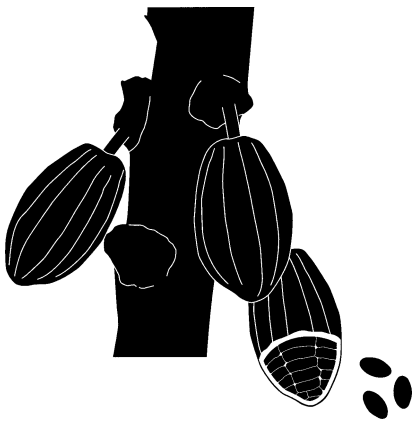
Oryza

ORYZA OIL & FAT CHEMICAL CO., LTD.

COCOA EXTRACT

Ingredient for weight management with natural antioxidants

- **COCOA EXTRACT-P**
  ((Powder, Food Grade)
- **COCOA EXTRACT-WSP**
  ((Water Soluble Powder, Food Grade)
- **COCOA EXTRACT-PC**
  ((Powder, Cosmetic Grade)
- **COCOA EXTRACT LC**
  (Liquid, Cosmetic Grade)
- **COCOA EXTRACT WSPC**
  (Liquid, Cosmetic Grade)



ORYZA OIL & FAT CHEMICAL CO., LTD.

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Health Ingredient for
Weight Management with
Natural Antioxidants

COCOA EXTRACT

1. Introduction

Historically, Cocoa (*Theobroma cocoa*) exist since BC 4000 years ago. It was first cultivated in the central region of Mexico since BC 2000 years ago. In Greek, Cocoa or Theobroma means "Food for God". Since ancient, cocoa has been regarded as precious food for preserving healthy life and longevity. It is usually consumed by the royalty. Meanwhile, the Spaniards consume cocoa by pounding of cocoa beans into powder which has evolved as popular chocholate consumption trend in the modern society.

Cocoa is cultivated tropically at southern latitude 20° northward of equator having average temperature of 27°C and high humidity. The characteristic of cocoa plant is that it flowers and fruits are produced on stem of plant. The fruit of cocoa is similar to the shape of a rugby ball, with a length of approximately 20cm. The flesh of cocoa is white in color embedded with cocoa seeds.

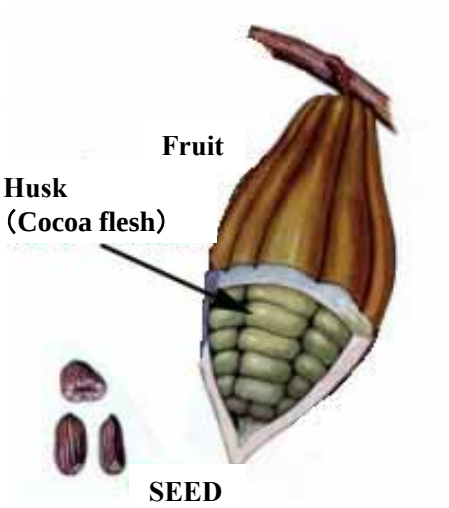


Fig 1. Cocoa Fruit & its components

Oryza Oil & Fat Chemical Co., Ltd. with its own extraction patent has successfully commissioned the production of cocoa extract with high concentration of theobromine (~10%) and polyphenols (~20%). In-house researches revealed that theobromine inhibits fat accumulation while cocoa polyphenols increases fat metabolism.

Recent finding indicated that cocoa extract contains naturally occurring γ-butyric acid (GABA) which is beneficial in lowering elevated blood pressure and calming nervousness. The health promoting effects of cocoa extract are contributed by the synergisms of theobromine and polyphenols. In addition, the relaxation effect of cocoa extract is believed to be an attribution of GABA. Cocoa extract is suitable for cosmetics and nutraceutical applications.

2. Functional components of Cocoa

The Japanese Chocholate and Cocoa Association constantly review and discuss on the researches of the functional uses of cocoa. Recent reviews suggest that cocoa is potentially useful as natural anti-depressant, enhances blood circulation, prevents atherosclerosis and H.pylori infections.

Oryza Oil & Fat Chemical Co., Ltd. prompted researches on Cocoa Husk and results confirmed that Cocoa husk extract is rich in health promoting polyphenols as shown in Fig. 2. Theobromine, a member of the methylxanthine family is identified as the principal component of cocoa extract. Theobromine was found to achieve peak plasma concentration 2 hours after oral administration with similar biological activity to caffeine on adenosine receptors. Nevertheless, it is inhibitory against phosphodiesterase leading to increase in c-AMP level manifesting symptoms like vasodilatation (but vasoconstriction in the brain), palpitation, diuresis and bronchodilatation. In contrast, unlike other methylxanthines, theobromine lacks effect on central nervous stimulation. Hence, Cocoa Extract is calming with its high theobromine content.

Further, theobromine promotes fat metabolism, it is strongly believed to be synergistic with caffeine in accelerating fat breakdown.

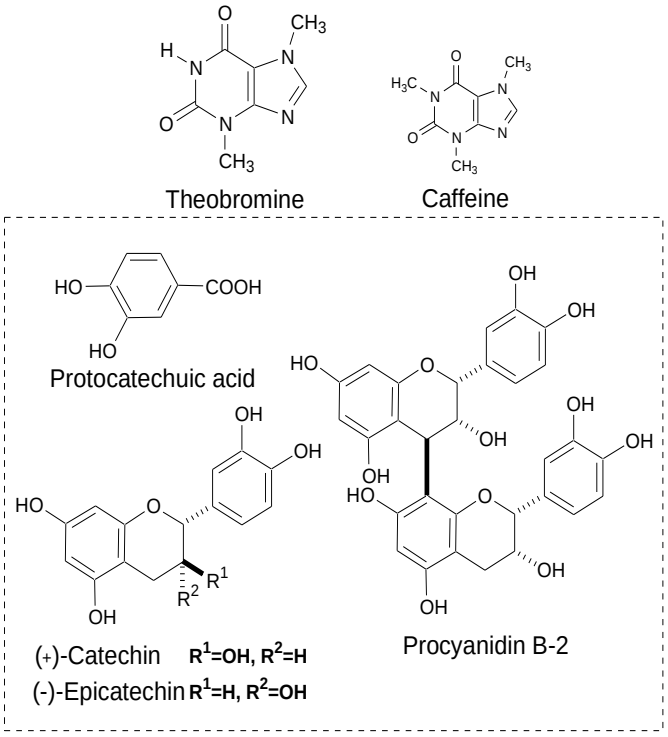


Fig. 2. Functional Components of Cocoa Extract

1) Smit H. J., Gaffan E. A., Rogers P. J. Methylxanthines are the psycho-pharmacologically active constituents of chocolate. Psychopharmacol. **176**, 412-419. (2004).
2) Fredholm B. B., Lindgren E. The effect of alkylxanthines and other phosphodiesterase inhibitors on adenosine-receptor mediated decrease in lipolysis and cyclic AMP accumulation in rat fat cells. *Acta. Pharmacol. Toxicol.* **54**, 64-71 (1984).
3) Hayashi S., Sakaguchi T. Studies on 3,7-dimethyl-1-(5-oxo-hexyl)-xanthine (BL 191). II. Effect of BL 191 on lipolysis in rat epididymal adipose tissue. *Chem. Pharm. Bull.* (Tokyo) **23**, 3119-3124. (1975).

