Phenolic Antioxidants Richly Contained in Corn Bran Are Slightly Bioavailable in Rats

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Phenolic acids (PAs) have been shown to be beneficial to human health and are found most abundantly in corn bran (∼4%, w/w), one of the main dietary fibers. This study therefore evaluated the bioavailabilities of phenolic antioxidants ferulic acid (FA) and *p*-coumaric acid (PCA) in refined corn bran (RCB) by determining their recovery in the plasma, urine, and feces of rats fed a single meal of a RCB diet containing 5% RCB or adapted to the RCB diet for 10 days. In both studies, 0.4−0.5% of ingested FA and 1.2−2.3% of ingested PCA were recovered in rat urine. By contrast, ∼81% of FA and ∼64% of PCA ingested with the single meal were excreted through the rat feces within 3 days after the ingestion. On the other hand, after rats were fed the RCB diet, total FA (all forms of FA) was recovered in plasma at a concentration of 35.0 ± 2.0 µg/L, total FA and total PCA were excreted through urine at levels of 155.4 ± 5.8 and 50.9 ± 6.6 µg/day, respectively. These parameters showed no significant change ($P = 0.93$, 0.09, and 0.66, respectively) after rats were fed the RCB diet continuously for up to 10 days. These results suggest that the PAs in RCB are bioavailable in rats. Their bioavailabilities, however, are relatively low compared with their high content in RCB and not improved by the adaptation for 10 days to the enriched RCB diet. Additionally, comparison with the results of other studies revealed that high contents of FA and, especially, diferulic acids in cereal bran, which act as cross-links between bran cell wall polysaccharides, may not improve but, rather, limit the bioavailabilities of PAs in vivo.

KEYWORDS: Bioavailability; corn bran; *p*-coumaric acid; ferulic acid; phenolic aicd; antioxidant

INTRODUCTION

The absorption, metabolism, and bioavailability of phenolic acids (PAs) are receiving increasing interest from many researchers mainly because of two characteristics of PAs: their ubiquitous existence in plant foods such as cereals, fruits, and vegetables (*1, 2*) and their proposed physiological functions in health protection and disease prevention (*3−5*).

For free PAs, such as ferulic acid (FA), *p*-coumaric acid (PCA), and caffeic acid, their absorption and metabolism have been well documented in the past decade (*6−14*). Free PAs have shown good absorbability, and they were recovered in plasma mainly in the form of conjugates such as glucuronides and/or sulfates. The PAs in plant foods, however, are mostly bound forms esterified or etherified to cell wall polymers (*15*). Accordingly, recent studies in vivo have been focusing on the bioavailabilities of the PAs in spinach cell walls (*16*), tomatoes (*17*), low-alcohol beer (*18*), pine bark extract (*19*), red wine (*20*), and coffee (*21*). More recently, the bioavailabilities of PAs contained in wheat bran, a main dietary fiber, in rats (*11*) and in humans (*22*) were also investigated.

Another main dietary fiber, namely, corn bran, is shown to be the most abundant source of PAs among the foods and

Table 1. Summary of the Contents of Main Phenolic Acids in Some Foods and Foodstuffs (Grams per Kilogram)[a]

	FA	PCA	di-FA	ref
refined corn bran (RCB)	30.1	2.9	0.9	determined in this work
corn bran	26.1–33.0	3–4	7–13.3	*23, 24*
fine wheat bran	5.3–5.4	0.17	0.81	*25–27*
rye bran	2.78	0.19	0.46	*26, 27*
whole wheat	1.27	–[b]	–	*28*
popcorn	3.13	–	–	*28*
sweet corn	0.42	–	–	*29*
rice endosperm cell wall	9.1	2.5	0.56	*30*
brown rice	0.42	–	–	*28*
barley	0.34	–	–	*28*
sugar-beet pulp	8	–	1.4	*31*
tomato (fresh)	0.06	–	–	*17*
low-alcohol beer[c]	0.0024	0.0011	–	*18*
pine bark extract[d]	2.4	0.4	–	*19*
roasted coffee[e]	2.38	0.23	–	*21*

