For improving cold sensitivity/stiff shoulders and preventing angiopathy

- BUCKWHEAT LEAF EXTRACT-P
- BUCKWHEAT LEAF EXTRACT-PC
1. Introduction

Cold sensitivity is a frequent complaint in females, and is caused by autonomic imbalance, abnormal secretion of female hormones, or peripheral hypesthesia, but is most closely associated with the stagnation of peripheral blood flow in the hands and feet. To reduce symptoms of cold sensitivity, improvement in microvascular blood flow is effective, and natto kinase, Japanese apricot extract, ginkgo leave extract, and Pycnogenol™ are already commercially available for blood flow improvement. These supplements improve blood flow by affecting blood cells (platelets and erythrocytes) and blood components (such as chylomicron and cholesterol) and improving blood fluidity.

The blood vessels as blood pathways also markedly affect blood circulation. With age, the elasticity of the vascular inner walls decreases, and the vascular ability to flexibly contract and relax in response to changes in temperature and blood pressure decreases. Therefore, to maintain normal blood circulation, care for both the blood and blood vessels is necessary.

Oryza Oil & Fat Chemical Co., Ltd. noted buckwheat containing rutin as a flavonoid that strengthens the blood vessels. Rutin has been long called vitamin P and known as a nutrient indispensable for strengthening the blood vessels and maintaining their elasticity. Buckwheat noodles eaten in Japanese daily life also contain rutin, but in a very small amount. We measured rutin in buckwheat and found a higher content in the leaves than in the seeds. In addition, in a human monitor test using a microchannel array flow analyzer (MC-FAN) and thermography, BUCKWHEAT LEAF EXTRACT improved blood fluidity and restored peripheral body temperature that had been reduced under cold conditions.

BUCKWHEAT LEAF EXTRACT-P contains a standardized amount of rutin (≥ 5%) and can be used not only for supplements that aim at improving cold sensitivity, stiff shoulders, and swelling but also for those that aim at preventing disorders associated with vascular aging such as atherosclerosis, hypertension, strokes, and myocardial infarction.
2. What is buckwheat leaf?

The history of buckwheat leaf as food is long. In 984, the “Ishinpo”, the oldest medical book in Japan edited by Yasuyori Tanba, mentions that buckwheat washes away dirty wastes in the 5 major visceral organs and connects the spirit and God, its leaves can be boiled and eaten as a vegetable, and buckwheat markedly improves the function of the ears and eyes and reduces excessive spirit. Thus, buckwheat has been recognized as a food that is good for health.

In China, buckwheat leaves are also used as a medicinal supplements. Concerning efficacy, the Great Chinese Medical Dictionary mentions that buckwheat leaves used for hypertension due to fragile capillaries prevent cerebral bleeding, and they also prevent various types of bleeding due to fragile capillaries and non-tuberculosis pulmonary bleeding, and can be used for the treatment of diabetic retinopathy. Thus, buckwheat leaves have effects on impaired circulation.

There are some kinds of buckwheat. Our BUCKWHEAT LEAF EXTRACT is made from *Fagopyrum esculentum* Moench and *Fagopyrum tataricum* L.
3. Components of buckwheat leaves and blood flow improving effects

The principle component of buckwheat leaves is rutin, a flavonoid glycoside (Fig. 1, left). Recent studies on the effects of rutin on blood flow shown below\(^1\)-\(^4\) have clarified that rutin improves blood flow by acting on the vascular smooth muscle and blood components. In clinical practice, oxerutin (Fig. 1, right), as a rutin derivative, is used for disorders associated with impaired blood circulation, and its effects on economy class syndrome have been reported\(^5\).

The absorption of rutin in humans is relatively slow, and metabolites appear in the blood 4-8 hours after its intake. The metabolites reach peak concentrations after 8-12 hours and disappear after 20-35 hours\(^6\)-\(^7\).