3. Weight Management Effect of Cocoa Extract

i. Prevention against weight gain

The effect of theobromine-rich fraction (theobromine 32.0%, polyphenols 12.1%) and polyphenols-rich fraction (theobromine 5.3%, polyphenols 53.6%) of cocoa extract on mice body weight and body fat was examined. As illustrated in Fig. 3, Table 1, theobromine-rich fraction (1%) effectively suppressed weight gain while polyphenol-rich fraction has no effect on body weight changes.

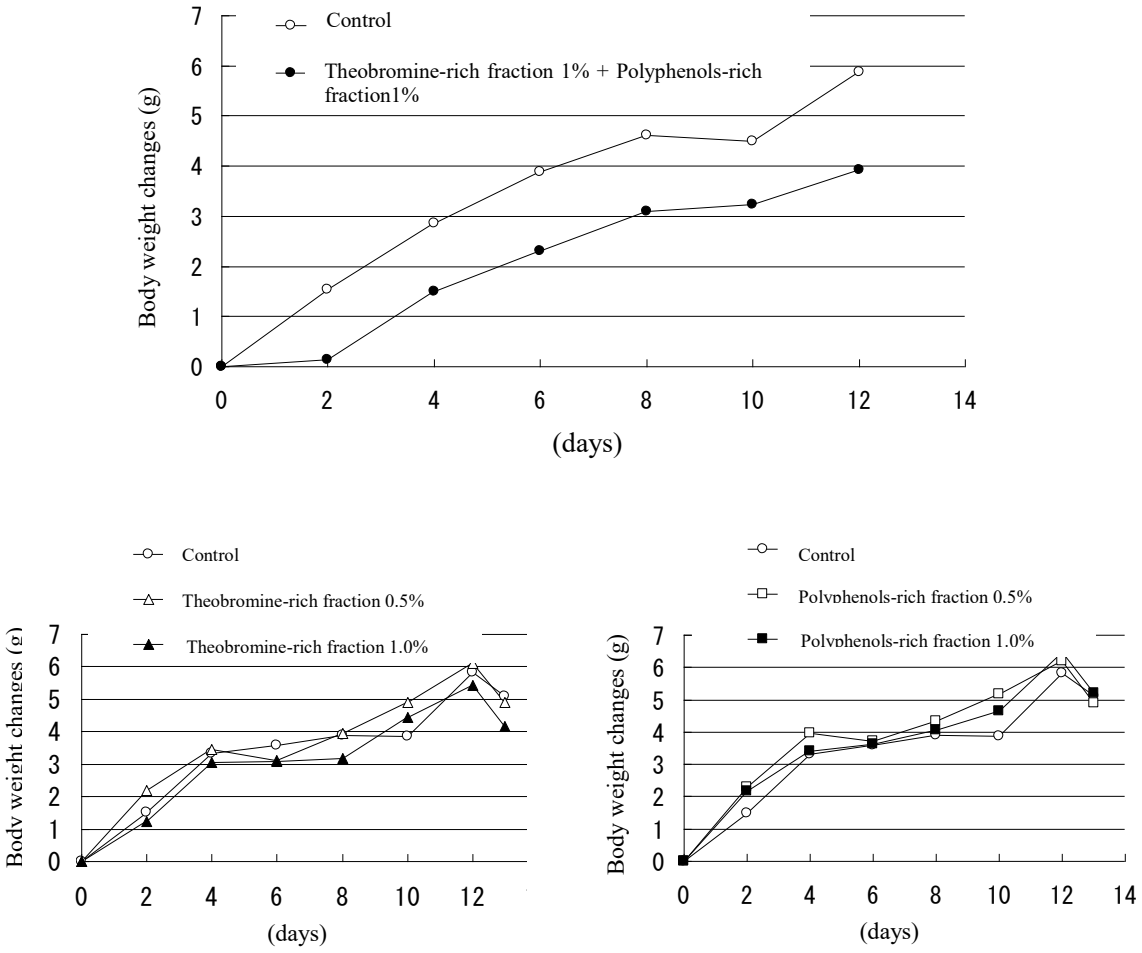


Fig. 3 The effect of fractions of Cocoa Extract on mice body weight (n=6)

However, in group fed with 1% theobromine-rich fraction + 1% polyphenols-rich fraction significantly suppressed weight gain as compared with theobromine–rich fraction only. Polyphenols is synergistic with theobromine in prevention against weight gain.

Table 1: The effect of fractions of Cocoa Extract & amount of feed on mice body weight

	Blend (%)	Initial Weight (g)	Final Weight (g)	Quantity Weight Gain (g)	Amount Feed Intake (g)
Control	-	30.7±0.6	35.8±0.9	**5.1±0.4**	74.8
Theobromine-rich fraction	0.5	30.6±0.4	35.5±0.8	**4.9±0.5**	75.2
	1.0	30.4±0.5	34.5±0.7	**4.2±0.4**	74.5
Polyphenols-rich fraction	0.5	30.1±0.4	35.0±0.9	4.9±1.1	77.2
	1.0	30.0±0.5	35.2±0.8	5.2±0.6	76.7
Control		29.1±0.4	35.0±0.9	**5.9±0.5**	65.0
Theobromine-rich fraction+ polyphenols-rich fraction	1.0+ 1.0	30.5±0.5	34.4±0.4	**3.9±0.3**	67.4

Average±S.E., n=6

[Experimental Method]

5-week old male ddY mice (by CLEA Japan) were breeded for 4 days followed by free access to powder feed containing test samples (MF, by Oriental Yeast) for 12-13 days. Mice body weight and amount of feed intake were recorded respectively.

These mice were then given free access to fractions of cocoa extracts for 12 days. Meanwhile, exercise stress was added to examine the effect of fractions of cocoa extract on body weight. It was found that weight gain was suppressed significantly in group fed with theobromine-rich fraction (1%) coupled with exercise as shown in Fig. 4. On the other hand, weight suppression effect of polyphenols-rich fraction required longer duration to be effective.

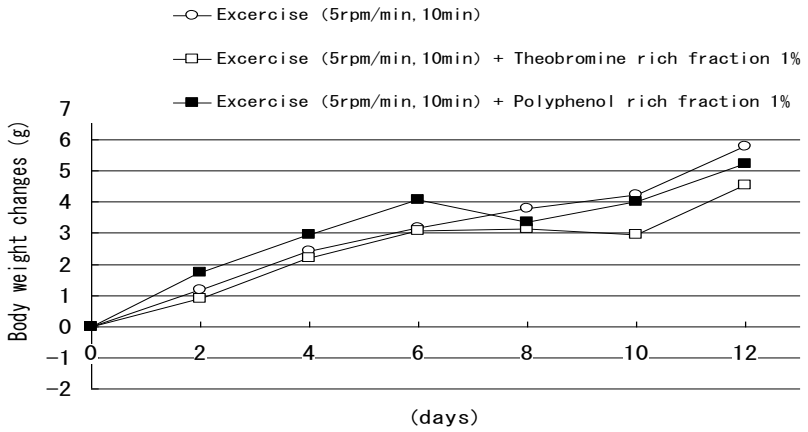


Fig. 4. The effect of fractions of Cocoa Extract on mice weight coupled with exercise stress. (n=6)