[a] Some values were calculated from the data of the references. The foods and foodstuffs except for the tomato, low-alcohol beer, and pine bark extract were calculated as dry matter. FA, ferulic acid; PCA, *p*-coumaric acid; di-FA, diferulic acids. [b] Not determined or reported. [c] Unit is g/L. [d] Also contains another main phenolic acid, i.e., caffeic acid, 1.8 g/kg. [e] Also contains caffeic acid, 13.8 g/kg.

foodstuffs reported in the literature (**Table 1**). A diet containing 5% corn bran could effectively lower the rat plasma cholesterol

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concentration (*32*). Similarly, a low-fat diet supplemented with 5% corn bran was effective in reducing the serum lipid concentration in men with hypercholesterolemia (*33*). The consumption of corn bran food also lowered the plasma very low-density lipoprotein cholesterol level in type II diabetes patients (*34*). PAs are considered as active ingredients in whole grains, which have shown the potential to reduce the risk of some cancers and coronary heart disease (*35*). Because antioxidant PAs are richly contained in corn bran, they are also presumed to contribute to the proposed physiological functions of corn bran. To prove this point, it is necessary to investigate the bioavailabilities of PAs in corn bran in vivo.

The purpose of the present study was therefore to evaluate the bioavailabilities of the phenolic antioxidants richly contained in refined corn bran (RCB) in vivo, by determining their recovery in the plasma, urine, and feces of rats fed a single meal of a RCB diet containing 5% RCB or adapted to the RCB diet for 10 days. The results show that PAs from RCB are bioavailable in rats. Their bioavailabilities, however, are relatively low compared with their high contents in RCB. Additionally, the bioavailabilities of PAs from RCB in rats were not improved by the adaptation for 10 days to the 5% RCB diet.

MATERIALS AND METHODS

Chemicals. β-Glucuronidase (EC 3.2.1.3 1) type H-2, β-glucuronidase type B-1, and D-saccharic acid 1,4-lactone were from Sigma Chemical Co. (St. Louis, MO). FA, PCA, and salicylic acid were of analytical or HPLC grade purchased from Wako Pure Chemical Industries (Osaka, Japan). Diferulic acid (5,5′-) was a present from Dr. Ohta (Department of Bioproduction Science, Faculty of Horticulture, Chiba University). RCB (Nihon Shokuhin Kakou Co., Ltd.) was mechanically refined by the wet-milling process, which contained ∼89% insoluble dietary fiber and <2% starch on the dry basis. The contents of main PAs in RCB were determined by use of HPLC (**Table 1**).

Animals and Diets. Wistar male rats (7 weeks old) from CLEA Japan (Tokyo, Japan) were housed in metabolic cages in an air-conditioned room (22 ± 2 °C) with a dark period from 7:00 p.m. to 7:00 a.m. Before the experiment, they were fed a commercial diet (type CE-2, CLEA Japan) for 3 days to acclimatize to the environment. A purified standard diet, containing little PAs, was prepared as previously described (*36*). Another diet, that is, the RCB diet, was prepared by replacing the 5% cellulose in the standard diet with 5% RCB. The rats were fed according to the methods described in the following sections and allowed free access to water during the entire experimental period. The care and treatment of the rats were carried out according to the guidelines prescribed by the Faculty of Horticulture, Chiba University.

