Role of Vegetable Protein in the Preparation of Weight Loss-Promoting Oil

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Abstract: We have reported that oil thermally processed with protein promoted safe and steady weight loss in animal experiments. In the present study, an oil for use in weight control was prepared by heating fresh oil with wheat gluten or soybean protein to determine the influence of protein differences on the weight loss-promotion effect. The 2 kinds of oil obtained, which differed neither from commercial fresh oil (starting oil) nor from one another in appearance, chemical properties, and aroma, were mixed (7%) with powdered AIN93G no-fat, defined standard diet and fed to 10-week-old Wistar rats ad libitum. After a 12-week feeding period, the rats were sacrificed to obtain blood and organs. There were no differences in amounts ingested, body weight increases, fecal excretion, organ weights, serum biochemical analyses, contents and fatty acid compositions of lipids of retroperitoneal fat tissue, or organ observations. Aspartate aminotransferase (AST), alanine aminotransferase (AST), and histological analysis supported the safety of the oil. In conclusion, the differences between wheat gluten and soybean protein in amino acid composition, both of the proteins and as free amino acids, were unrelated to the weight loss-promoting effect of the oil. Minor components in the vegetable proteins may have contributed to the effect on body weight.

Key words: wheat gluten, soybean protein, weight loss-promoting, serum triacylglycerol, fat tissue

1 INTRODUCTION

In our previous paper¹,², we reported that oil thermally processed with vegetable protein produced safe and steady weight loss effects in animal experiments. Some oils presently marketed for their weight control benefits² have chemical structures different from natural triacylglycerol in their fatty acid moieties, and thus differences in the metabolism of the oil explain their ability to promote weight loss. In contrast, the oil used in our studies ¹ is composed mainly of triacylglycerol. Coexisting polymerized oil was hydrolyzed minimally by lipase, and remarkably increased amounts of lipids were detected in feces. As polymerized oil decreases the nutritional supply of oil and fat, it is clearly a factor in the inhibition of body weight increases. However, it is presumed that polymerized oil and increased amounts of fecal lipids do not fully account for the weight loss–promoting effects, as described in our previous paper².

The effects on body weight were discovered while we were studying the influence of frying foodstuffs on the oxidation of oil during deep-frying. Sucrose, wheat starch, amino acids, and wheat gluten were chosen as components of frying foodstuffs²; wheat gluten is contained in wheat flour, which is frequently used as a batter coating for fried foods. As the wheat gluten employed is not 100% protein and contains substantial amounts of non–protein components besides water (data shown later), there might be a chance to prepare a more effective weight loss–promoting oil with other proteins. Besides wheat gluten, proteins from soybean², corn, barley, potato, sweet potato, sunflower, palm, and other sources are available. For comparison with wheat gluten, we selected soybean protein⁴, which is not of grain origin and for which scientific information is abundant.
2 EXPERIMENTAL

2.1 Materials

2.1.1 Protein

Gluten from wheat (Nacalai Tesque, Inc., Kyoto, Japan), and soybean protein (trial product, Fuji Oil Co., Ltd., Osaka, Japan) were obtained and analyzed for composition of free amino acids and amino acid composition of the protein at Japan Food Research Laboratories, Osaka, Japan.

2.1.2 Oil

One liter of fresh frying oil (Fuji Oil Co., Ltd., Osaka, Japan) was heated at 180°C for 10 h in a 2-L four-neck round-bottom flask under air or reduced pressure with 1wt% gluten from wheat and soybean protein, respectively. Protein was denatured and turned from pale yellow to yellow/brown during heating. Oils heated with protein were filtered over filter paper and the two oils heated under reduced pressure (wheat gluten oil, soybean protein oil) employed for an animal experiment.

2.1.3 Oil Analyses

Peroxide value (POV), carbonyl value (COV), acid value (AV), iodine value (IV) and Lovibond color were measured according to the Japan Oil Chemists’ Society’s Standard Methods for the Analysis of Fats, Oils, and Related Materials. The content of polar compounds (PC) was analyzed by a PC tester, 3M, Saint-Ouen l’Aumone, France. Triacylglycerol contents (TG) were determined by applying samples on Chromarods followed by development in a solvent mixture, hexane/diethyl ether/acetic acid, 50:10:1 v/v/v, and quantification with Iatroscan MK–6s, Iatron Laboratories, Inc., Tokyo, Japan. The fatty acid compositions of the fresh oil analyzed as previously were as follows: myristic acid (C14:0) 0.1%; palmitic acid (C16:0) 11.8%; palmitoleic acid (C16:1) 0.1%; stearic acid (C18:0) 3.1%; oleic acid (C18:1) 36.1%; linoleic acid (C18:2) 42.0%; and Ｅα–linolenic acid (C18:3) 6.4%; eicosanoic acid (C20:1) 0.2%; others 0.2%.