[Experimental Method]

5-week old male ddY mice were given free access to food (MF, by Oriental Yeast) containing fractions (1%) of Cocoa Extract for 12 days. Treadmill exercise (MK-770M, Muromachi Kikai) was performed on mice at 5rpm for 10 minutes daily.

ii. Prevention against fat accumulation

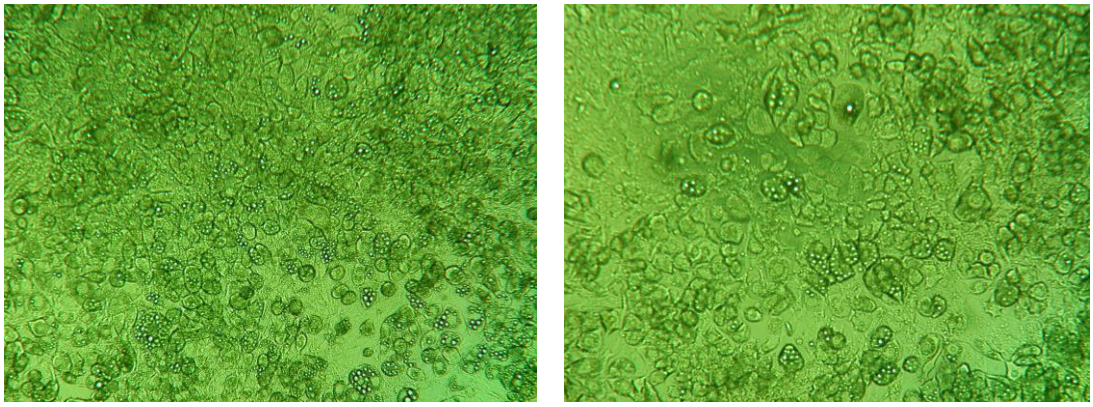
a. Inhibition on differentiation of 3T3-L1 fat cells (*in vitro*)

The effect of theobromine-rich fraction (theobromine 32.0%, polyphenols 12.1%) and polyphenols-rich fraction (theobromine 5.3%, polyphenols 53.6%) of Cocoa Extract on the differentiation of 3T3-L1 fat cells was examined. The levels of triglyceride and glycerol-3-phosphohydrolase (GPDH) of 3T3-L1 cells were measured as fat accumulation index. GPDH is a rate limiting enzyme involved in the conversion of glucose to triglyceride. 3T3-L1 cells treated with theobromine-rich fraction demonstrated a dose-dependent inhibition on triglyceride accumulation (as illustrated in Fig. 5, Table 2). However, there was no physical change observed in the cells. On the other hand, no changes observed in triglyceride level and activation of GPDH in cells treated with polyphenols-rich fraction of Cocoa Extract. Meanwhile, the single effect of isolated theobromine on 3T3-L1 fat cells was examined. It was found that isolated theobromine demonstrated similar dose-dependent inhibitory effects against triglyceride accumulation (Table 2). Hence, Cocoa's theobromine is inhibitory against fat accumulation.

Table 2: The effect of fractions of Cocoa Extract on differentiation of 3T3-L1 cells.

	Concentration (μg/mL)	Triglyceride (μg/well)	GPDH Activity (Unit/well)
Control	—	**180±9**	0.121±0.011
Theobromine-rich fraction	1	**160±9**	0.100±0.023
	3	**165±9**	0.156±0.004
	10	**157±9**	0.125±0.012
	30	**137±9**	0.120±0.021
	100	**125±9***	0.117±0.016
Control	—	170±14	
Isolated Theobromine	1	161±6	
	3	146±13	
	10	144±17	
	30	146±18	
	100		
Control	—	116±9	0.118±0.009
Polyphenols-rich fraction	1	136±9	0.121±0.025
	3	147±9	0.092±0.034
	10	145±9	0.063±0.031
	30	139±9	0.152±0.013
	100	133±9	0.101±0.033

average±S.E., n=4, *: $p<0.05$



Control　　Theobromine-rich fraction（100 μg/ mL）

Fig. 5. Microscopic illustration of 3T3-L1 cells after induction of differentiation in theobromine-rich fraction medium

[Method]

3T3-L1 cells ($5x10^4$ cells/ml) was inseminated in a 24-welled plate (500μl) followed by incubation for 24 hours in DMEM culture medium containing 10% bovine fetus serum (high glucose). Culture medium was replaced with insulin (1g/ml), dexamethasone (0.25M), and isobutyl methyl xanthine (0.5mM) to induce differentiation. 2 days later, culture medium was replaced by medium containing samples and insulin (1g/ml) followed by further incubation for 6 days. The culture medium was replaced every alternate day. Triglyceride concentration and GPDH activation was measured using Triglyceride E-Test Wako (Wako Pure Chemical Industries,Ltd.) and GPDH Activation Measurement Kit (Primary Cell) respectively.

iii. Accelerate fat breakdown

Cytochrome C oxidase – an enzyme present in the inner membrane of mitochondria. Together with Coenzyme Q 10, it is responsible for energy production in the electron transfer system. Fig. 6 shown the activity of cytochrome C oxidase in the Electron Transfer System of mitochondria. Cytochrome C oxidase converts nutrients into water via electron transfer from TCA cycle. The effect of Cocoa Extract on fat metabolism and activation of cytochrome C oxidase was examined.

As illustrated in Fig. 7. theobromine-rich fraction & isolated theobromine have no effect on activation of cytochrome C oxidase but a dose-dependent inhibition against triglyceride accumulation. In contrast, polyphenols-rich fraction significantly accelerated the activity of cytochrome C oxidase but no effect on triglyceride level.

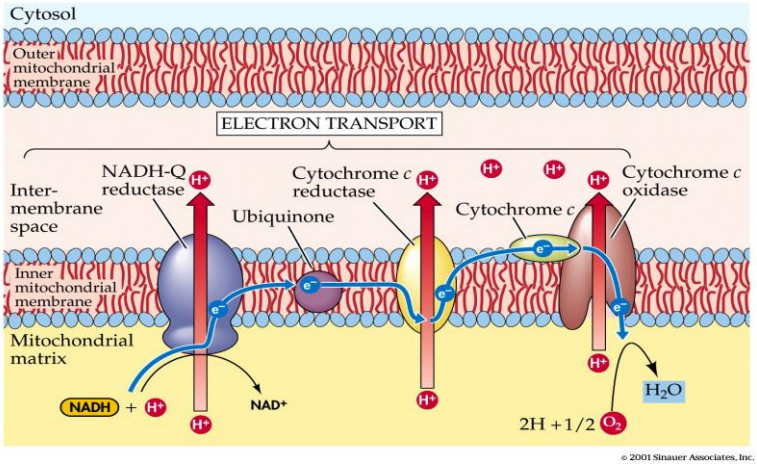


Fig. 6. Activity of Cytochrome C Oxidase in the electron transfer system.

NADH-Q oxidase, cytochrome C oxidase and cytochrome C reductase are the enzymes involved in the electron transfer system. Electrons generated in TCA cycle and beta-oxidation from glucose and fat metabolism respectively are transferred through REDOX path where oxygen is utilized yielding water and carbon dioxide.

