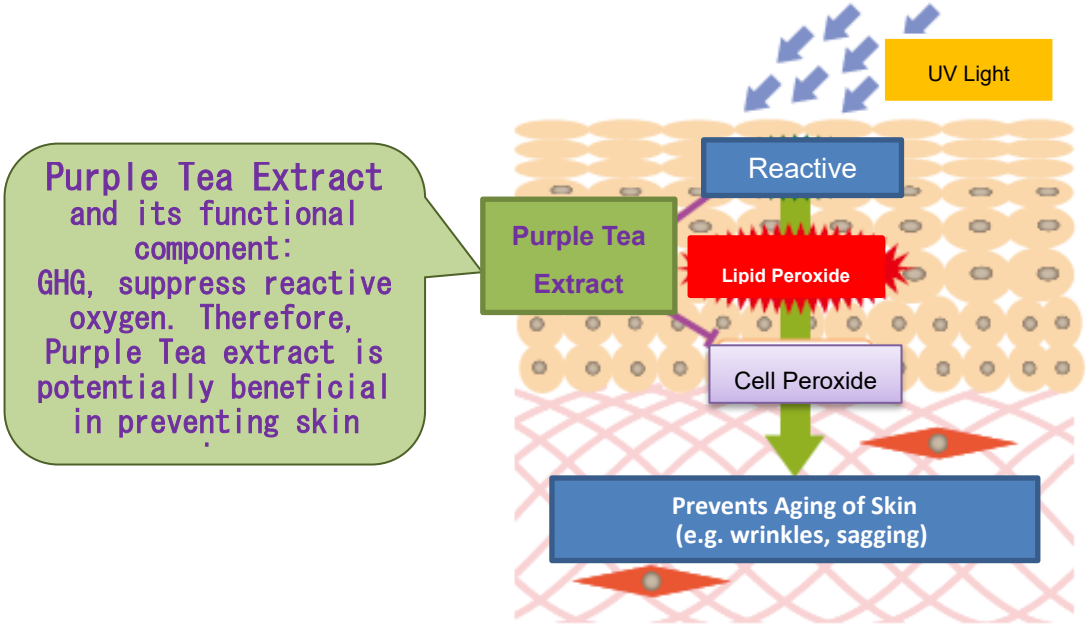

Figure 17: The mechanism of Purple Tea Extract on ageing of skin

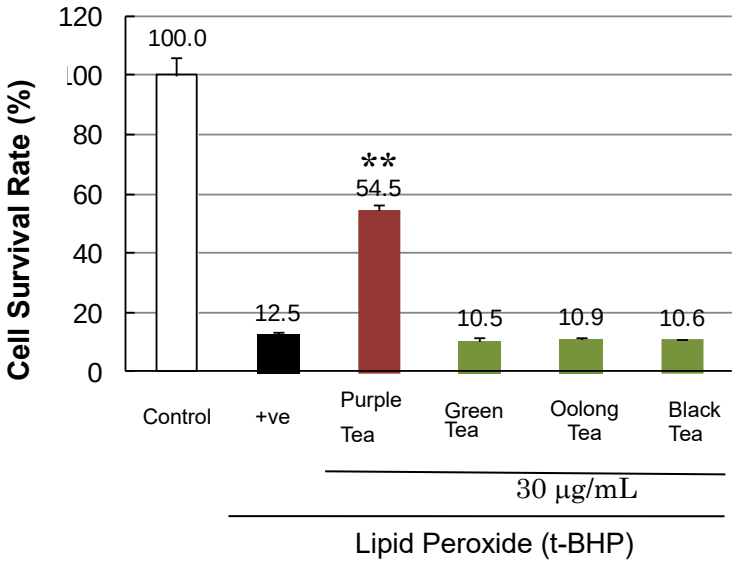

Figure 18: The effect of Purple Tea Extract on Lipid Peroxidation
(N=6, mean±S.S., **p<0.01, vs solvent)

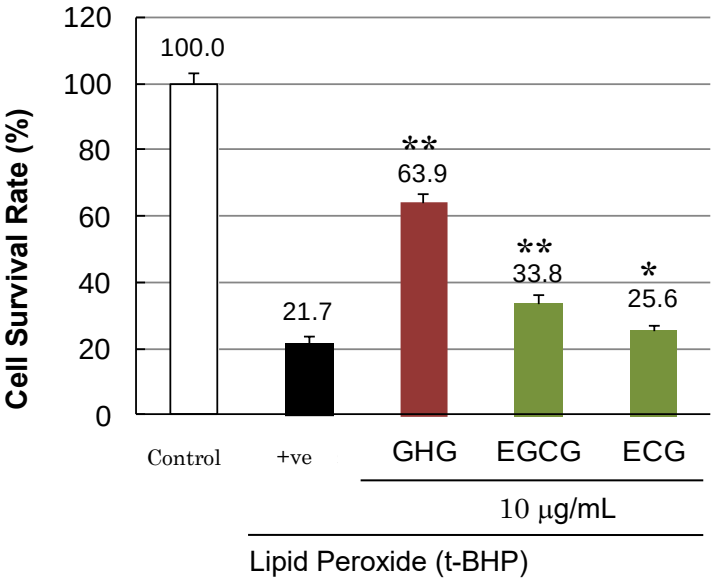

Figure 19: The effect of GHG, from Purple Tea Extract on lipid peroxidation
(N=6, mean±S.S., *p< 0.05, **p<0.01, vs solvent)