Experiment I: Determining the Recovery of FA and PCA in Plasma, Urine and Feces of Rats Fed a Single Meal of the RCB Diet. Twelve rats were fed according to the method of "meal feeding" (two meals per day, during 7:00−8:00 a.m. and 7:00−8:00 p.m.) as previously described (*36*). After the rats had been fed the standard diet for 7 days, they were given 3 g of the RCB diet or the standard diet at 7:00 p.m. before the evening meal on day 8 [i.e., d (−1), **Figure 1**]. Because the rats had been acclimated to the feeding method, they finished the given diets within 10−40 min. Thereafter, the rats were fed the standard diet continuously for 7 days. To investigate the effects of the experimental treatment on the growth of rats, another six rats (control) were fed the standard diet according to the normal method (rats were allowed free access to diet and water). For all rats, feces and urine were collected every day as described previously (*12*). The samples of urine and feces collected within 24 h before the RCB administration were referred to as the samples of day (−1). Those samples collected from 7:00 p.m. on day 8 until 7:00 p.m. the following day were referred to as the samples of day (+1). Blood (∼0.5 mL) was collected from the tail vein before and after the administration (at 11:00 a.m. on days 8 and 9, respectively). After the collection, all of the samples were treated as previously described (*12, 36*) and stored frozen at −30 °C until required for analysis.
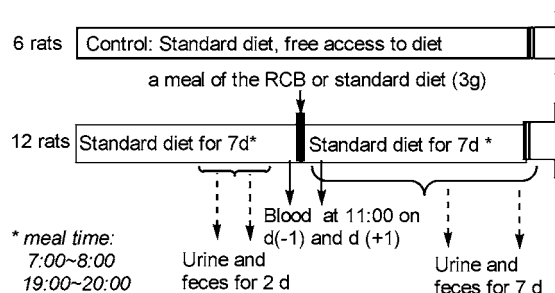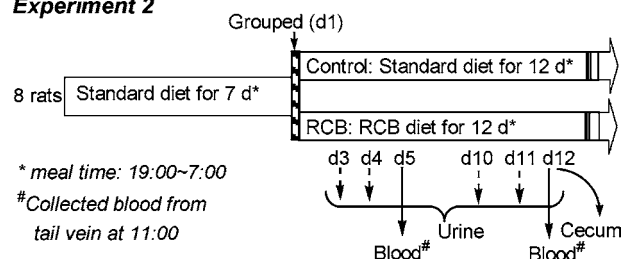

Figure 1. Design of the experiments. In experiment 1, urine and feces were collected every day for 2 days before and 7 days after rats were orally administered 3 g of the RCB or standard diet. Blood was collected from the tail 8 h before [at 11:00 a.m. on day (–1)] and 16 h after [at 11:00 a.m. on dat (+1)] the administration, respectively. To eliminate the influence of individual difference on diet ingestion and urinary and fecal excretion, the rats were fed by using the method of "meal feeding". To investigate the influence of the experimental treatment on the growth of rats, another six rats (control) were fed with the standard diet by using the normal method. In experiment 2, daily urine was collected after the grouped rats were fed with the RCB diet or the standard diets for 2, 3, 9, and 10 days (collected on days 3, 4, 10, and 11), respectively. Blood was collected at 11:00 a.m. on days 5 and 12, respectively. Cecum together with the content was removed after blood sampling.

Experiment II: Determining the Recovery of FA and PCA in Plasma and Urine of Rats Adapted to the RCB Diet. To investigate whether adaptation to the RCB diet could improve the bioavailabilities of the PAs in rats, the recovery of the PAs in the plasma and urine was determined after rats were fed the RCB diet containing 5% RCB for 2, 3, 9, and 10 days (**Figure 1**). In this experiment, the rats were allowed access to the diet only in the nighttime (7:00 p.m.−7:00 a.m.) as previously described (*12*). They were first fed the standard diet for 7 days and then divided into two groups. The rats of the control group were continuously fed the standard diet; the rats of the other group were changed to feed on the RCB diet. The samples of urine and feces were collected and treated according to the same methods as described for experiment I. The day when the rats were first fed the appointed diets was referred to as "day 1". The sample of urine collected from 7:00 p.m. on day 3 until 7:00 p.m. the following day was referred to as the sample of day 3. After blood sampling on day 12, ceca were removed from rats under pentobarbital anesthesia. The cecal wall and cecal content were weighed, respectively.