[Method]
Rat brown adipocytes incubation kit from Takara Bio was used. Brown adipocytes (19th generation, $4x10^4$ cells/ml) were inseminated in a 24-welled plate (500µl) and incubated for 4 days. Differentiation was induced when 90 percent confluence achieved. 2 days later, culture medium was replaced with medium containing samples and further incubated for 6 days. At the end of experiment, concentration of triglyceride and activation of cytochrome C oxidase was measured using Sigma's Kit.

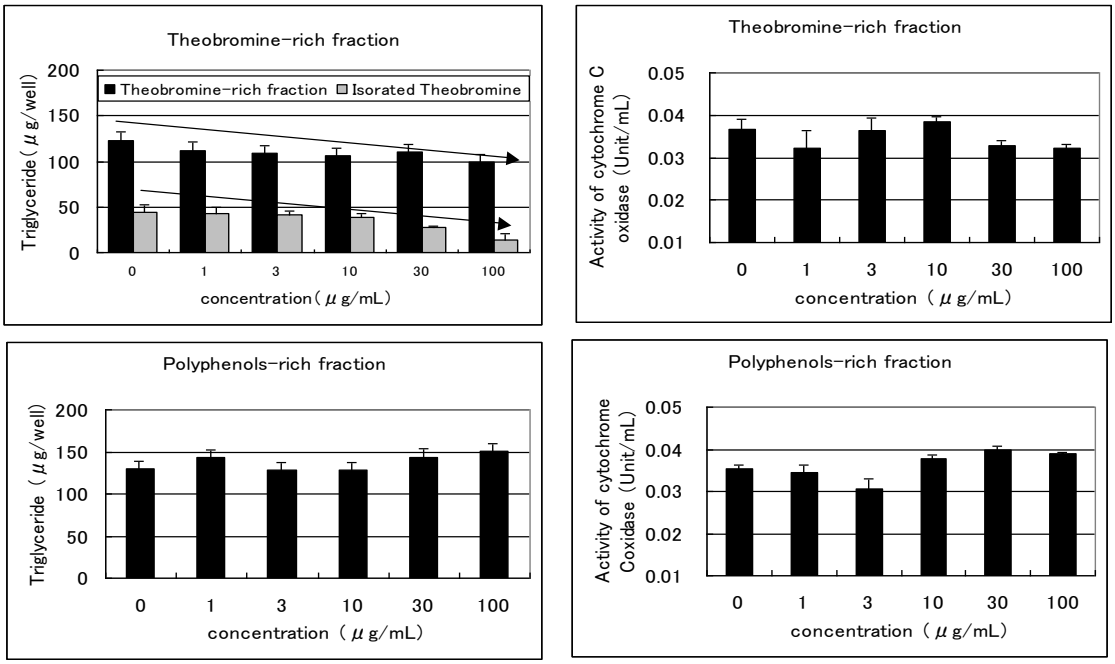


Fig. 7: The effect of theobromine-rich fraction and polyphenols-rich fraction of Cocoa Extract on the accumulation of triglyceride and cytochrome C oxidase in rat's brown adipocytes. (Average ±S.E., n=4)

As revealed in the experiment, Cocoa Extract effectively prevented the accumulation of triglyceride in adipocytes. It is strongly believed that Cocoa's theobromine is the active ingredient that prevents accumulation of triglyceride. On the other hand, Coffee Bean Extract, another popular ingredient for weight management from Oryza, acts on a different mechanisms, e.g. UCP-uncoupling and activation of CPT. Meanwhile, Cocoa's polyphenols has been shown to promote fat breakdown by accelerating fat metabolism in electron transfer system. Healthy weight management is achievable through different mechanisms as illustrated in Fig. 8. Effective weight loss is best achieved with synergism from different ingredients in the metabolism cascade.

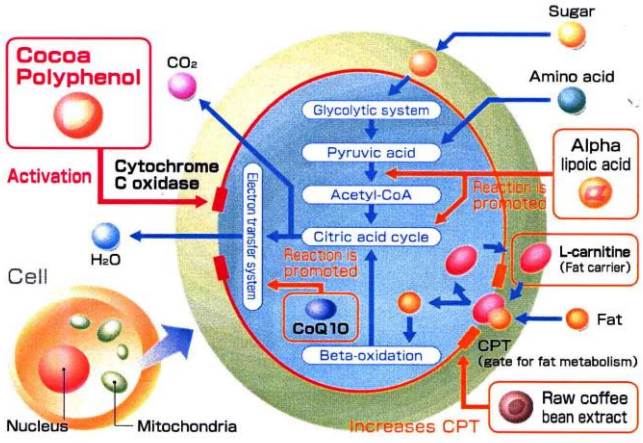


Fig. 8. Mechanism of Action of Cocoa Extract and other food ingredients in the mitochondria metabolic cascade

iv. Prevent fat absorption

Previous researches illustrated the high content of Cocoa theobromine and polyphenols are preventive against fat accumulation in adipocytes. Besides, Cocoa Extract contains approximately 5% caffeine which is documented to be effective against fat absorption.

Further research was prompted on olive oil loaded mice to verify the inhibitory effect of Cocoa Extract against fat absorption. Results reviewed that cocoa extract (theobromine 13%, polyphenols 23%, caffeine 5%) is preventive against olive oil induced elevated blood triglyceride level. As shown in Fig. 9, caffeine significantly inhibited olive oil induced elevated blood triglyceride level.

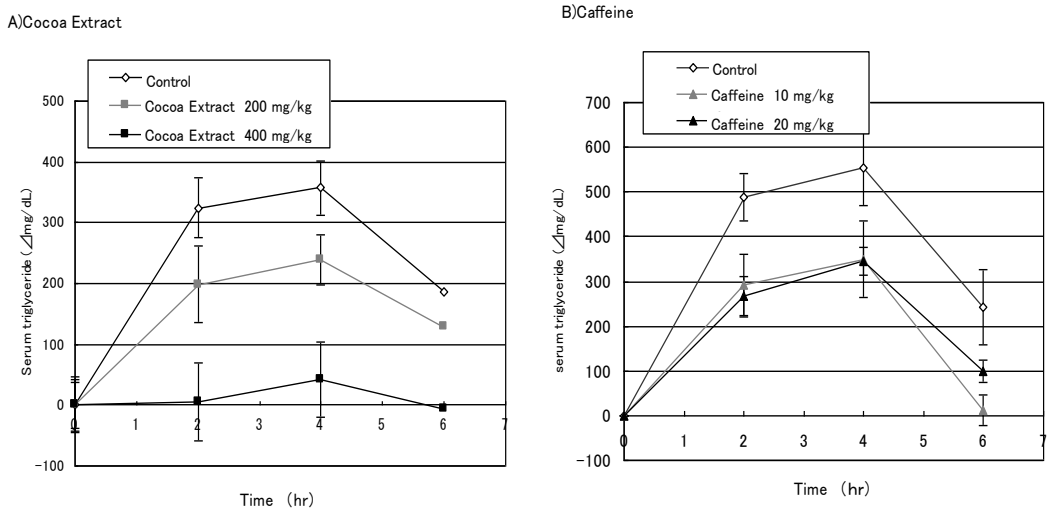


Fig. 9. Effect of Cocoa Extract and caffeine on serum triglyceride elevation in olive oil-loaded mice. (Mean with the SE)

[Method]

Animals & Treatment:

6-week old ddy mice were fasted for 20 hours and blood samples was collected as initial reading. 30 minutes later, Cocoa Extract (10ml/kg) in 5%w/v Acacia gum suspension were orally given to mice. Mice were loaded with olive oil 1 hour later.