5. Antioxidant Effect

Reactive oxygen species (ROS) are generated through normal metabolism. However, environmental stress such as UV exposure and oxidative stress due to modern lifestyle may increase levels of ROS. Figure 20 & 21 showed that Purple Tea Extract demonstrated strong antioxidant effect on DPPH & SOD model.

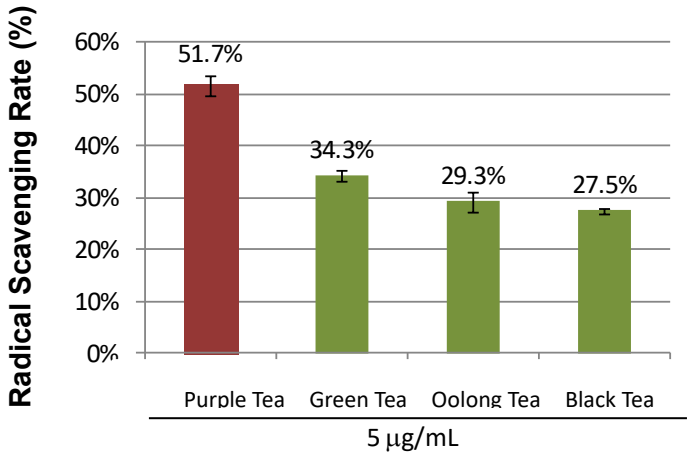

Figure 20: The effect of different tea extracts on DPPH radical scavenging model.

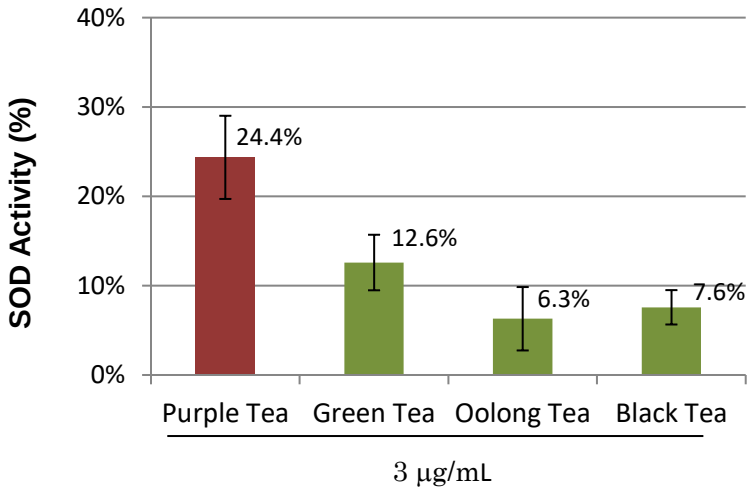

Figure 21: The effect of different tea extracts on SOD model.

[Method of experiment]

Different type of tea extracts was dissolved in DPPH solution, DPPH radical scavenging activity was measured by absorbance of faded DPPH. Meanwhile, SOD activity was measured using SOD Test Kit Wako (by Wako Pure Chemical Industries).

6. Whitening Effect

Effect on Tyrosinase

Melanin is responsible for the formation of freckles and dark spots on the skin upon UV exposure. Tyrosinase is the enzyme that catalyzes the production of melanin. As illustrated in Figure 22, Purple Tea Extract inhibited the activity of tyrosinase in a dose-dependent manner. Therefore, it is suggestive that Purple Tea Extract may have skin lightening effect.

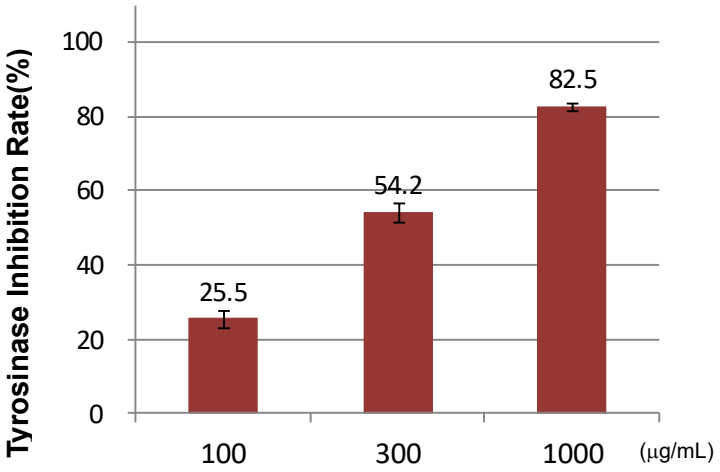

Figure 22: The effect of Purple Tea Extract on Tyrosinase activity

[Method of Experiment]

40mM of phosphate buffer solution (pH:6.8) 1360μL and 0.4mg/mL L-DOPA (from ACROS Co.) 500μL was dissolved in DMSO followed by mixing with test samples 40μL. Then, tyrosinase (300units/mL) (Sigma Co., mushroom derived) 100μL was added and allow to stand for reaction at room temperature for 5 min. Activity of tyrosinase was measured at absorbance wavelength 490nm.