Determination of Total FA and Total PCA. The amount of total FA (all forms of FA) and total PCA (all forms of PCA) in RCB and feces was determined as the free form by use of HPLC after the samples were hydrolyzed with NaOH aqueous solution according to the same method used in the previous study (*12*). The amounts of total FA and total PCA in urine and plasma were determined also as the free form by use of HPLC after the samples (100 μL for plasma and 50 μL for urine) were treated with β-glucuronidase type H-2 solution (with β-glucuronidase and sulfatase) as previously described (*12, 36*).

Determination of Free FA and Its Metabolites in Urine. Free FA and its metabolites in urine were determined with HPLC after the sample was prepared by use of a combination of enzymatic hydrolysis as previously described (*12*).

Table 2. Daily Excretion of Total *p*-Coumaric Acid (PCA) and Total Ferulic Acid (FA) in the Urine and Feces of Rats Administered a Single Meal (3 g) of the Refined Corn Bran (RCB) Diet Containing 5% RCB[a]

time[b]	in urine (μg/day)		in feces (μg/day)	
	total PCA	total FA	total PCA	total FA
day (−2)	19.8 ± 3.3	29.3 ± 1.9	9.8 ± 4.9	25.2 ± 5.8
day (−1)	21.3 ± 1.6	29.1 ± 1.7	8.2 ± 3.3	21.3 ± 9.7
day (+1)	31.2 ± 1.6* (2.3%)[c]	46.6 ± 1.9** (0.4%)	124.8 ± 8.2** (27%)	1289.4 ± 93.2** (28%)
day (+2)	19.7 ± 1.6	33.0 ± 1.9	127.8 ± 11.3** (28%)	1763.2 ± 35.0** (39%)
day (+3)	18.1 ± 1.6	31.1 ± 3.9	46.0 ± 8.2** (9%)	497.3 ± 31.1* (11%)
day (+4)	−[d]	−	8.2 ± 3.3	135.9 ± 25.2 (3%)
day (+5−7)	−	−	6.6 ± 3.3[e]	17.5 ± 5.8[e]
control[f]	18.1 ± 4.9	29.1 ± 4.0	8.2 ± 1.6	25.2 ± 3.9

[a] Values are means ± SE, $n = 6$. The means with ** ($P < 0.01$) or * ($P < 0.05$) differ from that on day (−2) in the column determined by Dunnett's multiple-range test. [b] The day before (marked with "−") or after (marked with "+") the rats were administered a single meal of the RCB diet. [c] Percentage of the total amount of daily excretion of the phenolic acids relative to the dosage (4515 μg of total FA and 435 μg of total PCA were included in the 3 g of RCB diet, respectively). The value was adjusted by subtracting that of control before the percentage calculation. [d] Not determined. [e] Average of the total amount of excretion from day 5 to day 7. [f] Amount of total PCA or total FA daily excreted by control group rats did not change significantly with days (one-way ANOVA, $P < 0.01$). The values on day (+1) are shown here as representative.

HPLC Analysis. An L-7100 intelligent pump (Hitachi, Tokyo, Japan), a Nova-Pak C18 column (4.6 × 250 mm; Waters Chromatography Division/Millipore, Milford, MA) with a guard column, and a UV detector system (Hitachi) were used for HPLC analysis. The conditions for HPLC analyses were the same as those used previously (*36*). Concisely, the mixing program of mobile phases was as follows (at a flow rate of 1 mL/min): solvent A (20% methanol in 5 mmol/L HCl) and solvent B (acetonitrile) were mixed using a linear gradient apparatus by changing solvent B as 0% (0 min) → 3% (5 min) → 15% (15 min) → 25% (22 min) → 0% (26 min) → 0% (30 min). Identifications of the compounds were confirmed by comparing retention times and absorption spectra to those of standard materials. Quantification was accomplished using calibration of the standards. The detection limits for FA, PCA, and diferulic acids were 15, 25, and, 46 μg/L, respectively.

