Antioxidative Activity of Corn Bran Hemicellulose Fragments

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Antioxidative compounds were solubilized from an insoluble dietary fiber, refined corn bran (RCB), by enzymatic hydrolysis. Antioxidative activities (AOA) of 5-*O*-feruloyl-L-arabinofuranose (FA) and *O*-(5-*O*-feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (FAXX) were slightly stronger than that of ferulic acid. AOA of high molecular fraction were stronger than those of ferulic acid, FA, and FAXX. Diferulic acid was an active constituent among the alkaline hydrolysate of high molecular fraction. AOA of ferulic acid sugar esters, FA, and FAXX were stronger than that of free ferulic acid in the microsomal lipid peroxidation system.

INTRODUCTION

Cereals are a main supplier of dietary fiber (DF), and it has been reported that hemicellulose fractions obtained from cereals, such as wheat bran and corn bran, have physiological functions. Burkitt et al. (1972) reported that cereal dietary fiber rich in pentosan caused an increase of fecal volume and improved the microflora of enteric bacteria. Walker and Arvidson (1954) reported that cereal dietary fiber intake decreased atherosclerosis in the epidemiological research. Neutral detergent fiber (NDF) prepared from cereal bran decreased the cholesterol level in serum in rats, which is regarded as a risk factor of arteriosclerosis (Ayano, 1992). Refined corn bran has hepatoprotective activities against the hepatic injury caused by galactosamine (Ayano, 1992), orotic acid (Miyasaka et al., 1992), and ethanol (Ebihara et al., 1992). In spite of various physiological functions of cereal DFs reported (Ikeda et al., 1982), the mechanisms of these functions, except the mechanism described by the aspects of physical chemistry, have not been well studied.

On the other hand, recently the structures of the cell wall of cereals have been studied in detail. Phenolic acids such as ferulic (FH) and *p*-coumaric acids (PCA) which are known to be naturally occurring antioxidants (Cuvelier et al., 1992; Onyeneho and Hettiatachchy, 1992) have been proved to exist as carbohydrate esters (Harris and Hartley, 1980; Ishi, 1985; Fry, 1986; Kato, 1992) in the Gramineae cell wall such as rice endosperm (Shibuya, 1984), *Zea* shoot (Kato and Nevins, 1985), barley straw (Mueller-Harvey and Hartley, 1986), and bamboo shoot (Ishii et al., 1990; Ishii and Hori, 1990).

In the previous paper (Ohta et al., 1992), it was reported that sake extract had a greater antioxidative activity (AOA) than that of polished rice extract. Crude antioxidants in Sake were adsorbed on Amberlite XAD-2 and eluted by 50% methanol solution. The most active fraction was further fractionated into four subfractions by a Bio-Gel P-2 gel filtration chromatography. Two fractions, which had large molecular weights, were presumed to be ferulic acid glycosides which came from rice endosperm cell wall, because alkaline or enzymatic hydrolysis liberated ferulic acid.

The objectives of this study were to (1) solubilize the antioxidative compounds from an insoluble dietary fiber, refined corn bran (RCB), and (2) measure the antioxidative

activities of RCB hemicellulose fragments, mainly phenolic acid arabinoxylane esters, in the several lipid peroxidation systems.

MATERIALS AND METHODS

Preparation of Corn Hemicellulose Fragments. Refined corn bran (RCB) Celluphar (Nihon Shokuhin Kakou Co., Ltd.), which had not been chemically treated, was used as a corn hemicellulose sample. It contained 89.75% insoluble dietary fiber on the dry basis. Ten grams of hemicellulose sample was hydrolyzed by 30 mM oxalic acid for 3 h over the boiling water. Antioxidative compounds in the filtrate of hydrolysate were adsorbed on Amberlite XAD-2 and eluted by 50% methanol. This hydrolysate was further hydrolyzed by hemicellulase contained in the commercial enzyme Cellulase Amano-T (*Trichoderma* sp.). The hydrolysate was adsorbed on Amberlite XAD-2 and elute by 50% methanol and concentrated.

Resolution of the Soluble Fraction by Chromatography on Sephadex LH-20. A portion (10 mL) of the soluble fraction was applied to a column of Sephadex LH-20 preequilibrated with 50% methanol and eluted with 50% methanol; fractions of 5 mL were collected and assayed for phenolic compounds. Tubes 11–16, 17–21, 25, 27–28, 31, 35, 43–49, and 51–60 were separately combined and concentrated to dryness to give fractions A–H.

