JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY



# Health-Promoting Effects of a Dietary Fiber Concentrate from the Soybean Byproduct Okara in Rats

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Okara (a byproduct of the soy milk industry) is rich in proteins (24.5−37.5 g/100 g of dry matter (dm)), lipids (9.3−22.3 g/100 g of dm), and dietary fiber (DF) (14.5−55.4 g/100 g of dm). It also contains isoflavones (0.14 g/100 g of dm). In the present study we fed female healthy Wistar rats either a standard rat diet or a supplemented 10% DF-rich okara (DFRO) diet for 4 weeks, and then we assessed several health parameters in the serum and the cecum compartments. In comparison to the control group, rats fed DFRO showed a significant decrease in weight gain (5.00 ± 1.22 g vs 2.00 ± 1.46 g, $P < 0.03$, during week 4) and in total cholesterol (65 ± 8 mg/dL vs 51 ± 5 mg/dL, $P < 0.05$) and a significantly increased antioxidant status (36.71 ± 15.31 μmol of Trolox equivalents (TEs)/g vs 69.75 ± 16.11 μmol of TEs/g, $P < 0.0003$) and butyrogenic effect (39.37%, $P < 0.003$) in the cecum. In addition, a significant enhancement in the apparent absorption (41.89 ± 1.64% vs 47.02 ± 2.51%, $P < 0.004$) and in the true retention (41.62 ± 1.60% vs 46.68 ± 2.55%, $P < 0.005$) of calcium was appreciated. In summary, these findings show for the first time that a concentrate DF from a soybean byproduct protects the gut environment in terms of antioxidant status and prebiotic effect. These results may highlight the development of an innovative soybean byproduct rich in DF which could be useful as a functional ingredient with health-promoting attributes.

**KEYWORDS: Okara; soybean; *Glycine max*; byproduct; dietary fiber; rats; prebiotic; reduction power; cecum; cholesterol; butyrate; minerals; calcium**

## INTRODUCTION

Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases. As a consequence, an increased consumption of products from vegetable origin, which contain high levels of dietary fiber (DF) and phytochemical constituents, has been recommended (*1, 2*).

Obesity is considered a worldwide public health problem showing a high prevalence in developed countries, with urgent need for new and more efficient strategies (*3*). Overweight is associated, among other factors, with lower DF intake (*4*). In contrast, the per capita intake of DF in Western countries does not cover the recommendations of DF intake given by health institutions (*5*). For example, adequate DF intake in the U.S./Canada is set at 31 g/day by the Food and Nutrition Board (FNB), whereas the DF daily recommended intake given by the FAO/WHO and the EURODIET project is about 25 g/day.

Prebiotics are dietary carbohydrates which escape digestion in the small intestine, but undergo bacterial fermentation in the large intestine and beneficially affect the intestinal microbiota (*6–8*). The products of fermentation in the colon, mainly short-chain fatty acids, have a role in the improvement of mineral absorption (*8*). Fermentable carbohydrates could favor mineral absorption in the distal part of the digestive tract in several ways: hypertrophy of the cecal wall and greater surface area, increase in soluble minerals, and accelerated blood flow (*9*).

Okara is a byproduct of the soy milk industry. Raw okara, also called soy pulp, is a white-yellow material consisting of insoluble parts of the soybean seed remaining in the filter sack when pureed soybean seeds are filtered in the production of soy milk. This byproduct has been used in the vegetarian diets of Western countries since the 20th century (*10*). Okara is rich in proteins (24.5−37.5 g/100 g of dry matter (dm)), lipids (9.3−22.3 g/100 g of dm), and DF (14.5−55.4 g/100 g of dm) (*10–13*). It also contains isoflavones (0.14 g/100 g of dm) (*12*). Due to this peculiar composition, okara might have a

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10.1021/jf800792y CCC: $40.75 
Published on Web 07/18/2008

potential use in the food industry as a functional ingredient. In this sense, okara could be used to increase the DF content in cereal products (*10*) and in low-fat beef patties (*14*), but as others authors state, there is little information on the production of a DF concentrate from okara (*12*).

Several health effects of okara have been assessed. Recently, in vitro experiments have indicated that okara is a potential source of antioxidant components (*15*), showing that protease hydrolysate from okara yielded antioxidant activity (*16*). Interestingly, it has been suggested that okara has beneficial effects in preventing obesity in a mouse model, through a marked reduction in the expression of leptin genes in adipose tissue (*3*). Another study has been conducted to evaluate the blood cholesterol levels in a hypercholesterolemic rat model (*11*), indicating the suppression of the increase in plasma cholesterol after 4 weeks of intake. Our group has previously examined the effects of okara in rats, concluding that okara might be useful as a weight-loss dietary supplement (*13*).

In our study, to increase the relative content in DF and to enhance the potential use of okara in preventing obesity, okara was treated to reduce its water and oil content. No studies dealing with the health-promoting attributes of a DF concentrate of a byproduct from soybean processing were found.

The evaluation of the protective effect of the DF-rich okara product in the serum and cecal compartments using an animal model could clarify the health-promoting attributes of okara. These compartments are a systemic one and a partial representation of the gut environment, respectively.

Therefore, the aim of the study was to evaluate the effect of a DF-rich okara diet on the biochemical parameters (total protein, albumin, uric acid, glucose, total cholesterol, LDL and HDL cholesterol, atherogenic index, triglycerides), mineral balance, prebiotic effect, and antioxidant status in healthy rats.