![Fig. 1. Chemical structures of rutin (left) and oxerutin (right)](image)


4. Function of BUCKWHEAT LEAF EXTRACT

(1) Improvement of blood flow

MC-FAN method (humans)

The effects of ingestion of BUCKWHEAT LEAF EXTRACT (120 mg) on blood fluidity were evaluated by a microchannel array and an MC-FAN in 9 male and female volunteers (age, 26-60 years) of our company. As a parameter of blood fluidity, slit passage time (whole blood passage time)/100 μl blood was used.

The whole blood passage time was 54-103 minutes before ingestion of BUCKWHEAT LEAF EXTRACT but 42-100 seconds 1 hour after ingestion, showing a reduction in 7 subjects. After consecutive extract ingestion for 1 week, the whole blood passage time was 48-71 seconds, showing a reduction in 8 subjects (Fig. 2).

![Fig. 2. Whole blood passage time before and after ingestion of BUCKWHEAT LEAF EXTRACT in each subject.](image)

The mean whole blood passage time 1 hour after ingestion of BUCKWHEAT LEAF EXTRACT in the 9 subjects was decreased by about 4.7% compared with the value before ingestion, but that after consecutive extract ingestion for 1 week was significantly decreased (22.4%) compared with the value before ingestion ($p < 0.01$, Student $t$-test; the same test was used below) (Fig. 3).

Fig. 4 shows microchannel images of a subject (S.H. in Fig. 2) who markedly responded to BUCKWHEAT LEAF EXTRACT. Platelet aggregates around the slit observed before
ingestion of BUCKWHEAT LEAF EXTRACT were absent 1 hour after ingestion, and leukocyte passage through the slip became smooth.

Fig. 3. Mean whole blood passage time before and after ingestion of buckwheat leaf extract ($N=9$, mean ± S.D.).

Fig. 4. Microchannel images before and after ingestion of BUCKWHEAT LEAF EXTRACT (upper, before ingestion; lower, 1 hour after ingestion).
One week after ingestion of BUCKWHEAT LEAF EXTRACT, blood components were measured. The total cholesterol and triglyceride levels after ingestion were slightly lower (5.5 and 8.7%, respectively) than those before ingestion. The cyclic AMP (c-AMP) concentration significantly ($p < 0.05$) increased (10.6%) (Fig. 5). The blood c-AMP has been reported to be increased by some phosphodiesterase inhibitors (peripheral vessel dilating effect) that improve renal blood flow. It is possible that BUCKWHEAT LEAF EXTRACT has similar effects.

![Graphs showing changes in blood components](image)

**Fig. 5.** Changes in blood components after ingestion of BUCKWHEAT LEAF EXTRACT ($N = 9$, mean ± S.D.).

**【Test methods】**

In the BUCKWHEAT LEAF EXTRACT ingestion test, blood fluidity was measured using blood treated with 5% heparin (1,000 units/mL). A microchannel array (Bloody 6-7, Hitachi Haramachi Electronics Co., Ltd.) and an MC-FAN (Hitachi Haramachi Electronics Co., Ltd.) were used to measure the passage time of 100 μL blood.

The subjects were 9 males and females of our company (age, 26-60 years; mean, 40.8 years). On the first day of the test, the subjects had 3 bread rolls and water (200 mL) for breakfast, and water (100 mL) 2 hours after breakfast. After 1 hour, blood was collected via a cubital vein, and whole blood passage time was measured as the value before ingestion of buckwheat leaves extract. Subsequently, BUCKWHEAT LEAF EXTRACT (120 mg) corresponding to 30 mg rutin was ingested with water (100 mL). After 1 hour, blood was collected, and whole blood passage time was measured as the value 1 hour after ingestion. From 6 days from the next day of the test, the subjects freely ingested BUCKWHEAT LEAF EXTRACT (120 mg/day). On the 7th day, whole blood passage time was measured by a method similar to that on the first day.
of the test as the value 1 week after consecutive ingestion.

