Eur Food Res Technol (2007) 225:925–928
DOI 10.1007/s00217-006-0497-4

SHORT COMMUNICATION

The effects of okara on rat growth, cecal fermentation, and serum lipids

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Received: 6 July 2006 / Revised: 13 September 2006 / Accepted: 1 October 2006 / Published online: 15 November 2006
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Abstract Okara, a soymilk residue, was characterized and used as a supplement to enrich dietary fiber in rats. Okara comprised 49% total dietary fiber, of which only 0.55% was soluble, protein (33.4%), fat (19.8%), and ash (3.5%). Okara as a diet supplement had no influence on food intake, but the growth rate and feeding efficiency were lower in the okara-fed group than in the control group. Okara increased fecal weight and moisture. In okara-fed rats, in vivo colonic fermentation of okara resulted in a lower pH, but a higher cecal weight and higher total short chain fatty acid production, compared to controls. There were no significant differences ($P \leq 0.05$) between groups in albumin, protein, uric acid, bilirubin, or glucose content in rat serum. The okara-supplemented diet produced a nonsignificant reduction in HDL-lipids and triglycerides. Okara, a rich source of low-cost dietary fiber and protein, might be effective as a dietary weight-loss supplement with potential prebiotic effect.

Keywords Okara · Dietary fiber · Prebiotics · Soymilk residue · Tofu byproduct · Soybean

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Introduction

Soybeans, which belong to the Leguminiaceae family, come from East Asia and are consumed as a base-food in many Eastern countries. The consumption of soy foods is on the increase, even in Western societies, due to their nutritional and health benefits.

Three different types of soy fiber are obtained from soybean: soy-bran, isolate fiber, and okara, all of which are high-quality and inexpensive. Soy bran is made from soybean hulls and soy isolate fiber is a fibrous form of soy protein [1]. Okara, a Japanese word meaning "honorable hull" or soy pulp, is a byproduct of soymilk and tofu manufacturing. On average, 53% of the initial soybean dry mass is recovered in tofu and 34% in okara [2]. Okara is rich in dietary fiber (50–60%), protein, and fat [2–5]. It has a nut taste (e.g., almond, coconut) and low solubility in water. The protein content of okara is approximately 30%, with a good essential amino acid profile and in vitro digestibility [3, 4], making it a low-cost vegetable protein. The fat that remains in okara is approximately 10% [2, 3], with a high polyunsaturated fatty acid content. Small amounts of starch, sugars, potassium, and significant levels of B group vitamins are also recovered in okara [2].

Most reports in the literature deal with okara protein [3, 4, 6]. Nevertheless, due to its high fiber content, okara can be used as a supplement in human diets, especially occidental diets, which lack the necessary fiber. Okara has been used to

partially replace wheat flour for bread making [7] and also as a fermentation stock for the production of seasonings, spices, and tempeh [8]. The use of okara, however, is still in the developmental stage.

Our aim was to study the composition and effects of okara on growth, cecal fermentation, and serum lipids in rats, to assess the potential of okara as a food ingredient with a health-promoting effect.

Material and methods

Raw material

Okara, a byproduct of soymilk and tofu-making processes, was obtained from a local food processing (Toofu-Ya S.L., Arganda del Rey, Madrid, Spain). Okara was freeze-dried (Virtis Benchtop 3L freeze-drier) and milled to a particle size of less than 1.0 mm before analysis.

Analytical methods

All determinations were performed at least three times and are reported on a dry matter basis as mean values ± standard deviation. The moisture content of okara was determined by weight loss after oven-drying to a constant weight at 105 °C [9]. Total nitrogen was determined using the micro-Kjeldahl method [10], and protein was calculated as nitrogen × 5.71. Fat content was determined by extraction with petroleum ether using the Soxhlet method [11]. Dietary fiber was analyzed using the Association of Official Analytical Chemists enzymatic–gravimetric method, fractionating into insoluble and soluble residues [12, 13]. Ash content was measured as the residue obtained after incinerating at 550 °C for 3 h [14].