## MATERIALS AND METHODS

**Chemicals.** Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble analogue of vitamin E, was from Aldrich Co. (St. Louis, MO). All reagents used were of analytical grade.

**Materials and Preparation of a Dietary Fiber Concentrate from Okara.** Okara was provided as a fresh byproduct from soybean (*Glycine max* (L.) Merr.) by Toofu-Ya S.L., a local food processing company (Arganda del Rey, Madrid, Spain). Due to its high water content (~79%) okara was preserved at −18 °C until preparation of the DF concentrate. Afterward the byproduct was freeze-dried, ground (1.0 mm mesh), placed in sealed vacuum bags, and stored at − 18 °C. The freeze-dried okara was defatted by Soxhlet extraction of the oil. The resulting product is called throughout the paper dietary-fiber-rich okara (DFRO).

**Animals, Maintenance, and Experimental Design.** Female Wistar Hannover rats ($n = 12$; 193−210 g), $10^1/_2$ week old, were from the feeding animal center of Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, Madrid, Spain. The use of animals was conducted in compliance with the Laboratory European guidelines for the care and use of laboratory animals and approved by the Experimental Animals Committee at the Universidad Complutense de Madrid. The rats ($n = 12$) were housed individually in metabolic cages in a room at 22 ± 1 °C and 60−65% humidity with a controlled 12 h light/12 h dark cycle. The rats were fed a basal diet (Panlab S.L., ref A04, Barcelona, Spain), and after a 1 week adaptation period (week 0) the rats were divided into two groups: the control group ($n = 5$) and the DFRO group ($n = 7$). The DFRO group was fed the basal diet plus 10% of the DFRO product during the 4 weeks of the experiment. The diets and water were provided ad libitum. The composition of the diets given to the control and the treated groups is given in **Table 1**.

**General Procedure.** The body weight, food intakes, feces weight, and urine volume were recorded at the end of each week, including the adaptation week. The feeding efficiency (FE), a parameter which reflects the relation between body weight gain and food intake, was also calculated. Feces and urine samples from each animal were recorded separately on alternate days three times per week. Immediately after collection the samples were frozen at −20 °C, and at the end of each week the samples from each animal were pooled; as a result, a pooled sample per animal and per week was analyzed in triplicate. The rats were anesthetized with carbonic anhydride and killed by blood extraction via carotid puncture. Blood samples were collected into tubes, and the serum was separated by low-speed centrifugation (1500*g*, 4 °C, 10 min). Several organs (heart, kidneys, spleen, and liver) were rapidly removed and weighed. The gastrointestinal tract was removed and its longitudinal measure recorded; also the cecum, including the content, was weighed and recorded. Serum samples and the cecum were stored at −80 °C until analysis.

**Table 1.** Composition of Test Diets Used in the Feeding Experiment (g/kg)

| | control | DFRO |
|---|---|---|
| moisture | 120 | 108 |
| protein | 154 | 142 |
| fat and oil | 29 | 26 |
| carbohydrates | 605 | 545 |
| starch | 443 | 399 |
| total sugar | 25 | 23 |
| dietary fiber (cellulose) | 39 | 35 |
| DFRO[a] | 0 | 100 |
| vit A (U/kg) | 15000 | 13500 |
| vit $D_3$ (U/kg) | 1500 | 1350 |
| vit E | 0.020 | 0.018 |
| minerals | 48 | 47 |
| calcium | 9.26 | 9.09 |
| magnesium | 1.68 | 1.63 |
| zinc | 0.096 | 0.095 |
| phosphorus | 5.90 | 5.31 |
| copper sulfate | 0.012 | 0.011 |
| energy (kcal/kg) | 3173 | 3149 |

[a] DFRO = dietary-fiber-rich okara. Approximate composition of DFRO (g/100 g of dry matter): water content, 4.9 ± 0.08; total dietary fiber, 65.31 ± 1.5; soluble fiber, 2.96 ± 0.3; insoluble fiber, 62.35 ± 1.2; protein, 32.28 ± 0.13; fat, 2.46 ± 0.15; free sugars, 3.01 ± 0.09; ash, 0.87 ± 0.05.

**Composition of the DFRO Product.** Residual moisture was determined after the freeze-drying and delipidation processes by gravimetric analysis (final water content 4.90 ± 0.08%). Total nitrogen was analyzed with a thermal conductivity cell after combustion (975 °C) of the sample (Laboratory Leco Protein analyzer), and protein was calculated as nitrogen content × 6.25. The fat content was determined by extraction with petroleum ether using the Soxhlet method (*17*). After an extraction with ethanol 85% (70 °C, 10 min) free sugars were measured spectrophotometrically by the Anthrone method (*18*). Ashes were determined by incineration (550 °C, 4 h) in a microwave muffle furnace (Milestone MLS-1200 Pyro) (*19*). The DF was measured as follows: The AOAC enzymatic-gravimetric method (*20*) was used, modified by using dialysis against water, instead of ethanolic precipitation of soluble DF (SDF) (*21*). After enzymatic hydrolysis of digestible components, the insoluble DF (IDF) and SDF fractions were separated and chemically hydrolyzed. The remaining residue was gravimetrically quantified as Klason lignin (KL). Constituent neutral sugars (NSs) and uronic acids (UAs) were quantified in the corresponding hydrolysates of the IDF and SDF fractions (*22*). NSs were determined by gas−liquid chromatography (GLC) as alditol acetates. UAs were quantified spectrophotometrically by the Scott method (*23*). IDF was calculated as $NSs_{IDF} + UAs_{IDF} + KL$, and SDF was calculated as $NSs_{SDF} + UAs_{SDF}$. Total DF (TDF) was calculated as IDF + SDF (*22*).