Before and 1 week after ingestion of BUCKWHEAT LEAF EXTRACT, blood samples were also analyzed for the total cholesterol, triglyceride, and c-AMP concentrations.

Promoting effects on skin temperature recovery (thermography)

A skin temperature recovery test was performed by low-temperature loading on the hands and feet in 2 male volunteers of our company. After the subjects immersed their hands and feet in ice water (14°C) in a constant temperature room, skin temperature was serially measured. Subsequently, they ingested BUCKWHEAT LEAF EXTRACT (120 mg), and after 1 hour, a skin temperature recovery test was performed again in the constant temperature room by a method similar to that in the first test.

Fig. 6 shows thermographs with (right) and without (left) ingestion of BUCKWHEAT LEAF EXTRACT. A marked difference was observed in the fingers. Immediately after low-temperature loading, the temperature distribution was 21.9-25.3°C both with and without extract ingestion. After 15 minutes, the temperature distribution without extract ingestion was 25.3-32.1°C, but that with extract ingestion was 28.7-35.5°C, showing a slight increase. After 30 minutes, the temperature distribution without extract ingestion was 28.7-33.8°C, but that with extract ingestion was 32.1-35.5°C, showing a marked increase.

【Test methods】

A skin temperature recovery test was performed by low-temperature loading on the hands and feet in 2 male volunteers of our company. Two-three hours after breakfast, the subjects ingested 100 mL water and entered a constant temperature room (temperature, 25°C; humidity, 50%). After 1-hour acclimation, they immersed their hands and feet in water (14°C) for 1 minute. Immediately after the discontinuation of immersion, skin temperature was serially measured (at 6.4-second intervals for 30 minutes) using an infrared camera. After measurement, they had lunch, and after 2 hours, ingested BUCKWHEAT LEAF EXTRACT (120 mg) with water (100 mL). They entered the constant temperature room again, and a skin temperature recovery test was performed by a method similar to that in the first test.
Fig. 6. Thermographs after low-temperature loading [left, control; right, with ingestion of BUCKWHEAT LEAF EXTRACT (120 mg); upper, immediately after low-temperature loading; middle, after 15 minutes; low, after 30 minutes]
(2) Anti-oxidative activity

In the process of the development of atherosclerosis, damage of vascular endothelial cells by oxidized low-density lipoprotein (LDL) is involved. In addition, in ischemic areas in impaired circulation, various active oxygen species are produced, damaging the blood vessels. To reduce the risk of angiopathy, intake of food with anti-oxidation effects is effective. Therefore, the anti-oxidation effects of BUCKWHEAT LEAF EXTRACT were evaluated using superoxide dismutase (SOD) activity and the ability to scavenge 1,1-diphenyl 2-pycrylhidragil (DPPH) radicals as parameters. As a result, BUCKWHEAT LEAF EXTRACT had SOD activity and DPPH radical scavenging ability in the concentration rage shown in Fig. 7.

- **SOD-like activity**

- **DPPH radical scavenging activity**

Fig. 7. Anti-oxidation activity of buckwheat leave extract [(1) SOD Test Wako (Wako Pure Chemical Industries, Ltd.) was used.  (2) DPPH was used.]
5. Stability of BUCKWHEAT LEAF EXTRACT

(1) Thermal resistance
Evaluation of the heat stability of BUCKWHEAT LEAF EXTRACT showed no changes in the rutin content even after heating at 120°C for 1 hour.

Fig. 8. Thermal resistance of BUCKWHEAT LEAF EXTRACT (100% as initial value)

(2) pH stability
After storing of 16% alcohol solution of BUCKWHEAT LEAF EXTRACT without light shielding at room temperature for 1 week, the rutin content was measured. Rutin in BUCKWHEAT LEAF EXTRACT was found to be stable from neutral to acid field of pH.