Statistical Analysis. Data are shown as means ± standard error (SE). For the data of urinary and fecal excretion, Dunnett or Tukey's multiple-range test was used when significant differences were obtained by one-way ANOVA. When variances were unequal, data were log-transformed before ANOVA and reanalyzed (*37*). A two-sided *t* test was used to analyze the significant differences of plasma concentrations (paired two samples for means) of the cecal wall weights and of cecal content weights (two samples assuming equal variances). $P < 0.05$ was selected for experiments to reflect statistical significance except that P values were noted.

RESULTS

Effects of Feed or Feeding Method on Growth of Rats. Rats fed a single meal of a RCB diet containing 5% RCB or the RCB diet for 12 days did not differ in food intake, body weight gain, or eviscerated carcass weight from the control group rats (data not shown). Rats fed according to the method of the "meal feeding" or the limitation of the feeding time in the nighttime did not differ significantly either from those rats fed by using normal method (data not shown).

Recovery of FA and PCA in Plasma, Urine, and Feces of Rats Fed a Single Meal of the RCB Diet. Little of the FA and PCA was detected in either the urine or feces before the ingestion of the single meal of the RCB diet (**Figure 2A**; **Table 2**). Within the first day after the ingestion, the amount of the excretions of FA and PCA through urine significantly increased ($P < 0.01$ for FA and $P < 0.05$ for PCA, **Table 2**; **Figure 2**, part **B** versus part **A**). From the second day, however, the amount of the urinary excretion of FA and PCA returned to the previous level (**Table 2**). By contrast, ~81% of total FA and ~64% of total PCA in the ingested RCB were excreted by the rats through the feces, mainly within 3 days after the ingestion (**Table 2**). Over 90% of diferulic acid in the ingested RCB was excreted through feces (peak 3 in **Figure 2C**; data not shown
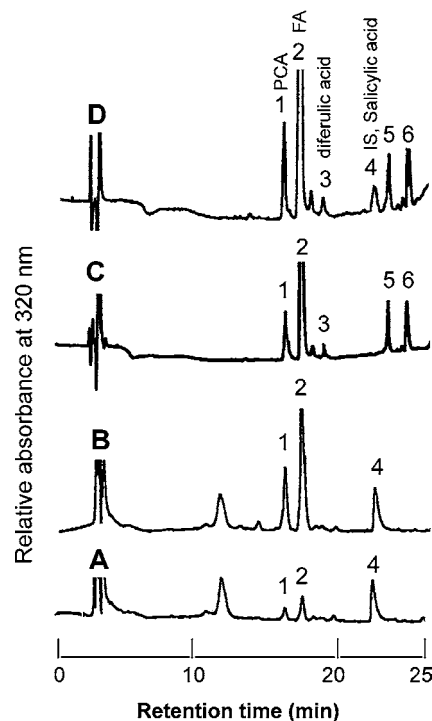

Figure 2. Typical HPLC-UV (320 nm) chromatogram of the extracts of the urine of the rats before (**A**) and after (**B**) being administered a single meal of a RCB diet, that of alkaline hydrolysate extracts of the feces from the rats fed the single meal (**C**), and that of the material RCB itself (**D**). IS, internal standard. The IS was not used in the determination of the phenolic acids in the feces (**C**).

in the tables). The diferulic acid was not detected in rat urine before or after the ingestion of the RCB diet (**Figure 2A,B**). None of PAs was detected in the plasma collected from the rats at 16 h after the ingestion of the RCB diet.

Recovery of FA and PCA in Plasma and Urine of Rats Adapted to the RCB Diet. About 1 g (0.9−1.1) of RCB was ingested by rats every day, which resulted in a daily ingestion of 30.1 (27.2−33.0) mg of total FA and 2.9 (2.6−3.2) mg of total PCA. The total FA concentrations of plasmas collected on days 5 and 12 were equal at 35 ± 2.0 μg/L each (**Table 3**). The proportions of free FA and its metabolites to the total FA in urine, the amount of daily urinary excretion of total PAs, or the urinary recovery of the PAs on day 11 did not differ from those on day 4 after the rats were adapted to the RCB diet. The weight of the cecal wall or the cecal content of rats fed the