**Mineral Composition in the Diets, Feces, and Urine.** To evaluate the digestibility of minerals, the control and DFRO diets, feces,and urine were analyzed for mineral composition. Mineral analysis was performed in each animal using the pool of feces and urine samples described above. Freeze-dried feces were milled, and the powered feces were ashed at a temperature increased linearly to 550 °C for 1 h and then at 550 °C for 24 h (Milestone MLS-1200 Pyro). The ashed samples, dissolved in 2 mL of HCl (50%)/$HNO_3$ (50%) (1:1, v/v) were diluted with water to 25 mL. The diets were ashed by the same method as the

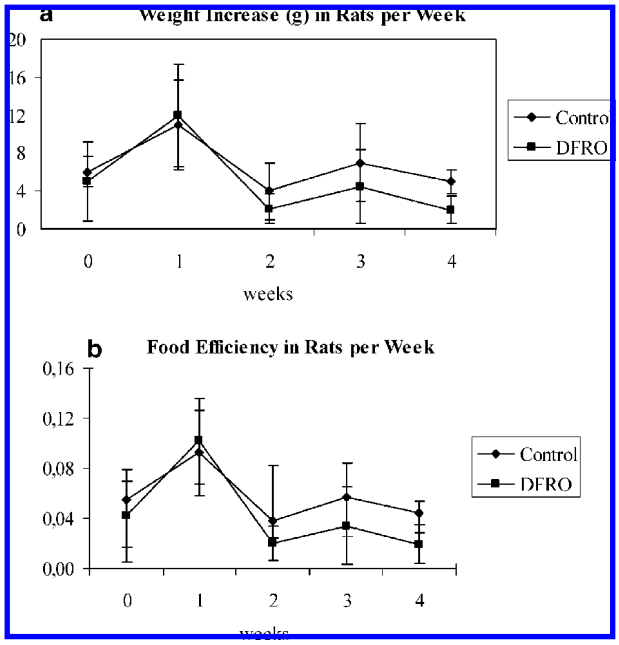


**Figure 1.** Effect of 4 week DFRO intake in healthy rats on (**a**) weight gain and (**b**) FE (FE = weight gain/food intake). The body weight and food intakes of each animal were recorded at the end of each week, including the adaptation week (0). Values are expressed as the mean value ± standard deviation of each group of animals each week.

**Table 2.** Effect of 4 Week DFRO Intake in Rats[a] on SCFAs[b] in the Cecum

| | control | | DFRO | |
|---|---|---|---|---|
| acid | mean | SD | mean | SD |
| acetic | 52.9 | 0.3[c] | 47.7 | 0.3[c] |
| propionic | 23.0 | 1.0[c] | 19.0 | 1.0[c] |
| isobutyric | tr[d] | | nd[e] | |
| butyric | 22.1 | 0.6[c] | 30.8 | 0.5[c] |
| isovaleric | tr | | tr | |
| valeric | tr | | tr | |
| total SCFAs (μmol/g of dm[f]) | 513 | 69 | 622 | 96 |

[a] Cecum samples from each animal were recorded at the end of the trial. SCFA analysis was done on each sample in triplicate. Values are expressed as the mean value ± SD of each group of animals. [b] Molar proportion. [c] Statistically significant difference between both groups of animals ($P < 0.05$). [d] tr = trace (<2% of the total SCFA value). [e] nd = not detected. [f] dm = dry matter.

feces. Urine was appropriately diluted with a 0.2% solution of $HNO_3$ in water (v/v) and subjected directly to atomization. Calcium, magnesium,and zinc concentrations in the control diet and DFRO diet, feces, and urine samples were measured using an atomic absorption spectrophotometer (Perkin-Elmer Analyst 200). Calcium and magnesium samples were previously diluted with a lanthanum oxide solution to 0.1% (w/v).

**Biochemical Parameters in the Serum.** The total protein, albumin, uric acid (UrA), glucose, total cholesterol (TC), triglycerides (TGs), and LDL-C and HDL-C levels were measured in the rat serum using an autoanalyzer (RA-500, Bayer, Spain).

**pH and SCFA Composition in the Cecum.** A portion of the cecal content was diluted 1:3 in water immediately after sampling; the pH was measured using a microelectrode (Crison, micro pH 2001), and the diluted sample was stored at −80 °C until analysis of the SCFAs. The untreated sample was also stored at −80 °C until analysis of the antioxidant status in the cecum.

To measure the SCFAs, the diluted samples were defrozen and centrifuged (9000*g*, 15 min, 4 °C) and the supernatants utilized for GLC. A 0.4 mL sample with 0.5 mL of internal standard, in 12% formic acid (4-methylvaleric acid, 2 μmol/mL) and made up to 1 mL with water, was centrifuged as above, and 1 μL of supernatant was injected

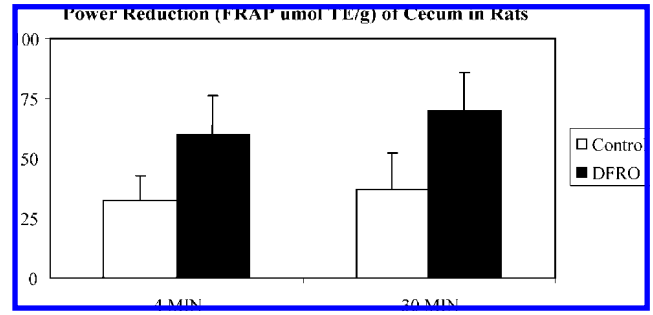


**Figure 2.** Effect of 4 week DFRO intake in healthy rats on RP toward Fe(III) in the cecum (FRAP value at 4 and 30 min). Cecum samples from each animal were recorded at the end of the trial. RP analysis was done on each sample in triplicate. Values are expressed as the mean value ± SD of each group of animals ($P < 0.05$).

into a GL 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 (*24*).