Fig. 9. Influence of pH on rutin contents
6. Daily dosage of BUCKWHEAT LEAF EXTRACT
   It is recommended to take 240 to 600mg/day of BUCKWHEAT LEAF EXTRACT-P.

7. Nutrition facts of BUCKWHEAT LEAF EXTRACT

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3.6g/100g</td>
</tr>
<tr>
<td>Protein *1</td>
<td>1.0g/100g</td>
</tr>
<tr>
<td>Fat</td>
<td>0.9g/100g</td>
</tr>
<tr>
<td>Ash</td>
<td>1.1g/100g</td>
</tr>
<tr>
<td>Carbohydrate *2</td>
<td>93.4g/100g</td>
</tr>
<tr>
<td>Energy *3</td>
<td>386 kcal/100g</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>&lt;0.5g/100g</td>
</tr>
<tr>
<td>Sodium</td>
<td>18.0 mg/100g</td>
</tr>
</tbody>
</table>

*1) N=6.25
*2) 100 - (moisture + protein + fat + ash + dietary fiber)
*3) Factors for calculating the energy value: protein, 4; fat, 9; available carbohydrate (carbohydrate + dietary fiber), 4

Test trustee: Japan Food Research Center Foundation
Date of issue of the test result report: December 5, 2002.
Research result issue number: No. 302110638-001

8. Safety profile of BUCKWHEAT LEAF EXTRACT

(1) Residual agricultural chemicals

<table>
<thead>
<tr>
<th></th>
<th>Results</th>
<th>Detectable limit</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>BHC</td>
<td>Not detected</td>
<td>0.02ppm</td>
<td>GC</td>
</tr>
<tr>
<td>DDT</td>
<td>Not detected</td>
<td>0.02ppm</td>
<td>GC</td>
</tr>
<tr>
<td>Aldrin</td>
<td>Not detected</td>
<td>0.01ppm</td>
<td>GC</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>Not detected</td>
<td>0.01ppm</td>
<td>GC</td>
</tr>
<tr>
<td>Endrin</td>
<td>Not detected</td>
<td>0.01ppm</td>
<td>GC</td>
</tr>
<tr>
<td>Diazinon</td>
<td>Not detected</td>
<td>0.05ppm</td>
<td>GC</td>
</tr>
<tr>
<td>Parathion</td>
<td>Not detected</td>
<td>0.05ppm</td>
<td>GC</td>
</tr>
<tr>
<td>Marathon</td>
<td>Not detected</td>
<td>0.05ppm</td>
<td>GC</td>
</tr>
</tbody>
</table>

Test trustee: Japan Food Research Center Foundation
Date of issue of the test result report: December 25, 2002.
Research issue number.: 302110748-001

(2) Acute toxicity (LD50)

BUCKWHEAT LEAF EXTRACT (5000 mg/kg) was orally administered to male and female ICR mice aged 5 weeks under fasting, and the mice were maintained and observed for 14 days. Neither deaths nor abnormal changes in body weight compared with the controls were observed. Autopsy performed after the discontinuation of the experiment showed no macroscopic abnormalities in organs. Therefore, in male and female mice, the LD50 of
BUCKWHEAT LEAF EXTRACT by oral administration is \( \geq 5,000 \text{ mg/kg} \).

(3) Buckwheat allergens

The presence or absence of buckwheat allergens in BUCKWHEAT LEAF EXTRACT was examined by two types of sandwich ELISA designated by the Ministry of Health, Labor and Welfare (Shokuhatsu No. 1106001). As shown in the below result table, no allergen was detected.

<table>
<thead>
<tr>
<th>Results</th>
<th>Unit</th>
<th>Detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not detected</td>
<td>µg/g</td>
<td>FASTKIT (Nippon Meat Packer, Inc.)</td>
</tr>
<tr>
<td>Not detected</td>
<td>µg/g</td>
<td>Specified material measurement kit [Morinaga Biochemical Research Laboratory Co. Ltd.]</td>
</tr>
</tbody>
</table>

*) The notification by the Ministry of Health, Labor and Welfare (Shokuhatsu No. 1106001) mentions that samples are considered to be positive when the protein content derived from the specified material (i.e., buckwheat antigen content) is \( \geq 10 \mu g/g \).