Animals and diet

A total of 20, 4-week-old, Wistar Hannover female rats weighing approximately 200 g, were used in the experiment. The rats were divided into two groups of 10. The control group was fed a standard rat chow (Panlab S.L., ref. A04, Barcelona, Spain), and the treated group was fed a mixture of the standard rat chow plus okara to give a final concentration of 10% (w:w) with 5% dietary fiber (Table 1). The rats were housed in cages in a room with controlled light (12 h, 08:00–20:00), temperature (22 ± 1 °C), humidity (60–65%), and free access to food and water. The animals were maintained in accordance with the laboratory guidelines (Facultad de Biología, Universidad Complutense de Madrid, Spain) concerning the care and use of animals. The body weight of the rats and feces were recorded weekly. The animals were fed for 4 weeks and were then humanely killed under anesthesia (diethyl ether). The whole organs (spleen, kidneys, heart, and liver) were rapidly removed and weighed. The gastrointestinal tract was also removed and cecal weight was recorded. After clotting the blood at room temperature, it was centrifuged at 1000 × g for 15 min and the serum was collected for further use.

Table 1 Composition of control and okara diets

	Control	Okara
Moisture (g/kg)	120	108
Protein (g/kg)	154	138.6
Fat and oil (g/kg)	29	26.1
Carbohydrates (g/kg)	605	544.5
Starch (g/kg)	443	398.7
Total sugar (g/kg)	25	22.5
Fiber (cellulose, g/kg)	39	35.1
Okara[a] (g/kg)	–	100
Minerals (g/kg)	53	47.7
Calcium (ppm)	9,100	8,190
Phosphorus (ppm)	5,900	5,310
Additives		
Vit A (U/kg)	15,000	13,500
Vit D_3 (U/kg)	1,500	1,350
Vit E (mg/kg)	20	18
Copper sulphate.$5H_2O$ (mg/kg)	12	10.8

Estimated energy intake of control diet by analysis: 3173 kcal/kg; estimated energy of the okara diet : 3167 kcal/kg.

[a]Composition of okara (% dry weight): total dietary fiber, 49.05 ± 1.35; protein, 33.42 ± 0.14; fat, 19.76 ± 0.04; ash, 3.49 ± 0.09.

Clinic analyses

Albumin, total protein, uric acid, bilirubin, and glucose were measured using commercial kits (Menarini, Firenze, Italy). High density lipoprotein lipids were measured in the serum after precipitation of apo-B-containing lipoproteins with phosphotungstic acid and magnesium (Böehringer Mannheim, Germany). Cholesterol, triglyceride, and choline-containing phospholipids were enzymatically measured in the serum (Menarini, Firenze, Italy). All the determinations were performed using a RA-1000 Technicon Autoanalyzer. The instrument and technique were adapted for use with rat serum.

Sampling and processing of cecal digesta

The cecum from each experimental group was separated and its content aseptically collected for the determination of pH, short-chain fatty acids (SCFA), and dry weight. A portion of the cecal content was diluted 1:3 in Milli-Q deionized water (Millipore, Iberica, Spain) immediately after sampling; the digesta pH was measured using a microelectrode (Crison, micro pH 2001), and the remaining sample was stored at −20 °C until analysis. When SCFA were measured, the diluted samples were thawed, centrifuged at 12000 rpm for

15 min at 4 °C, and the supernatants utilized for gas–liquid chromatography. A 0.4 mL sample with 0.5 mL internal standard in 12% formic acid (4-methyl valeric acid, 2 μmol/mL) and made up to 1 mL with Milli-Q water was centrifuged as explained earlier, and 1 μL of supernatant was injected into a gas–liquid chromatograph (5890 Hewlett Packard) equipped with a flame ionization detector and a fused silica column (Carbowax 20 M, 10 m × 0.53 mm × 1.33 μm film thickness). The carrier gas was nitrogen with a flow rate of 15 mL/min. The injector and detector temperature was 250 °C and the column temperature was isothermal at 120 °C [15].

Statistical analyses

The statistical study was performed using the SPSS program, using one-way analysis of variance with the statistical significance set at a probability level of less than 0.05. Posthoc analysis of the analysis of variance was performed using the Bonferroni test.

Results and discussion

Characterization of okara

Total dietary fiber of the okara comprised 49.05%, of which only 0.55% was soluble (Table 1). The protein content and fat content were higher than previously reported [2, 3, 5]. Ash and total fiber content were similar to that reported previously, but soluble fiber was much lower [2, 3].