7. Anti-oxidant Activity

Tea has been regarded as healthy beverage with known functionalities on health. One of them is its anti-oxidant activity. PURPLE TEA is grown in Kenya with rich content of 1,2-di-**G**alloyl-4,6-**H**exahydroxydiphenoyl-β-D-**G**lucose or GHG, a unique variety of polyphenol. GHG has excellent anti-oxidant activity and is not found in other type of teas. On the other hand, WHITE TEA is a lightly fermented tea harvested in China, and has high content of polyphenols. Study was conducted to compare the anti-oxidant activity between PURPLE TEA EXTRACT and WHITE TEA EXTRACT.

【Test Methods】

PURPLE TEA EXTRACT contained 56.7% polyphenols. WHITE TEA EXTRACT (containing 95.4% polyphenols) was diluted by dextrin to adjust the polyphenol content to 56.7% as PURPLE TEA EXTRACT for comparison. Anti-oxidant activity was determined using DPPH assay. Similarly, the anti-oxidant activity of polyphenols, namely, GHG and EGCG was compared.

【Test Results】

① Comparison of anti-oxidant activity between PURPLE TEA EXTRACT and WHITE TEA EXTRACT.

Both PURPLE TEA EXTRACT and WHITE TEA EXTRACT scavenged DPPH radicals. The scavenging effect was concentration-dependent in the concentration range of 1~30 μg/mL. And the anti-oxidant activity of PURPLE TEA EXTRACT was as strong as WHITE TEA EXTRACT. (Fig.23)

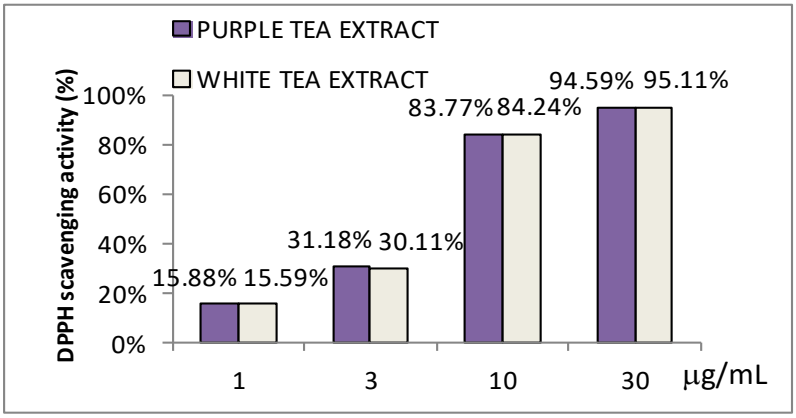

Figure 23: Comparison of DPPH scavenging activity between PURPLE TEA EXTRACT and WHITE TEA EXTRACT.

② Comparison of anti-oxidant activity between GHG and EGCG.

Both GHG and EGCG quenched DPPH radicals. The DPPH quenching effect of GHG was more potent than EGCG. Therefore, the anti-oxidant activity of GHG was higher than EGCG. (Fig.24)

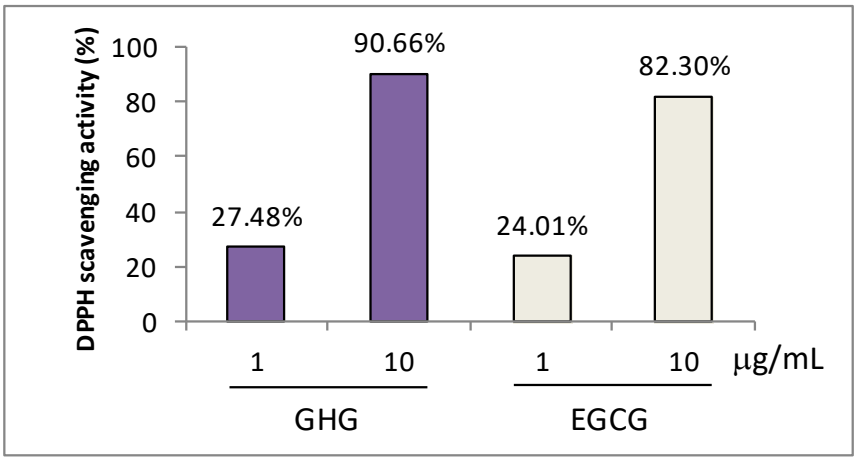

Figure 24: Comparison of DPPH scavenging activity between GHG and EGCG.