Samples collections and assay:

Further blood samples were collected at 2, 4 and 6 hour after oral administration of cocoa extract and olive oil. Serum was separated and triglyceride concentrations was determined by enzymatic method (Triglyceride E-Test Wako, Wako Pure Chemical Industries Co., Ltd.)

v. The effect of Cocoa Extract on Normal, Healthy Adult Males (*in vivo*)

Human study was prompted to evaluate the effect of Cocoa Extract on normal, healthy male adults. 16 healthy adult males (aged 24-60 years old) were given Cocoa Extract 100mg (containing theobromine 21.2% and polyphenols 30.7%) for 4 weeks. Obesity indexes and blood profile of test subjects were measured and determined prior to and at the end of the study.

4 weeks later, body weight, percentage of body fat, BMI, fat content, waist and hip circumference reduced. Body weight and fat level reduced significantly with $P<0.01$ while BMI reduced with $P<0.05$. No major changes observed on blood profile but blood sugar level and triglyceride level of test subjects were lowered. The results strongly suggest that Cocoa Extract prevents fat accumulation and promotes fat metabolism.

Table 3. Readings of Obesity Parameters Prior to and After 4-week oral administration of Cocoa Extract.

	Prior	After
Body Weight (kg)	**66.2±9.3**	**65.5±9.4** $p<0.01$
Percentage body Fat (%)	**20.0±5.1**	**19.8±5.4**
BMI (kg/m^2)	**22.6±2.8**	**22.4±2.8** $p<0.05$
Impedence (Ω)	481±63	488±65
Fat Content (kg)	**13.6±5.1**	**13.3±5.1**
Rate of Obesity (%)	**2.8±12.5**	**1.6±12.6** $p<0.01$
Waist circumference (cm)	**78.4±9.1**	**77.2±8.9**
Hip circumference (cm)	**95.1±6.5**	**94.6±6.3**
Waist / Hip ratio	0.82±0.06	0.81±0.05
Thickness of abdominal fat (mm)	13.7±5.0	15.9±6.5
Creatine(mg/dL)	0.82±0.11	0.80±0.10
Blood Glucose (mg/dL)	82.8±9.5	80.7±15.5
Triglyceride (mg/dL)	161.8±132.2	140.0±112.8
Phospholipids (mg/dL)	227.1±33.5	224.9±26.2
Total Cholesterol (mg/dL)	202.3±21.0	206.3±21.6
Leptin (ng/mL)	3.16±1.7	3.39±2.2
HDL-Cho (mg/dL)	58.6±18.7	58.4±14.3

Figures are average values and standard deviation of the 16 subjects

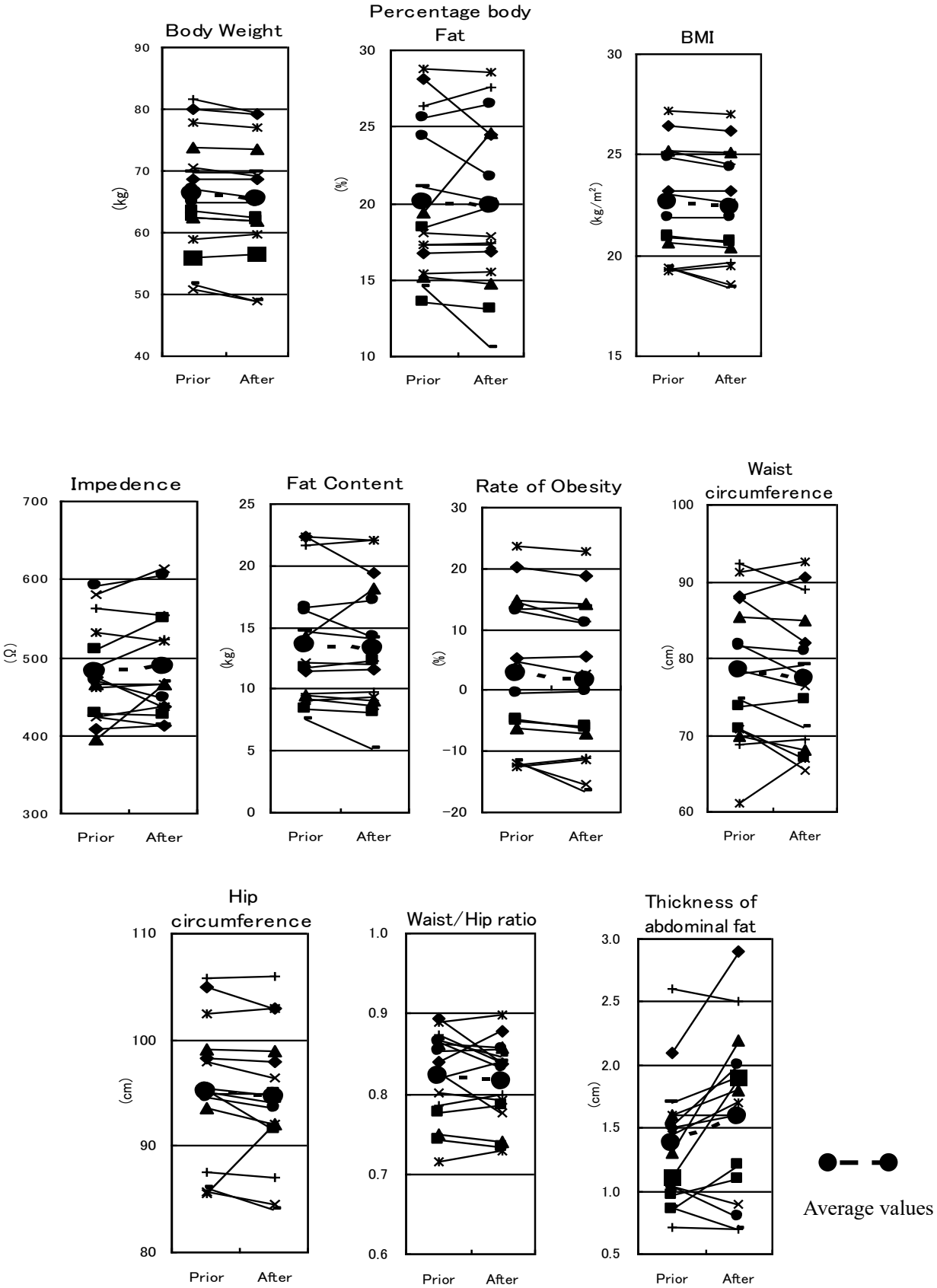


Fig. 10. Comparison of obesity indexes before and after taking cacao extract's fractions for four weeks