Table 3. Concentration of Total *p*-Coumaric Acid (PCA) and Total Ferulic Acid (FA) in Plasma and the Amount of Daily Excretion of Total PCA and FA in Urine and the Proportion of Free FA and Its Metabolites in the Urine of Rats Adapted to 5% of Refined Corn Bran (RCB) Diet [a]

	concn in plasma (μg/L)		daily excretion in urine (μg/day)		proportion of free FA and its metabolites in urine (%)			
	PCA	FA	PCA	FA	free FA	FA-sulfate	FA-glucuronide	FA-sulfoglucuronide
control[b]	ND	ND	19.7 ± 4.9b	27.2 ± 7.8b	–[c]	–	–	–
day 3	–	–	49.2 ± 4.9a (1.1%)[d]	149.5 ± 8.0a (0.4%)	–	–	–	–
day 4	ND	35.0 ± 1.9	50.9 ± 6.6a (1.2%)	155.4 ± 5.8a (0.5%)	3 ± 1	7 ± 2	9 ± 1	81 ± 3
day 10	–		54.2 ± 4.9a (1.2%)	186.4 ± 17.5a (0.5%)	–	–	–	–
day 11	ND	35.0 ± 2.0	54.2 ± 6.6a (1.2%)	145.6 ± 5.8a (0.4%)	6 ± 3	5 ± 1	7 ± 3	81 ± 2
P value		0.93			0.23	0.20	0.38	0.8

[a] Values are means ± SE, $n = 4$. Means in a column without a common letter differ ($P < 0.05$), Tukey's multiple-range test. Values of the plasma concentration and the proportion of free FA and its metabolites in a column were tested with two-sided *t* test (paired two sample for means). [b] The amount of total PCA or total FA daily excreted by control group rats did not change significantly with days of adaptation (one-way ANOVA, $P < 0.01$). The values on day 11 are shown here as representative. [c] Not determined. [d] Percentage of the amount of total PCA or total FA excreted in urine relative to that ingested. The value was adjusted by subtracting that of the control group before the percentage calculation.

RCB diet for 10 days did not differ from those of the control group rats fed the standard diet (0.58 ± 0.02 versus 0.54 ± 0.04 g, $P = 0.34$ for the cecal wall weight; and 2.09 ± 0.15 versus 2.27 ± 0.29 g, $P = 0.62$ for the content weight). For the control group rats, the levels of urinary excretion of FA and PCA were similar to those detected in experiment I (**Table 2**). Diferulic acids were not detected in the plasma or urine of rats fed either the standard diet or the RCB diet.

DISCUSSION

Although FA or PCA was not detected in the plasma of rats fed a single dose of the RCB diet (containing 4.5 mg of FA and 0.44 mg of PCA, experiment I), FA was detected at a concentration of 35.0 ± 2.0 μg/L in rat plasma after the rat ingested a larger amount of PAs daily (∼30.1 mg of FA and 2.9 mg of PCA, respectively, experiment II). That PAs were not detected in the rat plasma after a single ingestion of the RCB diet may be because only a single dose could not lead to a high enough concentration of PAs in plasma to be detected. In both experiments, however, the rats that ingested RCB excreted a significantly larger amount of total FA and total PCA through urine than those rats that ingested only the standard diet ($P < 0.01$ for FA, $P < 0.05$ for PCA, **Tables 2** and **3**). These results indicted that the phenolic antioxidants FA and PCA in RCB are bioavailable in rats.