**Antioxidant Activity.** *In Vitro Antioxidant Activity of DFRO. Preparation of the DFRO Extracts.* A 0.5 g sample of the DFRO product was placed in a test tube; 40 mL of methanol/water (50:50, v/v) was added, and the tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged (2500*g*, 10 min), and the supernatant was recovered. A 40 mL sample of acetone/water (70:30, v/v) was added to the residue, and shaking and centrifugation were repeated. Both extracts were mixed and made up to 100 mL with water. Extracts were produced in triplicate and used to measure the in vitro antioxidant activity (*22*).

*Total Polyphenols in DFRO.* The total polyphenols of the extracts was determined according to the Folin−Ciocalteu procedure (*25*), using gallic acid as the standard and expressing the results as gallic acid equivalents (GAEs).

*Reduction Power (RP).* The antioxidant activity, in terms of RP toward Fe(III), of an aqueous methanol−acetone okara extract was evaluated (*22, 26*). Briefly, 900 μL of ferric reducing power assay (FRAP) reagent, freshly prepared and warmed at 37 °C, was mixed with 90 μL of water and 30 μL of the test sample, standard, or appropriate reagent blank. The final dilution of the test sample in the reaction mixture was 1:34. The FRAP reagent contained 2.5 mL of a 10 mmol/L 2,4,6-tri-2-pyridyl-*s*-triazine (TPTZ) solution in 40 mmol/L HCl, plus 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$, plus 25 mL of 0.3 mol/L acetate buffer, pH 3.6. Readings at the absorption maximum (595 nm) were taken every 15 s using a Beckman DU-640 spectrophotometer thermostated at 37 °C. The readings at 4 and 30 min were selected for calculation of the RP values. Methanolic solutions of known Trolox concentrations were used for calibration, expressing the results as Trolox equivalents (TEs).

*Antioxidant Status in the Rat. Preparation of the Cecal Content Extracts.* The extracts of the cecal content were prepared in the same way as in the in vitro assay described above, using 0.300 g of fresh test sample.

*RP in the Serum and Cecum.* The antioxidant activity, in terms of RP, of the serum and cecum was estimated according to the procedure described above for the in vitro assay. In the case of serum samples 10 μL of the test sample was used.

**Mineral Balance.** Apparent mineral absorption and retention were calculated as follows: apparent mineral absorption (AMA) (%) = [(mineral intake − fecal excretion)/mineral intake] × 100, whereas apparent mineral retention (AMR) (mg/day) = [(mineral intake − fecal mineral excretion) − urinary mineral excretion], and the efficiency of mineral retention (EMR) (%) = (AMR (mg/day)/mineral intake) × 100.

**Statistical Analysis.** Results are expressed as mean values ± standard deviation. Comparison of the means of three measurements was performed by one-way analysis of variance (ANOVA). Differences

**Table 3.** Effect of 4 Week DFRO Intake on Mineral Metabolism in Healthy Rats[a]

| | | mineral absorption | | | | mineral retention | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AMA[b] | | | | AMR[c] | | | | EMR[d] | | | |
| | | control | | DFRO | | control | | DFRO | | control | | DFRO | |
| mineral | week | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD | mean | SD |
| calcium[e] | 0 | 41.94 | 5.84 | 40.25 | 6.13 | 59.76 | 7.10 | 57.48 | 12.64 | 40.95 | 5.79 | 39.60 | 6.17 |
| | 2 | 47.48 | 11.36 | 52.81 | 5.27 | 71.27 | 24.34 | 73.07 | 9.79 | 47.47 | 11.27 | 52.23 | 5.20 |
| | 4 | 41.89 | 1.64[f] | 47.02 | 2.51[f] | 62.61 | 2.63 | 65.56 | 4.78 | 41.62 | 1.60[g] | 46.68 | 2.55[g] |
| magnesium | 0 | 65.91 | 3.94 | 59.91 | 2.70 | 17.28 | 0.56 | 15.39 | 1.33 | 65.24 | 3.49 | 59.49 | 2.65 |
| | 2 | 62.81 | 9.94 | 64.25 | 4.50 | 16.56 | 5.35 | 15.93 | 1.58 | 62.21 | 10.11 | 63.52 | 4.45 |
| | 4 | 60.12 | 2.25 | 61.04 | 3.21 | 16.29 | 1.18 | 15.31 | 1.20 | 59.72 | 2.46 | 60.63 | 3.18 |
| zinc | 0 | 55.91 | 4.66 | 56.74 | 4.45 | 0.81 | 0.04 | 0.84 | 0.11 | 53.81 | 2.96 | 55.99 | 4.54 |
| | 2 | 38.28 | 15.89 | 47.02 | 7.40 | 0.59 | 0.32 | 0.80 | 0.06 | 37.63 | 15.99 | 46.29 | 7.46 |
| | 4 | 52.30 | 3.27 | 53.75 | 2.94 | 0.67 | 0.12 | 0.78 | 0.07 | 51.77 | 3.33 | 53.13 | 2.88 |

[a] Feces and urine samples from each animal were recorded separately on alternate days three times per week. As a result a pooled sample per animal and per week was analyzed for mineral balance in triplicate. Values are expressed as the mean value ± standard deviation (SD) of each group of animals. [b] Apparent mineral absorption (AMA) (%) = [(mineral intake − fecal excretion)/mineral intake] × 100. [c] Apparent mineral retention (AMR) (mg/day) = [(mineral intake − fecal mineral excretion) − urinary mineral excretion]. [d] Efficiency of mineral retention (EMR) (%) = (AMR/mineral intake) × 100. [e] Values differing significantly from the control. [f] $P < 0.0050$. [g] $P < 0.004$.

of $P < 0.05$ were considered to be significant (SPSS Inc., Chicago, IL, version 15.0).