【Discussion】

It has been shown that the anti-oxidant activity of PURPLE TEA EXTRACT and WHITE TEA EXTRACT was similarly strong. However, GHG has demonstrated higher potency in the anti-oxidant effect upon comparison with EGCG.

8. Stability

(1) Thermal Stability

① Thermal Stability of The Powder

Figure 25 showed the effect of heat on Purple Tea Extract-P and its polyphenols. After heating at 120°C for 2 hours, there is no reduction on the content of total polyphenols in Purple Tea Extract-P. Similarly, the content of GHG, main functional component of Purple Tea Extract, did not reduce after heating at 100°C for 2 hours.

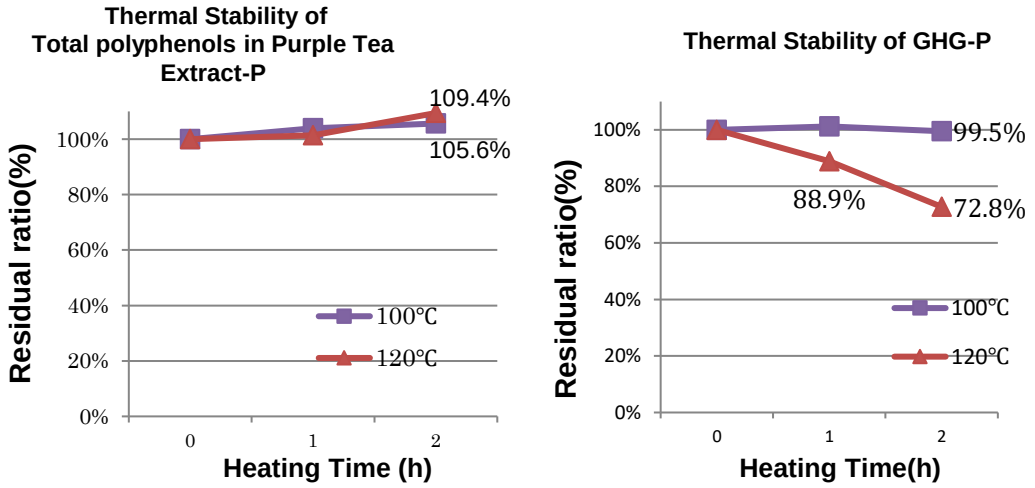

Figure 25: Thermal stability of Purple Tea Extract powder

② Thermal Stability of Purple Tea Extract in Aqueous Solution

Figure 26 showed the effect of heat on Purple Tea Extract in aqueous solution. Purple Tea Extract 0.1% in aqueous solution after heating at 100°C for 60 minutes did not show any reduction in its polyphenols content. The content of GHG did not reduced after heating at 80°C for 60 minutes.

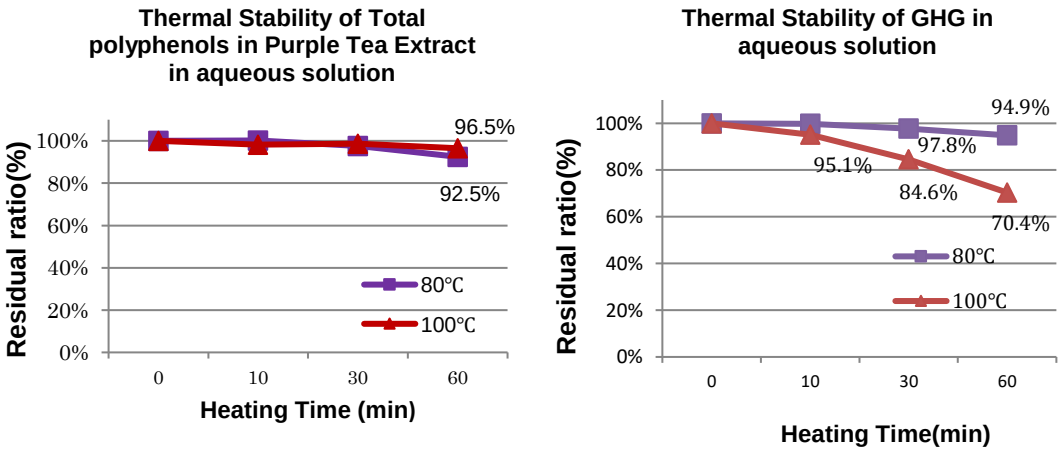

Figure 26: Thermal stability of Purple Tea Extract in aqueous solution

(2) pH Stability

① pH Stability of Purple Tea Polyphenols

The polyphenols of Purple Tea Extract in aqueous solution is highly stable at low pH environment, <pH5, after 1-week storage at room temperature and under refrigeration condition (4°C). There is no reduction in the content of polyphenols observed (Fig. 27).