Bioavailability of FA from RCB in Rats. Although ∼1 g of RCB intake daily could bring about urinary excretion of FA at a level of ∼155 μg/day, the urinary recovery of dietary FA from RCB was at a low level (0.4–0.6%, **Tables 2** and **3**). Such a urinary recovery level in rats is lower than that from wheat bran in rats (3.9%; *11*) and in humans (3.1%; *22*) and also lower than that from tomato in humans (11–25%; *17*). These results show that the bioavailability of FA from RCB in rats is relatively low compared with its high content in RCB. Moreover, because the adaptation to the RCB diet did not significantly change the plasma concentration of total FA ($P = 0.93$), the urinary recovery of total FA ($P = 0.09$), or the proportions of free FA and FA metabolites to the total FA in the urine ($P = 0.2–0.8$, day 11 versus day 4, **Table 3**), it was indicated that the bioavailability of FA from RCB in rats might be not improved by a short period adaptation to the RCB diet.

Corn bran has a phenolic acid-conjugated polysaccharide composition similar to that of wheat bran (*38*); nevertheless, the bioavailability of FA from corn bran is only ∼10% of that from wheat bran in rats (**Table 3** and ref *11*). These results in vivo are consistent with those in vitro: ∼57.2% of FA could be released from wheat bran by human colon content (*25*); in

contrast, only 2.3% of that could be released from destarched corn bran by a mixture of ferulate esterase and Driselase (*39*). Such results may be explained by the structural difference between the two types of cereal brans. Cereal bran contains heteroxylan mainly and some cellulose. The heteroxylans are further cross-linked through FA and diferulic acids (*24*). Corn bran contains a 6-fold larger amount of FA and a 6−10-fold larger amount of dehydrodiferulates than wheat bran (**Table 1** and ref *40*), which makes its structure more complex than that of wheat bran. The more complex structure of corn bran may more severely reduce the accessibility of the necessary enzymes (such as ferulate esterases, xylanases), which contribute to the release of FA, and this, in turn, limits the release of FA from RCB in rat intestine. To investigate if RCB could be degraded by intestinal microflora, during which FA could be released possibly by the enzymes of microorganisms, we compared the cecal weight of rats fed with the RCB diet and the standard diet. Feeding the two different diets made no significant differences in the weight of the cecal walls ($P = 0.34$) or the cecal contents ($P = 0.62$, results of experiment II), implying that a 10-day adaptation to the RCB diet could not increase the fermentation activity in rat cecum and that, accordingly, it may be difficult for RCB to be degraded or utilized by the intestinal microflora. Taken together, we can suppose that high contents of FA and, especially, diferulic acids in cereal bran, which act as cross-links between bran cell wall polysaccharides, may not improve but, rather, limit their bioavailabilities in vivo.

Free FA is shown to be absorbed in Caco-2 cells by monocarboxylic acid transporter (*41*), which accounts for the quick and almost absolute absorption of FA in the foregut after the oral administration of FA (*12, 13*). However, FA bound with mono- and oligosaccharides could not follow such a transport mechanism before free FA was released in advance by microorganisms in intestinal lumen (*36*). For the FA in RCB, it seems to be more difficult to be released from RCB in the gastrointestinal tract. Therefore, to improve the bioavailability of FA in RCB, RCB is required to be processed before ingestion such as by heat, steam, pressure, and/or enzymatic treatment. In fact, feruloyl-arabinoxylan, a high molecular weight fraction prepared from RCB, has been shown to be a higher urinary recovery of FA in rat (20 versus 0.4%, ref *12* and **Table 2**).

Bioavailability of PCA from RCB in Rats. Free FA and conjugated FA in the system circulation seem not to be metabolized into PCA because neither PCA nor its conjugated forms were detected in the plasma or urine of the rats administered FA (*12*). We could not predict whether any FA in RCB is metabolized into PCA by microflora in the gastrointes-

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tinal tract. Even if this would occur, such a metabolic pathway should not be the main way to contribute to those PCAs recovered in rat urine because most of the ingested FA in RCB (∼81%, **Table 2**) remained in the feces. In this study, accordingly, we presumed that the all of the PCAs detected in urine and feces were from the PCA in the diet ingested by rats.