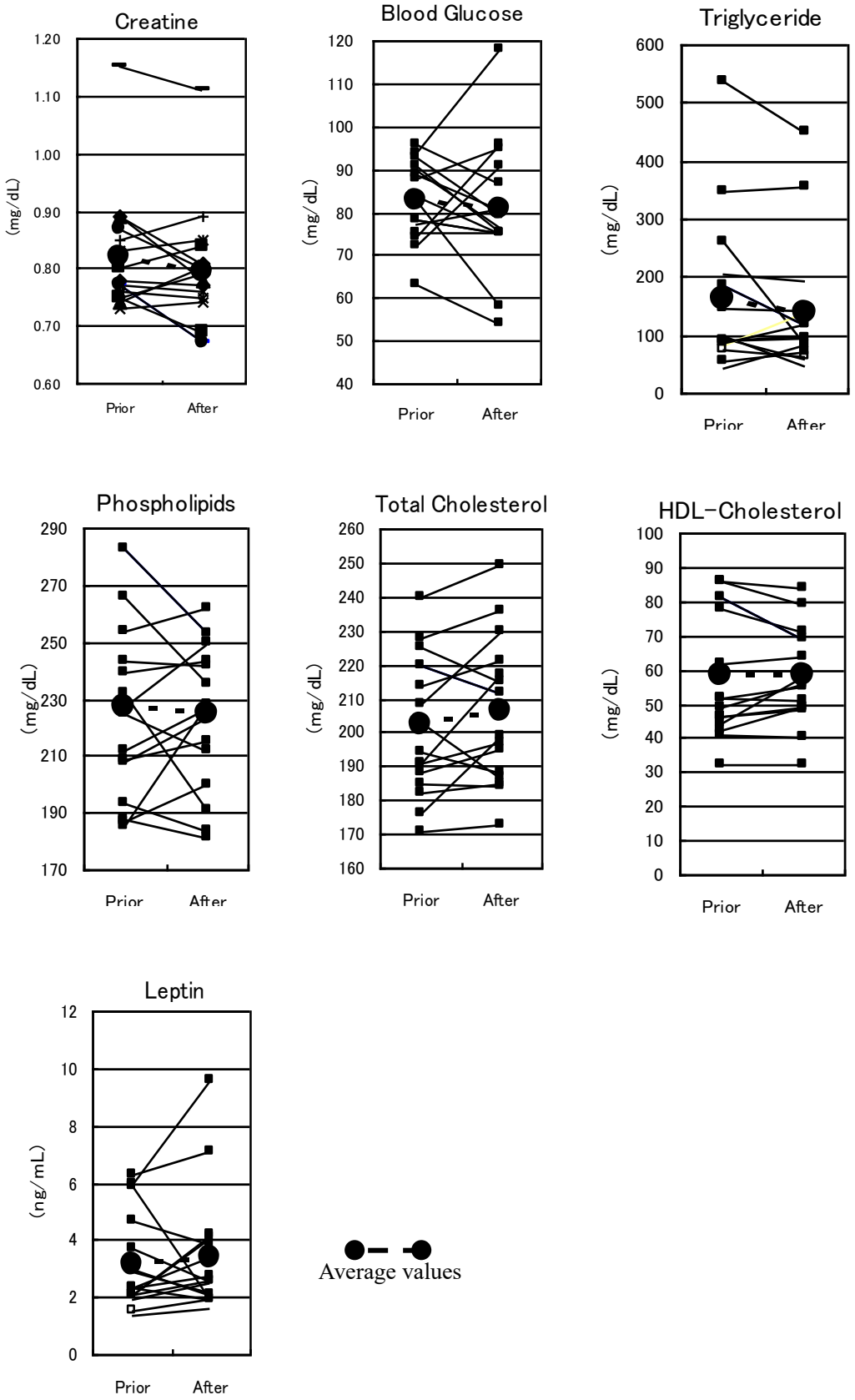


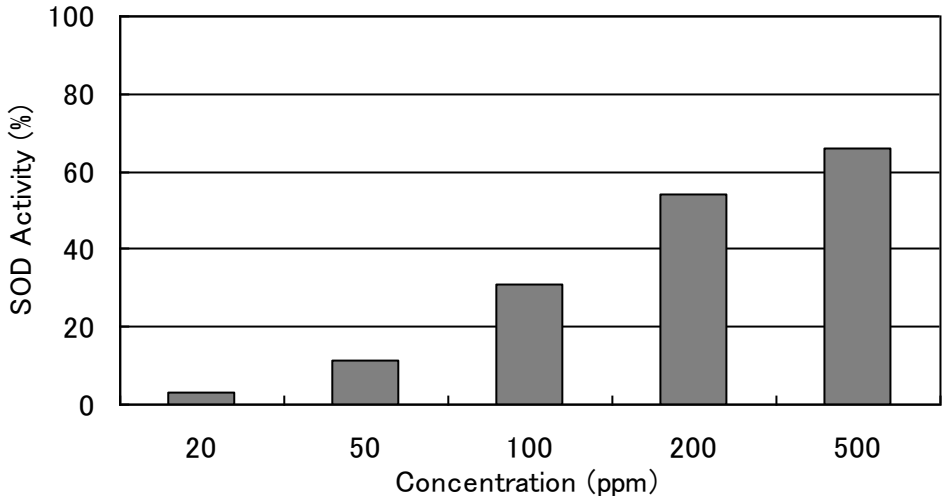
Fig. 11. Comparison of blood components before and after taking cacao extract's fractions for four weeks

4. Antioxidative Activity of Cocoa Extract

Reactive oxygen species (ROS) is generated as metabolic by product. ROS is highly damaging and causes various degenerative diseases of the modern society, e.g. aging.

The SOD mimicking activity and DPPH radical scavenging activity of Cocoa Extract was measured for its anti-oxidative capacity. As illustrated in Fig. 12, Cocoa Extract demonstrated a dose-dependent SOD mimicking activity and DPPH radical scavenging activity.

①SOD-mimicking Activity



②DPPH Radical Scavenger Activity

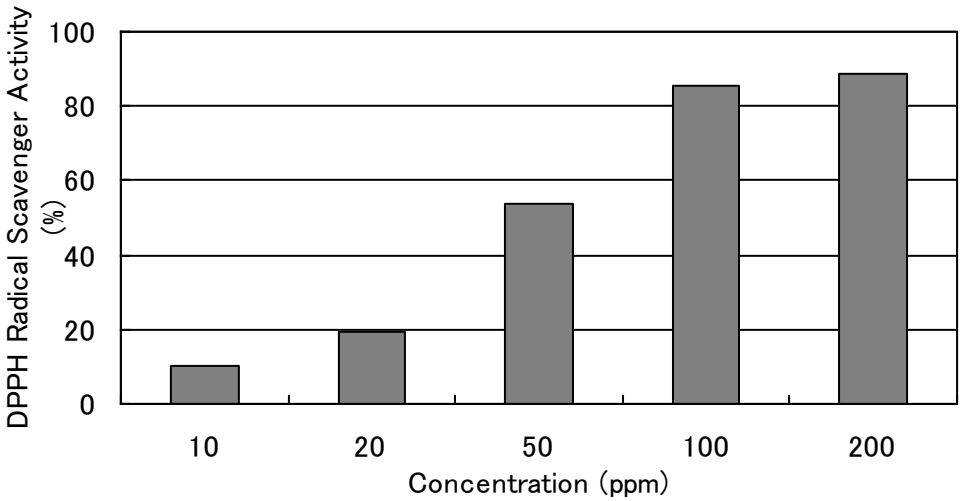


Fig. 12. Antioxidative Activity of Cocoa Extract

5. Stability of Cocoa Extract

i. Thermostability

Thermostability of Cocoa Extract (pure extract without excipient) was studied. Theobromine and polyphenols content of Cocoa Extract are highly stable upon heating at 100°C and 120°C. Cocoa Extract is stable at regular temperatures for food processing.