## RESULTS AND DISCUSSION

**Evaluation of the DFRO Product.** *Analytical Composition of DFRO.* The approximate composition of DFRO is given in **Table 1**. This product could be considered as a concentrate of DF (>65% TDF (dm)). Thus, a preliminary dehydration followed by delipidation of the raw okara led to a high-DF product. In this way a valorization of DF from raw okara was made. Some authors have remarked that the neutral taste and the absence of color of okara make it suitable for incorporation into food products without any change in their quality, unlike those from wheat fibers (*12*).

*In Vitro Antioxidant Activities.* The antioxidant potential of the DFRO aqueous methanol extracts was estimated from their ability to reduce the TPTZ−Fe(III) complex to a TPTZ−Fe(II) complex. This activity is called throughout the paper the RP. Amin and Mukhrizah (*15*) have tested the radical scavenging, the $\beta$-carotene blanching, and the RP toward Fe(III) of several vegetable byproducts, including raw okara. Among the three assays tested, okara shows the best relative response in the measure of RP toward Fe(III). On the basis of this support, we selected the activity of RP to evaluate the in vitro antioxidant activity of DFRO.

As a result, 1 g (dm) of DFRO had an RP equivalent to 1.78 mg of vitamin C. Thus, DFRO could contribute in terms of antioxidant action to the recommended dietary intake of this vitamin. The total polyphenol content in DFRO extracts was 0.24 ± 0.001 g of GAEs/100 g of dm. As a result, a specific RP was shown on the extract containing polyphenols. Although the quantity of isoflavones in okara represents almost 30% of the isoflavones in soybean seed (*27*), we cannot disregard that other bioactive components could be in part responsible for this activity.

**Evaluation of DFRO in the Rat Experiment.** *Weight Gain and Feed Consumption.* All animals treated with the DFRO diet were in good health throughout the experiment, and no side effects such as diarrhea were apparent. In addition, the rats appeared to behave normal throughout the study. Both diets—the control diet and the DFRO diet—showed similar energy values (**Table 1**).

During the 4 weeks of feeding, each rat consumed a similar amount of diet (control group, 450.86 ± 29.70 g; DFRO group, 460.40 ± 20.61 g). However, by the end of the second week, animals fed the DFRO diet showed a body weight gain and a feeding efficiency (FE) value lower (tendency) than those of the rats fed the control diet (**Figure 1**). In the case of body weight gain, this tendency of reduction becomes significant during week 4 (60%, $P < 0.035$) (**Figure 1a**). Consistently, Matsumoto et al. (*3*) show in a mouse model fed okara (10%) for 10 weeks a body weight gain lower than that of the untreated group at week 4, this difference being significant at the end of week 7.

*Effect on the Internal Organ and Cecal Weights.* There was no difference in the weight of the organs between both groups (data not shown). These measurements indicated that the animals were not adversely affected by the DFRO diet during the 4 weeks of experimental intake. No differences in the cecal weights were found.

*Effect on the Fecal Weight and Water Content.* The weight and moisture of the feces was higher for the DFRO group than for the control group in the 7−28 day period, but no statistically significant differences were observed. It is known that the IDF fraction (62.35% (dm) in DFRO) is partially fermented (*28*). The higher fecal water content in the DFRO group might be related to the extensive degradation of the DF by intestinal bacteria.

*Effect on the Serum Biochemical Parameters.* No difference was observed in LDL-C, TGL, glucose, albumin, total protein, and UrA serum levels in the DFRO group as compared to the control group (data not shown) at the end of the experiment. However, a significant decrease in the TC level (control group, 65 ± 8 mg/dL; DFRO group, 51 ± 5 mg/dL; $P < 0.05$) was observed in the DFRO group. Consistently, when okara was given to rats fed a high-cholesterol diet, the increase in the TC level was suppressed (*11*). In our study, although a significant decrease in HDL-C in the DFRO group was found, no difference in the atherogenic index (TC/HDL-C) between both groups was observed (1.55 ± 0.10 and 1.57 ± 0.09 in the control and the DFRO groups, respectively).

*Effect on the Cecal pH and SCFAs and on the Gastrointestinal Tract Longitudinal Measure.* Colonic fermentation resulted in a similar pH value (6.2 ± 0.1) of the cecum in the control group compared to the DFRO group (6.2 ± 0.1). No significant difference was found in total SCFA values in the cecum between both groups (**Table 2**). It is interesting to note that the molar proportion of acetate and propionate was significantly lower

(9.83%, $P < 0.05$, and 17.39%, $P < 0.05$, respectively) in the DFRO group, whereas in the case of butyrate it was significantly higher (39.37%, $P < 0.05$). In the case of isobutyric, isovaleric, and valeric acids, the values were less than 2% of the SCFAs in both groups of animals (**Table 2**).