2.1.4 Diets

A commercial powdered AIN93G diet without fat (Japan Clea, Tokyo) was purchased. Using a blender, the diet was mixed uniformly with 7 wt% of experimental oils, respectively, and 140 g of diet for each rat was sealed in a plastic bag (Diamilon M, Mitsubishi Plastics, Tokyo, Japan) by vacuum sealer (Tospack V–380G, Tosei, Tokyo, Japan), kept in a cold room, and subjected to radio-sterilization of 10 kGy by Kohga Isotope (Shiga, Japan) prior to the animal experiment. When the diet in a bag was used up, the next bag was opened for feeding.

2.2 Animals

Sixteen weanling male Wistar rats aged 3 weeks were obtained from Japan SLC, Inc., Shizuoka, Japan, and were housed separately in aluminum flat cages at 24 ± 2°C and humidity 50 ± 10%, with light from 7:00 to 19:00 at Japan SLC, Inc., Animal Experiment Center, Shizuoka, Japan.

2.3 Procedure

2.3.1 Animal experiment

Rats were maintained for 7 weeks on commercial pellet-ed AIN93G, then divided into two groups (8 rats/group) by Statlight System, Yukms, Tokyo, Japan, housed separately and allowed ad libitum water and AIN93G no-fat powder added with 7 wt% gluten oil or 7 wt% soybean oil throughout 12 experimental weeks. Autoxidation of oil in the diet was avoided by supplying a fresh diet daily. Body weight, and the amount of feed ingested in 24 h were determined weekly. Daily feed amounts in 24 h were also determined in 10, 13, 16 and 19 weeks of age. When rats were 22 weeks of age, a fasting period of 18 h was imposed prior to the animal experiment. Serum was obtained from blood drawn from the abdominal aorta. Livers, kidneys, and retroperitoneal fat tissue were excised, weighed, and examined. Total feed ingestion of each rat was also measured. Animal care and handling were in accordance with the Ethical Agreement Concerning Care and Use of Laboratory Animals for Research and Education, Kobe–Gakuin University.

2.4 Analyses of retroperitoneal fat tissue lipids

Frozen retroperitoneal fat tissue was ground into fine pieces with a mill mixer MR–280, Yamazen, Tokyo, Japan, and subjected to lipid extraction using the chloroform/methanol (2:1 v/v) method. The solvent was then removed by a rotary evaporator from the samples and retroperitoneal fat tissue lipids were methyl–esterified for the fatty acid analysis by gas chromatography.

2.5 Histological evaluation

Fixed liver and kidneys were embedded with paraffin, and microscopic specimens were sliced, then subjected to hematoxylin and eosin stain according to conventional methods. Histological evaluation was made at a magnification on 100 and 400 depending on circumstances.

2.6 Serum biochemical analyses

Determination of serum AST, ALT, insulin, glucose (GLC), TG, phospholipids (PL), total cholesterol (T–CHO), and nonesterified fatty acid (NEFA) were made with Autosera S ALT, Autosera S ALT (Daichi–Kagaku–Yakuhin Co. Ltd., Tokyo, Japan), Morinaga Insulin Measurement Kit (Morinaga Institute of Biological Science Inc., Tokyo, Japan), Glucose CII–test Wako, Triglyceride E–test Wako, Phospholipid C–test Wako, Cholesterol E–test Wako, and NEFA C–test Wako (Wako Pure Chemical Industries, Osaka, Japan), respectively.

2.7 Statistical analysis

All the values obtained from animals are revealed as mean ± SD. Data from 8 animals each for two groups were
analyzed using Student's t-test for unpaired observations and results were considered significant at \( p<0.05 \).

### 3 RESULTS

#### 3.1 Protein analyses

Table 1 shows that the protein contents of wheat gluten and soybean protein were the same, although there were small differences in water and ash contents. Wheat gluten was typically high in glutamic acid and proline, and its glutamic acid level was 2 times higher than that of soybean protein (Fig. 1). The levels of lysine, arginine, and aspartic acid in soybean protein were higher than those in wheat gluten. The major free amino acids of wheat gluten and soybean protein were tryptophan and aspartic acid; and arginine, glutamic acid and tryptophan, respectively, although free amino acids accounted for only one one-hundredth of the amino acids in protein.

#### 3.2 Oil analyses

The chemical properties of oils heated with wheat gluten or soybean protein under air and reduced pressure are shown in Fig. 2. In all conditions tested, no increase was found in AV, as the oil was heated without contacting water. Peroxides formed during heating under air were rapidly transformed into carbonyl and polar compounds, resulting in elevation of COV and PC contents and deterioration of oil color. In contrast, wheat gluten and soybean protein had essentially no effect on the chemical properties or aroma of the oil. The protein difference was unrelated to the properties of the oil. The smell of the thermally processed oil was controlled by the presence of air, which creates an unpleasant odor. Thus, heating the oil with protein under reduced pressure was preferable.