The urinary recovery of PCA from RCB in rats was 3−6 times greater than that of FA (2.3 versus 0.4% in **Table 2**, 1.2 versus 0.5% in **Table 3**), although the amount of total FA is ∼10 times that of PCA (w/w) in the RCB diet. On the other hand, the fecal recovery of total PCA was ∼16% less than that of total FA (64 versus 81%, **Table 2**), suggesting a greater percentage of the PCA dosage disappeared from the lumen. These results suggested that PCA in RCB was digested and/or absorbed more easily in the intestinal tract and thereby had a higher bioavailability than FA. Nevertheless, all of these results could not change the same fate of PCA as that of FA from the ingested RCB in rat lumen. That is, most of the total PCA (∼64%, **Table 2**) from the ingested RCB diet was excreted through rat feces and only a few of the disappeared PCAs from the lumen were recovered in the urine (∼2.3% recovered in the urine versus ∼36% disappeared in the lumen, **Table 2**). Such a result indicates that the bioavailability of PCA from RCB in rats is also relatively low compared with its high contents in RCB. This may be because PCA, similarly to FA in RCB, is esterified to arabinoxylans (*30*, *42*). Konishi et al. showed that free PCA was absorbed by a monocarboxylic acid transporter in Caco-2 cells (*43*) and rapidly absorbed in rats (*14*), suggesting that free PCA has a high bioavailability. There is little information in the literature on the bioavailability of PCA from foodstuffs except that Virgili et al. (*19*) reported that the urinary recovery of dietary PCA was ∼50% after humans ingested a single dose of 200 mg of a pine bark extract. The authors made no mention of the existing form of PCA in the plant extract. Such a high urinary recovery implied that it might exist in the free form and/or simple structural forms in the bark extract.

Bioavailabilities of Diferulic Acids from RCB in Rats. It should be noted that we did not focus on the bioavailabilities of diferulic acids, an isomeric group of antioxidants in RCB, because we thought they were not the main phenolic antioxidants in RCB. Only ∼0.9 g of diferulic acid (peak 3 in **Figure 2D**) in 1 kg of RCB was determined in this study. The result was consistent with that of the previous study (*44*). Saulnier et al. (*23*, *24*), however, have determined four isomers of diferulic acids in saponified extracts of corn bran, and their total concentration is 7−13.3 g/kg, suggesting that diferulic acids are the second most abundant phenolic antioxidants in corn bran. Such a large difference might be because, in our experiments, the other two or three peaks in the HPLC chromatogram of RCB alkaline extract (**Figure 2D**, peaks 5 and 6) had not been identified and determined. Nevertheless, whether the determined diferulic acid (peak 3 in **Figure 2D**) or the unknown phenolic compounds (peaks 5 and 6 in **Figure 2D**), they were detected in the feces but not in the urine of rats fed with RCB diet (parts **C** and **B** of **Figure 2**). Andreasen et al. (*27*) studied the intestinal release and uptake of diferulic acids from wheat and rye bran in detail. They showed that 4−36% of diferulic acids was released from wheat bran by human fecal extracts after incubation at 37 °C for 18 h. Free diferulic acids were also shown to be absorbed by rats. Nevertheless, a more recent study (*22*) showed that the diferulic acids could not be detected in the plasma or urine after humans ingested a high-bran breakfast cereal (wheat). Because the structure of RCB is more complex than that of wheat bran as discussed above, it might be more

difficult for the diferulic acids in RCB to be released and absorbed than those in the wheat bran.

In conclusion, this work showed that (1) the phenolic antioxidants FA and PCA from RCB were bioavailable in rats, (2) the bioavailabilities of the phenolic antioxidants from RCB in rats were relatively low compared with their high contents in RCB, and (3) their bioavailabilities could not be improved by adaptation for 10 days to the RCB diet containing 5% RCB. Comparison with the results of other studies revealed that the bioavailabilities of PAs vary greatly from bran to bran in which they are contained.

ABBREVIATIONS USED

PAs, phenolic acids; FA, ferulic acid; PCA, *p*-coumaric acid; RCB, refined corn bran; total FA, all forms of FA, containing free FA and all of its derivatives; total PCA, all forms of PCA.

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