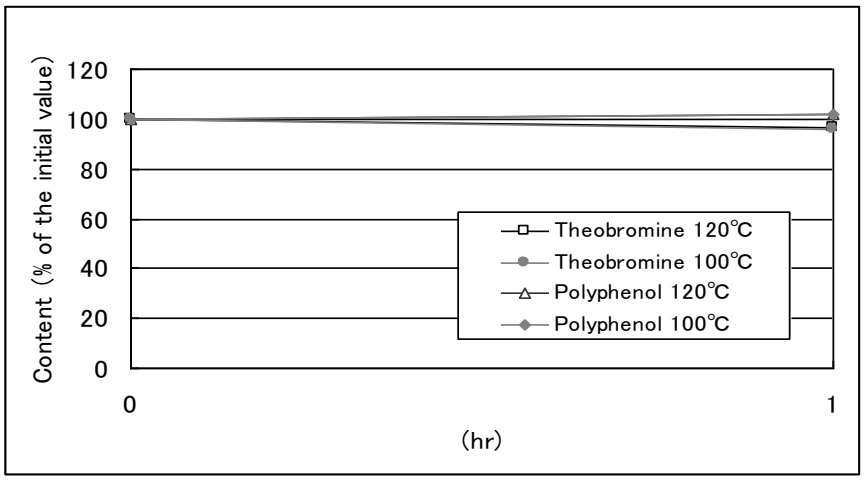


Fig. 13. Thermostability of Cocoa Extract

ii. pH Stability

pH stability of Cocoa Extract was studied. Cocoa Extract is dissolved in Ethanol 30%v/v yielding concentration of 0.5%. pH of Cocoa Extract solution was adjusted and stored at room temperature in a cool, dry dark place. Content of theobromine and polyphenol was measured on day 1 and day 7.
Results shown that Cocoa's theobromine is highly stable at all pH. In contrast, Cocoa's polyphenols stable only at acidic pH and easily decomposed at alkali pH. Cocoa polyphenols decomposes 10% on day 1 and further 10-25% one week later.

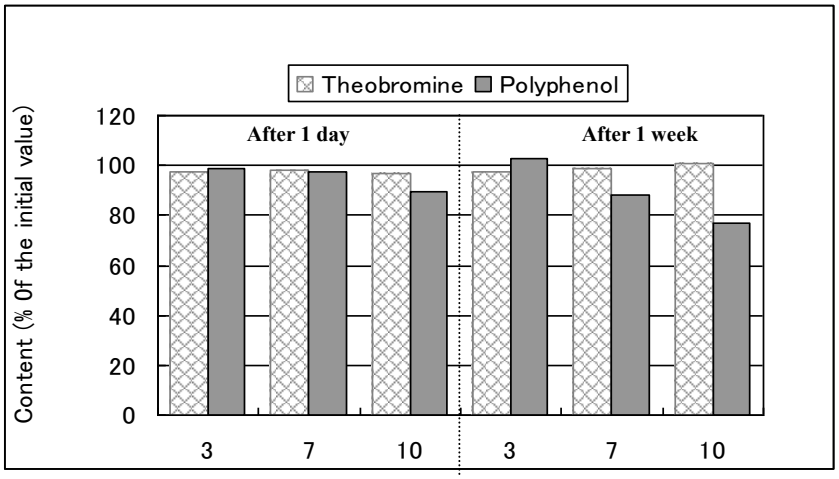


Fig. 14. pH Stability of Cocoa Extract

iii. Stability in aqueous state

Aqueous stability of Cocoa Extract was conducted. Concentration of 0.3% of Cocoa Extract-WSP at pH 3.5 was prepared. This aqueous solution was stored in a dark place at various temperatures: room temperature, 25°C, 40°C & 5°C for 4 weeks. Observation was carried out to detect precipitation, turbidity and colour changes. Results showed that Cocoa Extract-WSP is highly stable at its acidic aqueous state.

	Aqueous Stability (0.3% solution)			
Dark place	Room Temp	25°C	40°C	5°C
Precipitation, turbidity	Negative	Negative	Negative	Negative
Colour changes	Negative	Negative	Negative	Negative

6. Cocoa Extract - Nutritional Information

Description	P	WSP	Note	Analytical Method
Water	8.5 g/100g	3.8 g/100g		Heat-drying at atmospheric and reduced pressure
Protein	44.1 g/100g	2.3 g/100g	1	Kjeldahl Method
Fat	2.6 g/100g	0.7 g/100g		Acid degradation
Ash	7.0 g/100g	2.7 g/100g		Direct Incineration
Carbohydrate	37.8 g/100g	90.5 g/100g	2	
Energy	351 kcal/100g	378kcal/100g	3	Atwater Method (Revised)
Dietary Fiber	< 5.1 g/100	-		Prosky Method
Sodium	95 mg/100g	16 mg/100g		Atomic absorption photometry

Note:

1. Nitrogen, protein conversion factor: 6.25
2. Carbohydrate expression standard (Ministry of Health and Welfare's announcement No. 176)
   Calculation: 100 – (water + protein + fat + ash)
3. Energy expression standard (Ministry of Health and Welfare's announcement No. 176)
   Conversion factor: Protein 4, fat 9, sugar 4; dietary fiber 2

Test trustee: SRL, Inc
Date of analysis: September 17, 2007, February 24, 2010
Test No.: 200801310044, 201002180030

7. Cocoa Extract – Product Safety Profile

i. Residual Agricultural Chemicals

Cocoa is free from residual agricultural chemical as stipulated in table below compliance to the Food Hygiene Law by Ministry of Health, Labour and Welfare.

Description	Result	Detection limit	Method	Note
Captafol	Not detected	0.04 ppm	GC-MS	1
Chlorothalonil	Not detected	0.05 ppm	GC-MS	1
Dichlorvos	Not detected	0.5 ppm	GC-MS	1
Delta metlin	Not detected	0.05 ppm	GC-MS	1
Bio res metlin	Not detected	0.1 ppm	GC-MS	1
Pyrethrin	Not detected	1.0 ppm	GC-MS	1
Fenitrothion	Not detected	0.1 ppm	GC-MS	1
Amitrole	Not detected	0.03 ppm	LC-MSMS	1
Daminozid	Not detected	0.01 ppm	LC-MSMS	1
2, 4, 5-T	Not detected	0.1 ppm	GC-MS	2
Glyphosate	Not detected	0.2 ppm	LC	2
Cyhexatin	Not detected	0.02 ppm	GC	2

Note 1:
Test Trustee: Meiji Seika Co., Ltd.
Date of analysis: April 11, 2005
Reference No: 372674
Note 2:
Test Trustee: Kyusai Bunsiki Kenkyujo
Date of analysis: march 31, 2005
Reference No.: 20050315-6

ii. Acute Toxicity (LD_{50})

5-week old male ICR mice weighed approximately 30g were fed Cocoa Extract 2,000mg/kg body weight. Mice were given free access to food and water for 14 days. Experiment condition were maintained at temperature 23°C±2° and humidity at 50±10%. No fatal event nor abnormalities observed throughout the experiment. No apparent abnormalities observed upon randomized inspection post experiment. LD_{50} of Cocoa Extract is deduced to be higher than 2,000mg/kg.