![Fig. 1 Amino Acids in Wheat Gluten and Soybean Protein.](image1)

![Fig. 2 Chemical Properties of Oils Heated with Wheat Gluten or Soybean Protein under Air and Reduced Pressure.](image2)

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**Table 1** Composition of Wheat Gluten and Soybean Protein.

<table>
<thead>
<tr>
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<th>Wheat gluten</th>
<th>Soybean protein</th>
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<tr>
<td>Water (%)</td>
<td>8.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>1.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>82.7</td>
<td>83.3</td>
</tr>
<tr>
<td>Others (%)</td>
<td>7.5</td>
<td>7.4</td>
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</table>
3.3 Growth of animals

Body weight increases of the wheat gluten oil and soybean protein groups were similar, with the latter slightly lower without significant differences (Fig. 3). The total amount ingested per rat during the 12-week feeding experiment was (1446 ± 229) g for the wheat gluten group, and (1403 ± 178) g for the soybean protein group; with no significant difference. Fecal excretion also did not differ between the groups (Fig. 3). As rats fed ad libitum AIN93G no-fat powder containing 7% fresh oil excreted (0.4 ± 0.2) g/d of feces between 10 and 19 weeks of age, it is obvious that the fecal amounts of rats in the present study were high.

3.4 Analyses of liver, kidneys, and retroperitoneal fat tissue

No abnormalities were detected in the wheat gluten or soybean protein groups in histological analysis and observation of the liver, left kidney, right kidney, or retroperitoneal fat tissue, which weighed (8.586 ± 0.5518) g, (1.127 ± 0.0641) g, (1.072 ± 0.0475) g, and (9.715 ± 1.3215) g for the wheat gluten group and (8.552 ± 0.5274) g, (1.135 ± 0.0575) g, (1.075 ± 0.0558) g, and (9.794 ± 1.4968) g for the soybean protein group, respectively. Lipid contents of retroperitoneal fat tissue were (89.4 ±2.8) % for wheat gluten group and (88.9 ±1.4) % for soybean protein group also showing no statistical difference. In addition, there was no difference in fatty acid composition (Fig. 4), implying that wheat gluten and soybean protein oils did not cause any differences in lipid accumulation.

3.5 Serum biochemical analyses

No differences were observed in the parameters determined (Table 2; in our previous paper2), the TG level of the soybean protein group was significantly lower (p<0.01) than that of a control group fed the corresponding diet containing fresh oil. In the present study, only the GLC level of the wheat gluten group was higher than that of the soybean protein group, but

![Fig. 3](image)

**Fig. 3** Growth of Rats Fed a Diet Containing Wheat Gluten Oil or Soybean Protein Oil. Values are expressed as mean ± SD (n=8).

![Fig. 4](image)

**Fig. 4** Fatty Acid Composition of Retroperitoneal Fat Tissue Lipids from Rats Fed a Diet Containing Wheat Gluten Oil or Soybean Protein Oil. Values are expressed as mean ± SD (n=8).

<table>
<thead>
<tr>
<th></th>
<th>AST (U/L)</th>
<th>ALT (U/L)</th>
<th>TG (mg/dL)</th>
<th>GLC (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat gluten</td>
<td>82.9 ± 7.5</td>
<td>41.5 ± 9.7</td>
<td>60.5 ± 23.1</td>
<td>134.1 ± 20.9</td>
</tr>
<tr>
<td>Soybean protein</td>
<td>87.6 ± 11.2</td>
<td>45.3 ± 10.8</td>
<td>64.3 ± 16.5</td>
<td>126.0 ± 16.5</td>
</tr>
</tbody>
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<tr>
<th></th>
<th>PL (mg/dL)</th>
<th>T–CHO (mg/dL)</th>
<th>NEFA (μg/dL)</th>
<th>Insulin (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat gluten</td>
<td>115.6 ± 10.1</td>
<td>74.3 ± 5.9</td>
<td>57.3 ± 12.3</td>
<td>2.309 ± 1.123</td>
</tr>
<tr>
<td>Soybean protein</td>
<td>115.4 ± 10.1</td>
<td>75.4 ± 7.7</td>
<td>68.1 ± 12.0</td>
<td>2.528 ± 1.125</td>
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the difference was not statistically significant. AST and ALT were normal and not indicative of cytotoxicity.