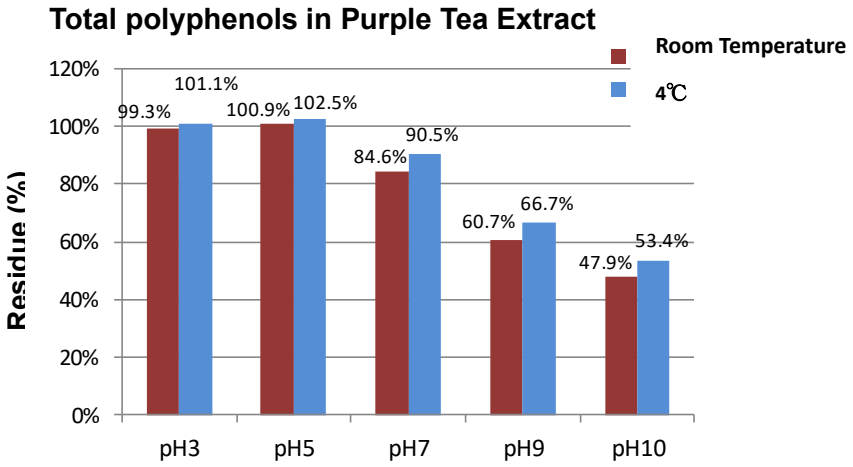

Figure 27: The pH stability of Polyphenols in Purple Tea Extract Aqueous Solution

② pH Stability of GHG

Further evaluation prompted to examine the pH stability of GHG of Purple Tea Extract in aqueous solution. Figure 28 showed that GHG remain stable at low pH condition, <pH5, after 1-week storage under refrigeration condition (4°C). There is no reduction in the content of GHG observed.

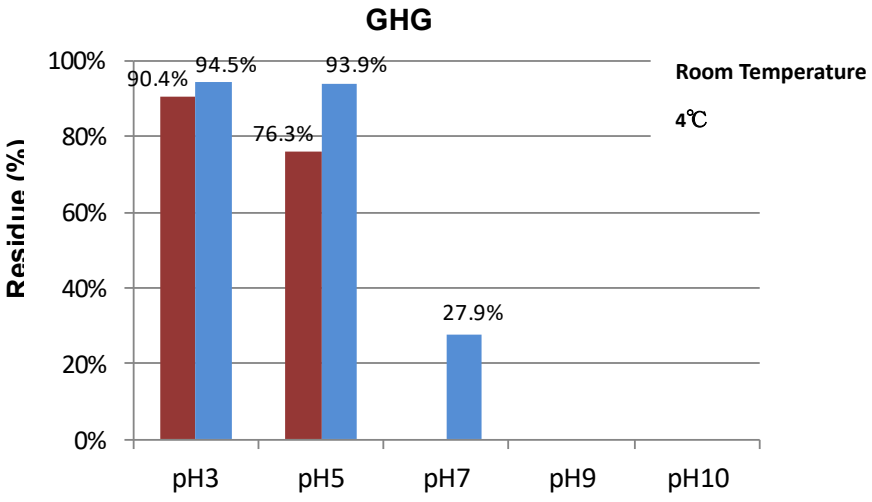

Figure 28: The pH stability of GHG of Purple Tea Extract aqueous solution (with light, 1 week storage)

③ Effect of pH on Colour Changes of Purple Tea Extract

Figure 29 showed the colour changes of Purple Tea Extract at different pH condition. Purple Tea Extract-P, (-PC) 0.1% in aqueous solution, normally has a light reddish brown colour at pH 4, the colour changed to pale red at pH 3. On the contrary, the colour become darker in brown colour with alkaline pH condition.

Figure 29: The effect of pH on colour changes of Purple Tea Extract-P, (-PC) 0.1% aqueous solution

Meanwhile, Purple Tea Extract-LC gives brown colour at pH 5. Similarly, the colour became darker with increasing pH, i.e. alkaline condition. The colour remain stable at weakly acidic condition, therefore, it may be incorporated into cosmetics application without any problem (Fig. 30, 31).