8. Cocoa Extract – Recommended Daily Dosage

The recommended daily dosage for Cocoa Extract – P is 100-250mg/day

9. Applications

	Applications	Claims	Products
Foods	Food for healthy weight management Beauty food	1. Healthy weight management 2. Prevent fat accumulation 3. Promotes fat metabolism 4. Promote healthy weight loss	Beverages, hard & soft capsules, tablets, candies, chewing gums, chocolates, wafers, jellies etc
Cosmetics	Anti-cellulite Topical slimming aids		Body lotions, body gel etc.

10. Packaging

Cocoa Extract – P & Cocoa Extract – WSP [Food grade, powder]
Cocoa Extract – WSPC [Cosmetics grade, powder]
5kg Inner Package: Aluminium bag
Outer Package: Cardboard

Cocoa Extract – LC (Cosmetics grade, liquid]
5kg Inner Package: Cubic polyethylene container
Outer Package: Cardboard

11. Storage

Store in cool, dry dark place.

12. Expression

Cocoa Extract – P
Expression: Cocoa Extract
Cocoa Extract – WSP
Expression: Dextrin, Cocoa Extract

Cocoa Extract – PC
INCI Name: Theobroma Cacao (Cocoa) Extract
Cocoa Extract – WSPC
INCI Name: Dextrin, Theobroma Cacao (Cocoa) Extract
Cocoa Extract – LC
INCI Name: Butylene Glycol
Water
Theobroma Cacao (Cocoa) Extract

Product Standard

Cocoa Extract-P

(Food)

This product is extracted from cocoa seed, the seed of *Theobroma cacao* Linn (*Sterculiaceae*) with aqueous ethanol. It guarantees minimum of 10.0% of theobromine and 20.0% polyphenols.

Appearance	Brown to dark brownish powder with unique cocoa aroma.	
Theobromine	Min. 10.0%	(HPLC)
Polyphenols	Min. 20.0%	(Folin-Denis Method)
Loss on Drying	Max. 10.0%	(Analysis for Hygienic Chemists, 1g, 105°C, 2hr)
Purity Test		
(1) Heavy Metal (As Pb)	Max. 30ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1ppm	(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 Chemist)
Moulds & Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemist)
Coliforms	Negative	(Analysis for Hygienic Chemist)

Composition

Ingredient	Content
Cocoa Extract	100 %

Product Standard

Cocoa Extract-WSP

(Food)

This product is extracted from seeds of cocoa seed, the seed of *Theobroma cacao* Linn (*Sterculiaceae*) with aqueous ethanol. It guarantees minimum of 0.1% theobromine and 0.2% polyphenol. This product is water-soluble.

Appearance	Light brown powder with slight unique aroma.	
Theobromine	Min. 0.1%	(HPLC)
Polyphenols	Min. 0.2%	(Folin-Denis Method)
Loss on Drying	Max. 10.0%	(Analysis for Hygienic Chemists, 1g, 105°C, 2 hours)
Purity Test		
(1) Heavy Metal (as Pb)	Max. 10ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1ppm	(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 Chemist)
Moulds & Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemist)
Coliforms	Negative	(Analysis for Hygienic Chemist)

Composition

Ingredient	Contents
Dextrin	75 %
Cocoa Extract	25 %
Total	100 %

Product Standard

Cocoa Extract-PC

(Cosmetic)

This product is extracted from seeds of cocoa seed, the seed of *Theobroma cacao* Linn (*Sterculiaceae*) with aqueous ethanol. It guarantees minimum of 10.0% theobromine and 20.0% polyphenols.

Appearance	Brown to dark brownish powder with slight unique aroma.	
Theobromine	Min. 10.0%	(HPLC)
Polyphenols	Min. 20.0%	(Folin-Denis Method)
Loss on Drying	Max. 10.0%	(1g, 105°C, 2 hr)
Purity Test		
(1) Heavy Metal (as Pb)	Max. 30ppm	(The Second Standard Method of The Japanese Standards of Quasi-Drug Ingredients)
(2) Arsenic (as As_2O_3)	Max. 1ppm	(The Third Standard Method of The Japanese Standards of Quasi-Drug Ingredients)
Standard Plate Counts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemist)
Moulds & Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemist)
Coliforms	Negative	(Analysis for Hygienic Chemist)

Composition

Ingredient	Content
Theobroma Cacao (Cocoa) Extract	100 %

Product Standard

Cocoa Extract-WSPC

(Cosmetic)

This product is extracted from seeds of cocoa seed, the seed of *Theobroma cacao* Linn (*Sterculiaceae*) with aqueous ethanol. It guarantees minimum of 0.1% theobromine and 0.2% polyphenol. This product is water-soluble.

Appearance	Light brown powder with slight unique aroma.	
Theobromine	Min. 0.1%	(HPLC)
Polyphenols	Min. 0.2%	(Folin-Denis Method)
Loss on Drying	Max. 10.0%	(Analysis for Hygienic Chemists, 1g, 105°C, 2 hours)

Purity Test

(1) Heavy Metal (as Pb)	Max. 10ppm	(Sodium Sulfide Colorimetric Method)
(2) Arsenic (as As_2O_3)	Max. 1ppm	(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 Chemist)
Moulds & Yeasts	Max. 1×10^2 cfu/g	(Analysis for Hygienic Chemist)
Coliforms	Negative	(Analysis for Hygienic Chemist)

Composition

Ingredient	Contents
Dextrin	75 %
Theobroma Cacao(Cocoa) Extract	25 %
Total	100 %

Product Standard

COCOA EXTRACT-LC

(Cosmetic)

This product is extracted from cocoa seed, the seed of *Theobroma cacao* Linn (*Sterculiaceae*) with aqueous ethanol and is dissolved in aqueous 1,3-butylene glycol.

Appearance Brown color liquid with slight unique aroma.

Certification Test

Polyphenols Dissolve 0.5 ml of this product in 2.0 ml water. Add 0.2 ml Folin-Denis reagent into the solution followed by 0.4 ml saturated Na_2CO_3. The solution will turn into blue color.

Purity Test

(1) Heavy Metal (as Pb) Max 10ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredients)

(2) Arsenic (as As_2O_3) Max 1ppm (The Second Method of The Japanese Standards of Quasi-Drug Ingredients)

Standard Plate Counts Max 1×10^2 cfu/g (Analysis for Hygienic Chemist)

Moulds & Yeasts Max 1×10^2 cfu/g (Analysis for Hygienic Chemist)

Coliforms Negative (Analysis for Hygienic Chemist)

Composition

Ingredient	Contents
Butylene Glycol	69 %
Water	30 %
Theobroma Cacao (Cocoa) Extract	1 %
Total	100 %

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

From product planning to OEM - For any additional information or assistance, please contact：

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Established Date: August 3, 2005
Revised Date: May 13, 2019

ORYZA OIL & FAT CHEMICAL CO., LTD.