Assay of Antioxidative Activity in the Autoxidation System. Antioxidative activity (AOA) assay was carried out by the autoxidation system (Ramarathnam et al., 1988, 1989), and hydroperoxide was measured by the thiocyanate method described by Wurzenberger and Grosch (1984). Each concentration of test compound in the reaction mixture was 0.4 mM determined as ferulic acid described under Phenolic Acid Analysis.

Lipid Peroxidation of Rat Liver Microsome. Antioxidative activities on CCl$_4$-induced lipid peroxidation was analyzed by the method described by Ito et al. (1990). The microsomal lipid peroxidation induced by CCl$_4$ was determined by the method of Kornbrust and Mavis (1980). Microsomes were prepared from the liver of Sprague–Dawley rat (male) according to the method of Kamataki and Kitagawa (1974). The reaction mixture contained 1 mL of the microsomal fraction, 0.1 mM nicotinamide adenine dinucleotide phosphate (NADP$^+$), 1.5 mM glucose 6-phosphate, 2.5 mM nicotine amide, 1.25 mM MgCl$_2$, phosphate-buffered saline, 5 mM CCl$_4$ (50 μL of DMSO) in a final volume of 2 mL. The concentration of test compound was 0.5 mM. After incubation at 37 °C for 60 min under N$_2$, the reaction was stopped by the addition of 0.5 mL of 10% trichloroacetic acid solution. Thiobarbituric acid reaction substance (TBARS, including malondialdehyde) was measured by the thiobarbituric acid method (Buege and Aust, 1978).

Phenolic Acid Analysis. Each fraction was extracted with 0.5 M NaOH at 60 °C for 90 min under N$_2$. The extract was acidified to pH 3.5 (6 M HCl) and then extracted with EtOAc. The amount of phenolic acids extracted was determined from the Abs (320 nm) value of the extract using ferulic acid as a

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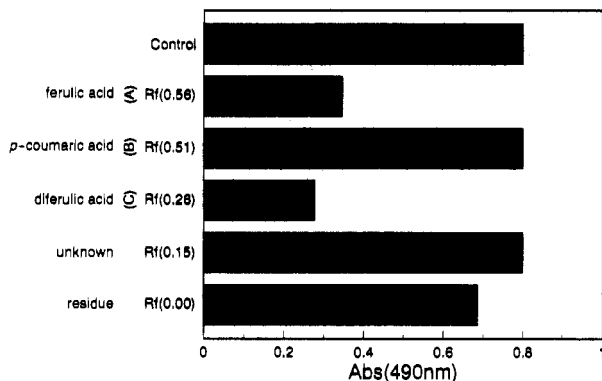
Figure 1. Antioxidative activity of alkaline hydrolysate of RCB. $R_f(0.00)$ not hydrolyzed residue, $R_f(0.15)$ unknown, $R_f(0.26)$ diferulic acid, $R_f(0.51)$ p-coumaric acid, $R_f(0.56)$ ferulic acid; control, no peak area equally prepared.

standard material described by Shibuya (1984). Thin-layer chromatography (TLC) of phenolic acids was performed on silica gel 60 plates (Merck) with the solvent system benzene–dioxane–HOAc (90:25:4). Spots were detected under UV illumination. HPLC was carried out with a Hitachi Model L-6200 liquid chromatograph equipped with UV detector. HPLC column (μ Bondapak C18 125 Å, 10 μm, 3.9 × 300 mm) were used for analytical purpose and for preparation. Phenolic acids were analyzed with a linear gradient of MeCN (0–25%) in 50 mM NaOAc buffer, pH 4.0, and detected by absorption at 280 nm. Flow rate was 1.0 mL/min. Qauntitative analysis was performed using peak area.

Radical Scavenging Effect. The radical scavenging effect was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Ito et al., 1990). DPPH (1 mM) dissolved in ethanol (1.2 mL) was added to a test compound (10 mM) dissolved in EtOH (200 μL). The mixture was incubated for 10 min at room temperature. The residual DPPH was determined by absorbance at 517 nm.

RESULTS

Antioxidative Activities of Alkaline Hydrolysate (AH) and 50% Methanol Extract Obtained from RCB. The concentration of free phenolic acids (2.55 mg/g of RCB) and antioxidants extracted from RCB by 50% methanol were negligible, being compared with those of alkaline hydrolysate on the RCB basis (32.59 mg/g of RCB). Antioxidative activity of AH was stronger than that of ferulic acid on the phenolic acid basis (data not shown). More than 85% of AH constituents was ferulic acid, suggesting that there was an unknown antioxidant among the AH constituents.