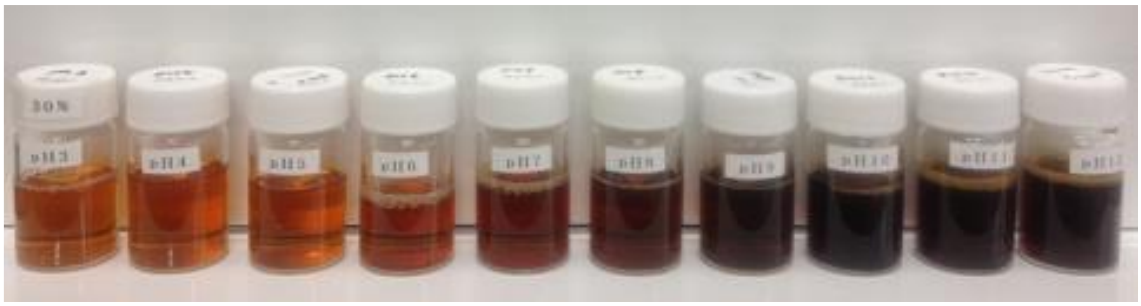

Figure 30: The effect of pH on colour changes of Purple Tea Extract-LC

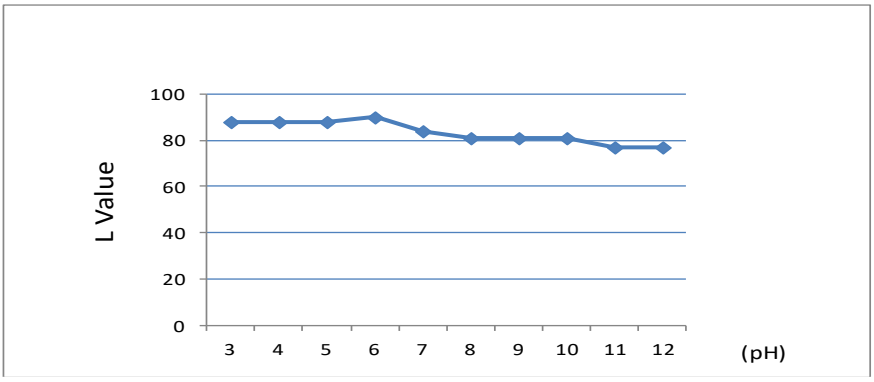

Figure 31: Colour changes of Purple Tea Extract-LC in Aqueous Solution of different pH after storing for one week (determined with a spectrophotometer, light, room temperature)

(3) Light Stability

① Light Stability of Purple Tea Polyphenols

Purple Tea Extract in Aqueous Solution was kept in 3 different packing: transparent bottle, brown bottle, aluminium bag. The content of total polyphenols was measured to determine the effect of light on Purple Tea Polyphenols. As shown in Figure 32, the content of total polyphenols in brown bottle and aluminium bag remained stable at pH 3.

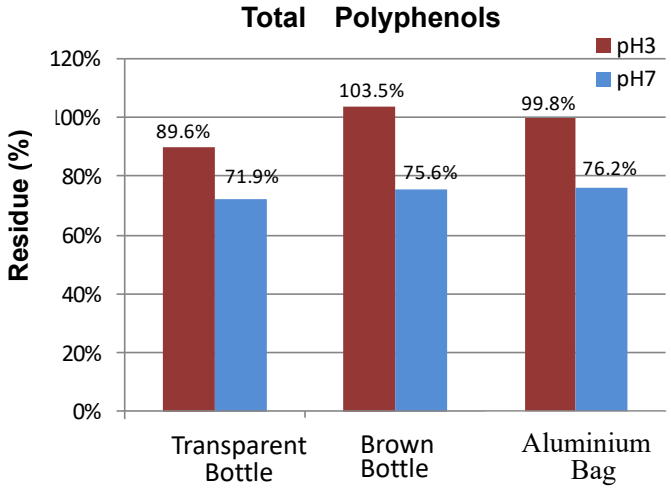

Figure 32: Light Stability of Polyphenols of Purple Tea Extract in Aqueous Solution.

(with light, room temperature, storage for 1 week)

② Light Stability of GHG

Figure 33 showed the effect of light on GHG content of Purple Tea Extract in aqueous solution. The degradation of GHG content in brown bottle and aluminium bag was prevented.

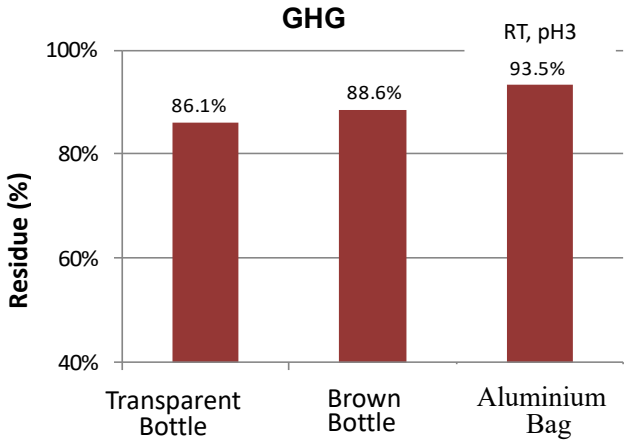

Figure 33: Light Stability of GHG of Purple Tea Extract in Aqueous Solution

(with light, room temperature, storage for 1 week)

(4) Storage Stability

The colour stability of Purple Tea Extract-LC under different storage conditions was determined by the spectrophotometer. The brown colour tended to become denser when stored by the window or at 40℃or at room temperature. Therefore, it is recommended to store and use in a dark place. (Fig.34)