Test trustee: Falco Life Science Ltd.

Date of issue of the test result report: April 4, 2003

Test result report number: AL130551

9. Practical Applications of BUCKWHEAT LEAF EXTRACT

<table>
<thead>
<tr>
<th>Applications</th>
<th>Health claim</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Healthy foods for women</td>
<td>1. Improving cold sensitivity/stiff shoulders</td>
<td>Hard and soft gel capsule, tablet, candy, chewing gum, cookies, chocolate, wafers, jelly, drink, etc.</td>
</tr>
<tr>
<td>2. Cure women’s diseases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. For diet and reducing edema</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Skin beauty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Healthy food for prevention of lifestyle associated disease</td>
<td>Prevention of atherosclerosis, hypertension, cerebral and myocardial infarction</td>
<td></td>
</tr>
</tbody>
</table>
10. **Packaging**

- **BUCKWHEAT LEAF EXTRACT –P** (Powder, for food)
- **BUCKWHEAT LEAF EXTRACT –PC** (Powder, for cosmetic)

5kg
- Interior packaging: aluminum-coated plastic bag
- Exterior packaging: cardboard box

11. **Storing Method**

Store in cool, dry place. Avoid humidity.

12. **Examples of the indications of buckwheat leaf extract**

The specified material “buckwheat” means buckwheat seeds and their processed materials and does not include buckwheat leaf extract. Therefore, the description, “A specified material is contained.” is not necessary as material indications. However, to avoid mistaking buckwheat leaf for buckwheat seeds, “buckwheat leaves” should be clearly mentioned.

Indication examples: buckwheat leaves extract, extract of buckwheat leaves
This product is extracted from buckwheat (*Fagopyrum esculentum* Moench. or *Fagopyrum tataricum* L.) leaves with ethanol. It includes more than 5.0% of rutin.

1. **Appearance**
   It is slightly brown or brown powder and has unique smell.

2. **Rutin content**
   Min. 5.0% (HPLC)

3. **Loss on drying**
   Max. 8.0% (Analysis for Hygienic Chemists, 1g, 105°C, 2h)

4. **Purity test**
   (1) **Heavy metals**
   Max. 10ppm (The Japanese Standards for Food Additives)
   (2) **Arsenic**
   Max. 1ppm (Standard Methods of Analysis in Food Safety Regulation)

5. **Standard plate counts**
   Max. 1 × 10³ cfu/g (Analysis for Hygienic Chemists)

6. **Moulds and Yeasts**
   Max. 1 × 10² cfu/g (Analysis for Hygienic Chemists)

7. **Coliforms**
   Negative (Analysis for Hygienic Chemists)

8. **Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Contents</th>
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</thead>
<tbody>
<tr>
<td>Buckwheat leaves extract</td>
<td>35 %</td>
</tr>
<tr>
<td>Dextrin</td>
<td>65 %</td>
</tr>
<tr>
<td>Total</td>
<td>100 %</td>
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   (1) Heavy metals
      Max. 10ppm
   (2) Arsenic
      Max. 1ppm

5. Standard plate counts
   Max. $1 \times 10^3$ cfu/g (Analysis for Hygienic Chemists)

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   Max. $1 \times 10^2$ cfu/g (Analysis for Hygienic Chemists)

7. Coliforms
   Negative (Analysis for Hygienic Chemists)

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We referred to the experiment methods of The Japanese Standards of Cosmetic Ingredient.
MEMO:
ORYZA OIL & FAT CHEMICAL CO., LTD., striving for the development of the new functional food materials to promote your health.

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

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