In vivo assay

Both diets had an equivalent caloric content (Table 1). Okara had no effect on food intake, which on average was 10 ± 0.1 g/day in both groups. Nevertheless, final weight, growth rate, and feeding efficiency were lower for the okara-fed group than the control group (Table 2). There were no significant differences ($P \leq 0.05$) in heart, spleen, kidneys, and liver weights between the treated and control rats, and the weights were within normal ranges (data not shown).

The okara diet increased fecal excretion. Thus, daily fecal weight was always higher for the okara-fed group (53.2 g f.w.) than the control group (21.5 g f.w) throughout the experiment. This was attributed to a higher moisture content of feces in the okara-fed group, because of a higher intake of insoluble dietary fiber. The contribution of okara protein cannot be ruled out, however, as soy protein holds three to four times its weight in water [16]. Fecal moisture in the okara-fed group (54.18 ± 7.53 g/100 g) was more than three times that of the control group (16.72 ± 1.89 g/100 g). Similar results were reported previously [17].

Table 2 Effect of okara on body weight gain, growth rate, feeding efficiency[a], and serum lipids

	Control	Okara
Initial weight (g)	203.8 ± 3.97	200.2 ± 4.80
Final weight (g)	227.7 ± 7.99	216.9 ± 5.26
Growth rate (g/day)	0.94 ± 0.15	0.69 ± 0.22
Feeding efficiency	0.093 ± 0.01	0.068 ± 0.02
Serum lipids(mg/dL)		
Triglycerides	80 ± 14	72.83 ± 25.15
Cholesterol	55.84 ± 10.75	64.10 ± 14.28
HDL-cholesterol	37.70 ± 5.69	34.84 ± 9.63
LDL-cholesterol	18.14 ± 9.42	29.25 ± 15.63
HDL-phospholipids	69.25 ± 7.18	67.52 ± 6.90

Mean values ± SD.

[a]Feeding efficiency $= \frac{\text{Body weight gain (g)/Time(d)}}{\text{Food intake (g/d)}}$.

In vivo colonic fermentation of okara

Cecal weight in okara-fed rats (3.9 ± 1.0 g) was slightly higher than that in the control group (3.7 ± 0.4 g). Fermentable carbohydrates affect cecal weight [15, 18]. Colonic fermentation resulted in a lower pH (6.0 ± 0.1) of cecal contents in okara-fed rats, compared to controls (6.3 ± 0.3). Accordingly, total SCFA of cecal contents were higher in okara-fed rats (823.3 ± 148.2 μmol/g), than in controls (631.7 ± 97.1 μmol/g), consistent with previous reports [15]. The molar proportion of acetate was slightly higher in the okara-fed group, but molar proportions of propionate and butyrate were similar [15, 19]. SCFA are fermentation products of nondigestible carbohydrates that are responsible for the low pH in the cecum. The low content of branched chain fatty acids indicated that okara proteins had not been fermented.

The effect of okara fiber on the rat gastrointestinal tract has been reported [17]. Apparent degradability of okara is twice that of wheat bran and there are also differences in the pH of the cecal contents. Moreover, okara fiber reduces transit time in the intestine [17].

Albumin, protein, uric acid, bilirubin, glucose, and serum lipids

There were no significant differences ($P \leq 0.05$) in albumin (43.93 ± 2.62 g/dL), protein (64.57 ± 4.53 g/dL), uric acid (3.67 ± 1.04 mg/dL), bilirubin (1.06 ± 0.54 mg/dL), or glucose (200 ± 46 mg/dL) contents between groups. Serum lipids are shown in Table 2. The okara-supplemented diet produced a reduction of HDL lipids and triglycerides, but there was no significant difference compared to controls. Normally, rats have higher HDL than LDL cholesterol levels. Serum lipids were close to previous results reported for tofu-fed rats [20].

Conclusions

There were no significant differences between the two groups in any of the analyses, with the exception of decreased body weight and increased cecal fermentation in the okara-fed group. Thus, okara might be useful as a dietary weight-loss supplement with potential prebiotic effect.

Acknowledgements This research was supported by the Spanish Ministry of Science and Education, through CICYT Projects AGL 2002-03221-ALI and AGL 2005-02447-ALI. Thanks to Mr. Takazumi of Toofu-Ya S.L. for the okara provided and to Mrs. Morcillo and Mrs. Gañán for assistance with the animal experiments. IEM acknowledges CSIC for her scholarship.

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