The gastrointestinal longitudinal measure tended to be higher in the DFRO group compared to the control group (control group, 0.48 ± 0.08 cm/g of body weight; DFRO group, 0.51 ± 0.04 cm/g of body weight). It is reported that these differences correspond to changes in the structure of the intestine wall (*29*). DF fermentation in the large intestine leads to the production of SCFAs, which are used as an energy source by colonocytes and have a trophic effect on the colon wall. Specifically, butyrate is the major energy source for the colonocytes because it is transported preferentially (*30*). We suggest that the increase of the gastrointestinal longitudinal measure of the DFRO animals could be due to the relatively higher molar proportion of butyrate to total SCFAs in the DFRO group as compared to the control group.

The relatively higher molar proportion of butyrate was likely to be due to the decrease in acetate. Consistently, a route of butyrate formation has been described in which acetate is used as a cosubstrate; this route might even help to detoxify excess acetate in the proximal colon (*31*).

Further studies in the feces are needed to evaluate the specific group of bacteria responsible for these findings. These results suggest the potential use of DFRO as a prebiotic, which modifies large intestinal fermentation to produce relatively more butyrate.

*Effect on the Antioxidant Status in the Serum and Cecum.* The need to protect cells from oxidation stress since an unbalanced oxidative stress could lead to apoptotic death or necrosis in cells is well documented. The sensitivity of cells to oxidative stress depends on their intrinsic antioxidant systems, in particular the levels of glutathione within the cell. It is suggested that a redox state, which involves gluthatione, could protect the cell from oxidative damage. Specifically, in the gut environment, the high exposure to exogenous reactive oxygen species is described. Thus, it is likely that an increase of the RP in this compartment would have a positive health influence (*32*).

As a consequence, we evaluated two biological compartments, the serum and cecum, using a global antioxidant measure such as the RP. We chose these compartments as a systemic one and as a partial representation of the gut environment.

The intake of DFRO did not lead to an increase in the RP value in the serum, neither the measurement at 4 min nor that at 30 min (control group, 120.45 ± 70.64 μmol of TEs/L; DFRO group, 97.59 ± 59.27 μmol of TEs/L; at 30 min). Since the major determinant of RP is the UrA concentration (*26, 33*), the specific UrA value in the serum was measured. No significant difference in UrA was found between the control group and the DFRO group (2.5−10.6 mg/dL vs 3.6−8.6 mg/dL, respectively). Thus, these data suggested that the DFRO diet did not alter the UrA concentration nor the RP measurement.

In contrast, in the case of the animals fed the DFRO diet, the value of RP in the cecum was significantly higher (i.e., at 30 min, $P < 0.0003$) than in the case of the animals fed the control diet (**Figure 2**). A similar effect has been found previously in artichoke, an edible vegetable rich in DF (*33*). To elucidate the bioactive compound responsible for this effect on the cecum, it is worth mentioning that our group has found a relatively high yield of resistant protein in native okara after the emulation of an enzymatic digestion (5.07 ± 0.96 g/100 g of dm) (*34*). Consistently other authors report a close association between DF and resistant protein in raw okara (*35*).

On the other hand, several protein hydrolysates from okara with antioxidant activity against the peroxidation of linoleic acid have been described (*16*). Thus, the antioxidant activity of DFRO in the cecum could be attributed in part to the peptides released from the resistant fraction.

Apart from that, the highly reduced environment in the cecum was likely to influence the increase in the formation of specific reduced products such as butyrate. This product shows a protective role against colorectal cancer in vivo (*36*). This is the first time that both the production of a fermentable substrate such as butyrate and the evaluation of the antioxidant status in the cecum have been linked in an animal model.

*Effect on the Mineral Balance.* Calcium and magnesium absorption takes place in both the small and large intestines. Some reports demonstrated that the cecum and colon have a large capacity for calcium and magnesium absorption. Certain polysaccharides may trap substantial amounts of cations and carry them to the large bowel (*37*). We evaluated the metabolism of calcium, magnesium, and zinc (**Table 3**). Our results in relation to the calcium balance showed at the end of week 4 significantly higher apparent absorption (12.24%, $P < 0.004$) and true retention (13.79%, $P < 0.005$) in rats fed DFRO in comparison to animals fed the control diet. The stimulation of mineral absorption seems to be caused by the increased solubility of minerals in the cecal contents (*38*). The apparent absorption and true retention of magnesium were 60% for both groups during the study. Magnesium absorption in animals and humans may vary between 35% and 70% of magnesium intake (*39*). The results obtained showed that, under our conditions, magnesium absorption and retention percentages were slightly higher in the second week of the experiment for both groups, but the total magnesium absorbed or retained did not differ significantly ($P > 0.05$) between the groups at the end of the study. A partial explanation of the lack of an effect of DFRO on intestinal magnesium absorption in the present study could be an increase in the turnover of epithelial cells as a consequence of fermentable DF, which may be responsible for the increase in endogenous magnesium excretion (*40*). The zinc apparent absorption and zinc mineral balance were not significantly different ($P > 0.05$) between the DFRO and control groups. Thus, feeding on the DFRO diet did not significantly affect the intestinal zinc balance.

Collectivelly, these investigations have revealed that the present level of consumption of the DFRO to 10% in the diet of a healthy rat model with a mean daily intake of 16.10 ± 1.06 g of DFRO lowered weight gain, lowered the total serum cholesterol levels, and led to an increased butyrogenic effect and antioxidant status in the cecum. In addition, enhancement of the apparent absorption and retention of calcium was appreciated. No adverse effects from ingestion of DFRO were identified in this study. Thus, this DFRO may be used as a functional ingredient with health-promoting effects. This works represents one of the first efforts to enhance the functionality of okara as a byproduct of the soymilk industry.