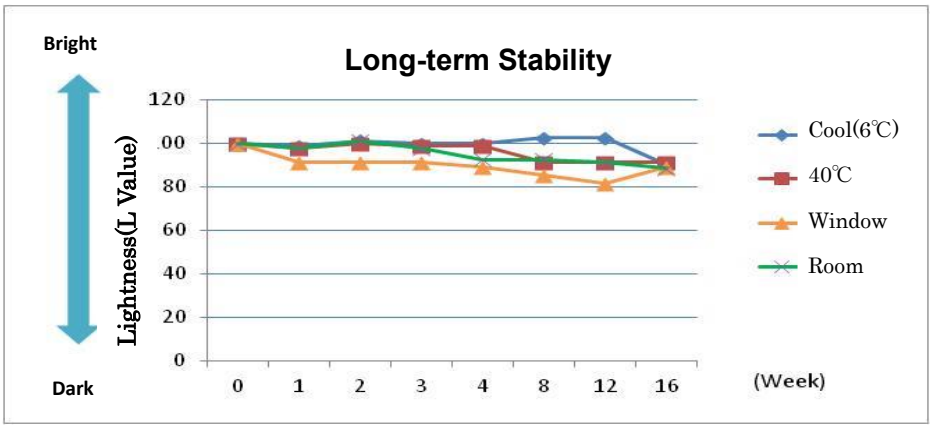

Figure 34: The colour stability of Purple Tea Extract-LC under different storage conditions

9. Nutritional Profile

Table 5: Nutritional Profile of Purple Tea Extract

Item	Per 100g edible portion	Analysis Method
	Purple Tea Extract-P	
Energy	377kcal	Modified Atwater method *
Protein	12.0g	Combustion method
Fat	2.2g	Acid degradation
Carbohydrate	77.0g	Calculation: 100 – (water + protein + fat + ash)
Sodium	22.4mg	Atomic absorption spectrophotometry
Sodium Chloride Equiv	<0.1g	Sodium equiv. value
Water	4.3g	Heat drying at atmospheric pressure
Ash	3.9g	Direct incineration
Fiber	0.6g	Prosky method

* Energy conversion: protein 4, fat 9, sugar 4, fiber 2

Test trustee: Food Analysis Technology Centre SUNATEC

Date of analysis: Sept 13, 2013 Test No.: 130902155-001-01

10. Safety Profile

(1) Residual Agricultural Chemicals

The raw material, Purple Tea leaves was collected from pesticides FREE plantation. No pesticides were used.

Purple Tea leaves : 166 pesticides items were not detected.
Test Trustee: Japan Ecotec Co., Ltd.
Date: June 20, 2013
Test No: 313288-1

(2) Acute Toxicity (LD_{50})

Acute Toxicity of Purple Tea Extract was conducted according to the Guidelines for Single-Dose Toxicity Tests for Pharmaceutical Products where Purple Tea Extract 2000mg/kg was orally given to mice (ICR, 5-week old, weight approximately 20-25g). And the mice were observed for 14 days. No abnormalities and fatal event observed at 2000mg/kg. No abnormalities of organs observed under macroscopic examination upon autopsy. Thus, LD_{50} of Purple Tea Extract is deduced to be >2000mg/kg.

(3) Mutagenicity

Ames test was conducted to evaluate the mutagenicity of Purple Tea Extract using Salmonella typhimurium TA98 and TA100. At concentration 19.5 - 5000μg/plate, no mutagenicity was observed.

11. Recommended dosage

Product	Claims	Recommended dosage
Purple Tea Extract-P	Anti-obesity, diet Anti-ageing Anti-oxidant, skin whitening	100mg/day

12. Recommended usage level

Product	claims	Recommended use level
Purple Tea Extract-PC	Anti-ageing of skin Anti-oxidant, skin whitening	0.003～0.03%
Purple Tea Extract-LC		0.5～5%

13. Applications

Uses	Applications	Claims	Examples
Foods	Nutraceuticals Beauty Foods	Anti-obesity Diet Anti-ageing Anti-oxidant, Skin Whitening	Beverages (soft drinks etc), hard and soft capsules, tablets, candies, chewing gum, cookies, chocolate wafers, jelly, *etc*.
Cosmetics	Beauty Cosmetics		Sunscreen, toner, lotion, body gel, shampoo, conditioner and bath salts, *etc*.

14. Packaging

Product	Packing	Weight
Purple Tea Extract-P (Water Soluble Power, FOOD grade)	Interior: Aluminium bag Exterior: Cardboard box	1 kg 5 kg
Purple Tea Extract-PC (Water Soluble Power, Cosmetics grade)	Interior: Aluminium bag Exterior: Cardboard box	1 kg 5 kg
Purple Tea Extract-LC (Water Soluble liquid, COSMETICS grade)	Interior ：Cubitainer Exterior：Corrugated packing	1 kg 5 kg 20 kg

15. Storage

It is recommended to avoid places with high temperature and high humidity, keep in dark at room temperature. It is recommended to finish using once opened. Desiccants may be used for keeping moisture away.

16. Expression

<FOOD>

Purple Tea Extract-P

Expression 1: Purple Tea Extract powder

Expression 2: Purple Tea Extract, Dextrin, Citric Acid

*It is advisable to check with Regional Agricultural Administration Office and Health Department for food labelling.