4 DISCUSSION

The compositions of wheat gluten and soybean protein were not substantially different (Table 1), but the amino acid compositions of the protein and free forms were characteristic (Fig. 1). The oil prepared with the protein was similar to fresh oil in chemical properties and aroma (Fig. 2). In addition, all of the parameters determined in the animal experiment showed no statistically significant differences between the wheat gluten and soybean protein groups; these parameters included ingestion amounts, body weight increases, fecal amounts (Fig. 3), observation of organs, organ weights, lipid contents in retroperitoneal fat tissue, fatty acid compositions of the lipids (Fig. 4), and serum biochemical analyses (Table 2). In addition, histological analysis showed no degeneration. The fatty acid composition of fat tissue lipids from rats fed the test oil prepared under air were C16:0 (29.7 ± 1.6)%, C18:1 (39.8 ± 1.5)%, C18:2 (17.4 ± 0.8)%, C18:3 (1.1 ± 0.3)%6). The oil prepared under reduced pressure showed high levels of C18:2 and C18:3 and a low level of C16:0; the percentage of C18:1 was unchanged (Fig. 4). As almost no oxidation and polymerization of polyunsaturated fatty acids occurred during thermal treatment under reduced pressure, wheat gluten and soybean protein oils seem to be digested and absorbed alike and in the same way as fresh oil.

These results imply that the difference between wheat gluten and soybean protein in amino acid composition, both of protein and of free amino acids, was unrelated to the effects of the oil on body weight. To our knowledge, ingestion of protein and/or amino acids does not create effects that promote weight loss; that is, increased fecal amounts, body weight increases, fecal amounts (Fig. 3), observation of organs, organ weights, lipid contents in retroperitoneal fat tissue, fatty acid compositions of the lipids (Fig. 4), and serum biochemical analyses (Table 2). In addition, histological analysis showed no degeneration. The fatty acid composition of fat tissue lipids from rats fed the test oil prepared under air were C16:0 (29.7 ± 1.6)%, C18:1 (39.8 ± 1.5)%, C18:2 (17.4 ± 0.8)%, C18:3 (1.1 ± 0.3)%6). The oil prepared under reduced pressure showed high levels of C18:2 and C18:3 and a low level of C16:0; the percentage of C18:1 was unchanged (Fig. 4). As almost no oxidation and polymerization of polyunsaturated fatty acids occurred during thermal treatment under reduced pressure, wheat gluten and soybean protein oils seem to be digested and absorbed alike and in the same way as fresh oil.

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There are several studies on the effects of legumes on body weight. Liao et al.12 investigated the effects of a soy–based low–calorie diet on weight control, body composition, and blood lipid profiles compared with a traditional low–calorie diet and found that the soy–based diet reduced body fat percentage to a greater extent than the traditional diet. Kwon et al.13 raised rats on a high–fat diet containing 0.037% black soybean anthocyanins. Body weight gain was significantly lowered in these rats when compared with rats fed a high–fat diet alone (p<0.05). The diet containing black soybean anthocyanins also suppressed weight gain in the liver; tended to decrease the weights of the epididymal and perirenal fat pads; and significantly reduced the levels of serum triglycerides and cholesterol (p<0.05). Cederroth et al.14, 15 studied the effects of soy isoflavones, a class of phytoestrogens on glucose and lipid metabolism and their possible mechanism of action, and suggested that they favorably altered glycemic control, improved weight and fat loss, and lowered triglycerides, low density lipoprotein cholesterol and total cholesterol. Shimoda et al.16 fed ddY mice a standard diet containing green coffee bean extract (GCBE) for 14 days and found that 0.5% and 1% GCBE reduced visceral fat content and body weight. Kim et al.17 injected obese rats with 50 mg/100 g body weight adlay seed crude extract (ACE) daily for 4 weeks. The group receiving a high–fat diet + ACE significantly reduced food intake and body weight, as well as weights of epididymal and peritoneal fat. The levels of triglyceride, total cholesterol, and leptin in blood serum were significantly decreased in this group compared to control group.

As described above, weight loss–promoting effects can be expected in components of legumes. Our previous study1 demonstrated such effects in oil heated with wheat gluten. Katcher et al.20 randomly advised obese adults with metabolic syndrome to either to avoid whole–grain foods or to obtain all of their grain servings from whole grains for 12 weeks. There were significantly greater decreases in percent body fat in the abdominal region in patients consuming whole grains than in those consuming refined grains. Kim et al.19 evaluated the physiologic consequences of using white rice or a mixture of brown rice and black rice in overweight Korean women (body mass index ≥25 kg/m²) over a period of 6 weeks. Meal replacement with mixed rice was superior to replacement with white rice in weight control. Thus, minor components in legumes and grains may have effects that are beneficial in weight control.

In conclusion, the differences between wheat gluten and soybean protein in amino acid composition of both protein and free amino acids were unrelated to effects on body weight. Minor components in the vegetable proteins may have contributed to the observed effects on body weight.
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References