## ABBREVIATIONS USED

AI, atherogenic index (TC/HDL cholesterol); AMA, apparent mineral absorption; AMR, apparent mineral retention; ANOVA, one-way analysis of variance; DF, dietary fiber; dm, dry matter; DFRO, DF-rich okara; EMR, efficiency of mineral retention; FE, feeding efficiency (weight gain/food

intake); fm, fresh matter; FNB, Food Nutrition Board; FRAP, ferric-reducing antioxidant power; GAE, gallic acid equivalent; GL, gas−liquid; GLC, gas−liquid chromatography; HDL-C, high-density lipoprotein cholesterol; IDF, insoluble dietary fiber; KL, Klason lignin; LDL-C, low-density lipoprotein cholesterol; NSs, neutral sugars; $NSs_{IDF}$, neutral sugars in the IDF fraction; $NSs_{SDF}$, neutral sugars in the SDF fraction; RP, reduction power toward Fe(III); SCFAs, short-chain fatty acids; SDF, soluble dietary fiber; SPSS, Statistical Package for the Social Sciences; TC, total cholesterol; TGLs, triglycerides; TDF, total dietary fiber; TE, Trolox equivalent; TPTZ, 2,4,6-tri-2-pyridyl-*s*-triazine; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; UrA, uric acid; UAs, uronic acids; $UAs_{IDF}$, uronic acids in the IDF fraction; $UAs_{SDF}$, uronic acids in the SDF fraction.

## ACKNOWLEDGMENT

We thank M. Takazumi from Toofu-Ya S.L. for the okara provided and E. Morcillo for assistance with the animal experiments.

## LITERATURE CITED

(1) Kris-Etherton, P. M.; Hecker, K. D.; Bonanome, A.; Coval, S. M.; Binkoski, A. E.; Hilpert, K. F.; Griel, A. E.; Etherton, T. D. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am. J. Med.* **2002**, *113*, 71S–88S.

(2) Willett, W. C. Diet and health: What should we eat? *Science***1994***264*532–537.

(3) Matsumoto, K.; Watanabe, Y.; Yokoyama, S.-I. Okara, soybean residue, prevents obesity in a diet-induced murine obesity model. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 720–727.

(4) Campagnolo, P. D. B.; Vitolo, M. R.; Gama, C. M.; Stein, A. T. Prevalence of overweight and associated factors in southern Brazilian adolescents. *Public Health* **2008**, *122*, 509–515.

(5) Lunn, J.; Buttriss, J. L. Carbohydrates and dietary fibre. *Nutr. Bull.* **2007**, *32*, 21–64.

(6) Blaut, M. Relationship of prebiotics and food to intestinal microflora. *Eur. J. Nutr.* **2002**, *41*, 11–16.

(7) Macfarlane, S.; Macfarlane, G. T.; Cummings, J. H. Review article: Prebiotics in the gastrointestinal tract. *Aliment. Pharmacol. Ther.* **2006**, *24*, 701–714.

(8) Louis, P.; Scott, K. P.; Duncan, S. H.; Flint, H. J. Understanding the effects of diet on bacterial metabolism in the large intestine. *J. Appl. Microbiol.* **2007**, *102*, 1197–1208.

(9) Younes, H.; Demigné, C.; Rémésy, C. Acidic fermentation in the caecum increases absorption of calcium and magnesium in the large intestine of the rat. *Br. J. Nutr.* **1996**, *75*, 301–314.

(10) O'Toole, D. K. Characteristics and use of okara, the soybean residue from soy milk production: A review. *J. Agric. Food Chem.* **1999**, *47*, 363–371.

(11) Fukuda, M.; Sugihara, Y.; Itou, M.; Horiuchi, R.; Asao, H. Effects of feeding with okara on plasma and liver lipid levels in rats. *J. Jpn. Soc. Food Sci. Technol.* **2006**, *53*, 195–199.

(12) Surel, O.; Couplet, B. Influence of the dehydration process on active compounds of okara during its fractionation. *J. Sci. Food Agric.* **2005**, *85*, 1343–1349.

(13) Préstamo, G.; Rupérez, P.; Espinosa-Martos, I.; Villanueva, M. J.; Lasunción, M. A. The effects of okara on rat growth, caecal fermentation, and serum lipids. *Eur. Food Res. Technol.* **2007**, *225*, 925–928.

(14) Turhan, S.; Temiz, H.; Sagir, I. Utilization of wet okara in low-fat beef patties. *J. Muscle Foods* **2007**, *18*, 226–235.

(15) Amin, I.; Mukhrizah, O. Antioxidant capacity of methanolic and water extracts prepared from food-processing by-products. *J. Sci. Food Agric.* **2006**, *86*, 778–784.

(16) Yokomizo, A.; Takenaka, Y.; Takenaka, T. Antioxidative activity of peptides prepared from okara protein. *Food Sci. Technol. Res.* **2002**, *8*, 357–359.

(17) James, C. S. *Analytical Chemistry of Foods*; Blackie Academic & Professional: London, 1995.

(18) Osborne, D. R.; Voogt, P. *Análisis de los Nutrientes en los Alimentos*; Acribia: Zaragoza, Spain, 1986.

(19) A.O.A.C. Method 923.03. In *Official Methods of Analysis*, 16th ed.; Cuniff, P., Ed.; AOAC International: Arlington, VA, 1995; pp 32−42.