<Cosmetics>

Purple Tea Extract-PC

INCI：CAMELLIA SINENSIS LEAF EXTRACT, DEXTRIN, CITLIC ACID,

Expression: Tea leaves extract, dextrin, citric acid

Purple Tea Extract-LC

INCI：WATER, BUTYLENE GLYCOL,
CAMELLIA SINENSIS LEAF EXTRACT

Expression: water, BG , tea extract

Expression of quasi-drugs: purified water, 1,3 - butylene glycol, tea extract (1)

PRODUCT STANDARD

PRODUCT NAME

PURPLE TEA EXTRACT-P

FOOD

This product is extracted with aqueous ethanol from the leaves of purple tea (*Camellia sinensis*).

Appearance	Reddish brown to reddish purple powder with slightly unique scent.	
Polyphenols	Min. 30.0 %	(Folin-Denis method)
GHG*	Min. 3.0 %	(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. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Purple Tea Extract	60%
Dextrin	30%
Citric acid	10%
Total	100%

Expiry date	3 years from date of manufacturing.
Storage	Store it in a cool, dry, ventilated area with desiccant. Keep it away from high temperature and sunlight, and store it in a closed container.

*1,2-di-Galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose

PRODUCT STANDARD

PRODUCT NAME

PURPLE TEA EXTRACT-WSP

FOOD

This product is extracted with water from the leaves of purple tea (Camellia sinensis). It contains a minimum of 10.0 % polyphenols and 2.0 % GHG*. This product is water soluble.

Appearance	Reddish brown to reddish purple powder	
Polyphenols	Min. 10.0 %	(Folin-Denis method)
GHG*	Min. 2.0 %	(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. 3×10^3 cfu/g	(Analysis for Hygienic Chemists)
Moulds and Yeasts	Max. 1×10^3 cfu/g	(Analysis for Hygienic Chemists)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Maltodextrin	56%
Purple Tea Extrac	34%
Citric acid	10%
Total	100%

Expiry date	2 years from date of manufacturing.
Storage	Store it in a cool, dry, ventilated area with desiccant. Keep it away from high temperature and sunlight, and store it in a closed container.

*1,2-di-Galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose

PRODUCT STANDARD

PRODUCT NAME

PURPLE TEA EXTRACT-PC

COSMETICS

This product is extracted with aqueous ethanol from the leaves of purple tea (*Camellia Sinensis*).

Appearance	Reddish brown to reddish purple powder with slightly unique scent.	
Polyphenols	Min. 30.0 %	(Folin-Denis method)
GHG*	Min. 3.0 %	(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	(Method 2, JSQI)
(2) Arsenic (as As_2O_3)	Max. 1 ppm	(Method 3, JSQI)
Standard Plate Counts	Max. 1×10^2 cfu/g	(General test, JP)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(General test, JP)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Camellia Sinensis Leaf Extract	60%
Dextrin	30%
Citric acid	10%
Total	100%

Expiry date	2 years from date of manufacturing.
Storage	Store it in a cool, dry, ventilated area with desiccant. Keep it away from high temperature and sunlight, and store it in a closed container.

This specification and test method conforms in Japanese Standard of Quasi-drug Ingredient

unless it is not specify.
*1,2-di-Galloyl-4,6-hexahydroxydiphenoyl-β-D-glucose

PRODUCT STANDARD

PRODUCT NAME

PURPLE TEA EXTRACT-LC

COSMETICS

This product is a water-soluble liquid obtained by dissolving the aqueous ethanol extract of the leaves of purple tea (*Camellia sinensis*) in butylene glycol and water.

Appearance	Brown to reddish brown liquid with slightly characteristic odor.	
Identification		
Green Tea Extract (1)	Add one or two drops of ferric chloride TS to the water solution of this product (1 in 10). The solution develops dark green color.	
Purity Test		
(1) Heavy Metals (as Pb)	Max. 20 ppm	(Method 2, JSQI)
(2) Arsenic (as As_2O_3)	Max. 2 ppm	(Method 3, JSQI)
Standard Plate Counts	Max. 1×10^2 cfu/g	(General test, JP)
Moulds and Yeasts	Max. 1×10^2 cfu/g	(General test, JP)
Coliforms	Negative	(Analysis for Hygienic Chemists)

Composition

Ingredient	Content
Water	69.5%
Butylene glycol	30.0%
Camellia Sinensis Leaf Extract	0.5%
Total	100.0%

Expiry date 1 years from date of manufacturing.

Storage Store it in a cool, dry, ventilated area with desiccant. Keep it away from high temperature and sunlight, and store it in a closed container.

This specification and test method conforms in Japanese Standard of Quasi-drug Ingredient unless it is not specify.

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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“The catalog was created based on academic data. For expressions of consumer products containing this product, follow the Health Promotion Law, Pharmaceutical Low, and other related laws and regulations.”

New additional data in this version

1) Purple tea anthocyanin. Page 7
2) Effect on clock gene. Page 12
3) Clinical trial data of purple tea leaves. Page 18

Established Date: October 9, 2013
Revised Date: May 31, 2019

ORYZA OIL & FAT CHEMICAL CO., LTD.