(20) Prosky, L.; Asp, N. G.; Schweizer, T. F.; DeVries, J. W.; Furda, I. Determination of insoluble, soluble, and total dietary fiber in foods and food products: interlaboratory study. *J. AOAC Int.* **1988**, *71*, 1017–1023.

(21) Mañas, E.; Saura-Calixto, F. Dietary fibre analysis: Methodological error sources. *Eur. J. Clin. Nutr.* **1995**, *49*, S158–S162.

(22) Jiménez-Escrig, A.; Rincón, M.; Pulido, R.; Saura-Calixto, F. Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *J. Agric. Food Chem.* **2001**, *49*, 5489–5493.

(23) Scott, R. W. Colorimetric determination of hexuronic acids in plant materials. *Anal. Chem.* **1979**, *51*, 936–941.

(24) Tortuero, F.; Fernández, E.; Rupérez, P.; Moreno, M. Raffinose and lactic acid bacteria influence caecal fermentation and serum cholesterol in rats. *Nutr. Res. (N.Y.)* **1997**, *17*, 41–49.

(25) Montreau, F. R. Sur le dosage des composés phénoliques totaux dans les vins par la methode Folin-Ciocalteau. *Connaiss. Vigne Vin* **1972**, *24*, 397–404.

(26) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.

(27) Jackson, C.-.C.; Dini, J. P.; Lavandier, C.; Rupasinghe, H. P. V.; Faulkner, H.; Poysa, V.; Buzzell, D.; de Grandis, S. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process. Biochem.* **2002**, *37*, 1117–1123.

(28) Jiménez-Escrig, A.; Sánchez-Muniz, F. J. Dietary fibre from edible seaweeds: Chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr. Res. (N.Y.)* **2000**, *20*, 585–598.

(29) Pirman, T.; Ribeyre, M. C.; Mosoni, L.; Rémond, D.; Vrecl, M.; Salobir, J.; Patureau-Mirand, P. Dietary pectin stimulates protein metabolism in the digestive tract. *Nutrition* **2007**, *23*, 69–75.

(30) Licht, T. R.; Hansen, M.; Poulsen, M.; Dragsted, L. O. Dietary carbohydrate source influences molecular fingerprints of the rat faecal microbiota. *BMC Microbiol.* **2006**, 6.

(31) Nugent, S. G.; Kumar, D.; Rampton, D. S.; Evans, D. F. Intestinal luminal pH in inflammatory bowel disease: Possible determinants and implications for therapy with aminosalicylates and other drugs. *Gut* **2001**, *48*, 571–577.

(32) Jackson, M. J.; Papa, S.; Bolaños, J.; Bruckdorfer, R.; Carlsen, H.; Elliott, R. M.; Flier, J.; Griffiths, H. R.; Heales, S.; Holst, B.; Lorusso, M.; Lund, E.; Moskaug, J. Ø.; Moser, U.; di Paola, M.; Polidori, M. C.; Signorile, A.; Stahl, W.; Viña-Ribes, J.; Astley, S. B. Antioxidants, reactive oxygen and nitrogen species, gene induction and mitochondrial function. *Mol. Aspects Med.* **2002**, *23*, 209–285.

(33) Jiménez-Escrig, A.; Dragsted, L. O.; Daneshvar, B.; Pulido, R.; Saura-Calixto, F. In vitro antioxidant activities of edible artichoke (*Cynara scolymus* L.) and effect on biomarkers of antioxidants in rats. *J. Agric. Food Chem.* **2003**, *51*, 5540–5545.

(34) Espinosa-Martos I.; Rupérez P. Indigestible fraction and physicochemical properties of okara a by-product from soymilk. *Book of Abstracts*; Dietary Fiber Congress. Helsinki, Finland. June 12−14, 2006.

(35) Yoshii, H.; Furuta, T.; Maeda, H.; Mori, H. Hydrolysis kinetics of okara and characterization of its water-soluble polysaccharides. *Biosci., Biotechnol., Biochem.* **1996**, *60*, 1406–1409.

(36) Avivi-Green, C.; Polak-Charcon, S.; Madar, Z.; Schwartz, B. Different molecular events account for butyrate-induced apoptosis in two human colon cancer cell lines. *J. Nutr.* **2002**, *132*, 1812–1818.

(37) Demigné, C.; Levrat, M.-; Rémésy, C. Effects of feeding fermentable carbohydrates on the caecal concentrations of minerals and their fluxes between the caecum and blood plasma in the rat. *J. Nutr.* **1989**, *119*, 1625–1630.

(38) Rémésy, C.; Levrat, M.-A.; Gamet, L.; Demigné, C. Cecal fermentations in rats fed oligosaccharides (inulin) are modulated by dietary calcium level. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **1993**, *264*, G855–G862.

(39) Coudray, C.; Demigné, C.; Rayssiguier, Y. Effects of dietary fibers on magnesium absorption in animals and humans. *J. Nutr.* **2003**, *133*, 1–4.

(40) Coudray, C.; Feillet-Coudray, C.; Grizard, D.; Tressol, J. C.; Gueux, E.; Rayssiguier, Y. Fractional intestinal absorption of magnesium is directly proportional to dietary magnesium intake in rats. *J. Nutr.* **2002**, *132*, 2043–2047.

**Received for review March 13, 2008. Revised manuscript received June 4, 2008. Accepted June 4, 2008. The Spanish Ministry of Science and Education through Project AGL2005-02447-ALI supported this research.**

JF800792Y