AOAs of Alkaline Hydrolysate Components. The thin-layer chromatogram showed that five major constituents were among the AH constituent (Figure 1). The amounts of component A (R_f 0.56), component B (R_f 0.51), and component C (R_f 0.26) were higher on the RCB basis. Considering their concentration in the AH, C had a strong AOA and B had no AOA. From their R_f values, retention times of HPLC, and UV spectrums, component A, B, and C were tentatively identified ferulic acid, p-coumaric acid, and diferulic acid, respectively. The amounts of these phenolic acids calculated using their relative amounts and the total amount (32.59 mg/g of RCB) of phenolic acids present in the RCB were 26.65, 5.10, and 0.84 mg/g of RCB for ferulic, p-coumaric, and diferulic acids, respectively.

AOAs of Corn Hemicellulose Fragments. Antioxidative compounds were solubilized from an insoluble dietary fiber, refined corn bran, by enzymatic hydrolysis. The gel filtration chromatogram of RCB hydrolysate is shown in Figure 2. Figure 3 showed that antioxidative activities of fractions F–H were stronger than that of ferulic
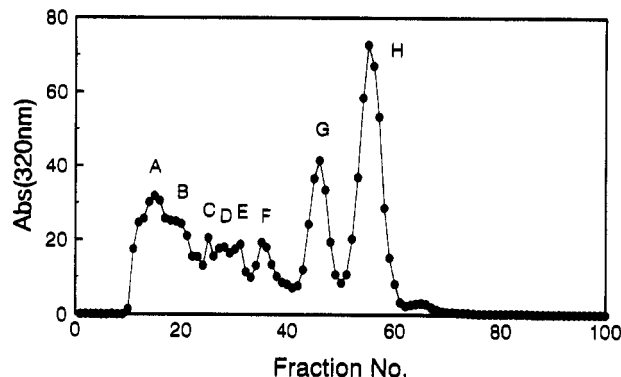
**Figure 2.** Chromatogram of enzymatic hydrolysate of RCB.

acid and that AOAs of the high molecular fractions (HMF) A–D were stronger than those of free ferulic acid and fractions F–H. Table 1 shows that HMF contained much more diferulic acid than low molecular fractions. Nuclear magnetic resonance spectroscopic (NMR) data and other analytical data indicated that fraction H was 5-O-feruloyl-L-arabinofuranose (FA). Confirmation of the structures of suspected geometrical isomers was obtained by NMR spectroscopy by comparison of the trans-ferulic acid with mixtures of two isomers obtained from it by treatment of solutions with daylight (Hartley and Jones, 1976). The NMR signals (270 MHz; Fourier transform) of FA in deuterated water were δ 3.86 (6H, s, OMe), 6.35 (2H, d, J = 15.94 Hz, trans-CH=CH), 6.90 (2H, d, J = 8.06 Hz, aromatic H), 7.10 (2H, d, J = 8.06 Hz, aromatic H), 7.16 (s, aromatic H), 7.60 (2H, d, J = 15.94 Hz, trans-CH=CH), 5.32 (arabinose H-1), 4.18 (arabinose H-2), 4.05 (arabinose H-3), 4.37 (arabinose H-4), 4.48 (arabinose H-5eq), 4.29 (arabinose H-5ax), confirming it as the trans isomer and that it contained a small amount of the cis isomer. Fraction F was tentatively identified as O-(5-O-feruloyl-α-L-arabinofuranosyl)-(1$\rightarrow$3)-O-β-D-xylopyranosyl-(1$\rightarrow$4)-D-xylopyranose (FAXX) from chromatographic data described by Kato and Nevins (1985).

Antioxidative Activities on CCl$_4$-Induced Lipid Peroxidation. Table 2 shows that AOA of ferulic acid sugar esters, such as FA, FAXX, and other high molecular fractions, are stronger than that of free ferulic acid in the microsomal lipid peroxidation induced by CCl$_4$. As shown in Figure 4, in the microsomal lipid peroxidation system, AOAs of 0.5 mM ferulic acid sugar esters are as strong as 0.5 mM α-tocopherol. AOA of FA was dose dependent between 0.5 and 1.5 mM.

DPPH Radical Scavenging Activities. Figure 5 shows that ferulic acid carbohydrate esters have DPPH radical scavenging activities. This finding indicated that AOA in an autoxidation system was caused by their radical scavenging activity. Fractions A–E could not be tested because of their insolubility in ethanol. DPPH radical scavenging activities of ferulic acid carbohydate esters were as strong as that of ferulic acid. The difference of AOA in the microsomal lipid peroxidation system between free acid and sugar esters could not be described by the radical scavenging activity.

DISCUSSION

In the previous paper, it was reported that sake extract had a greater antioxidative activity (AOA) than that of polished rice extract and that they come from the rice endosperm cell wall (Ohta et al., 1992). In the present study, we have demonstrated that the cell wall fragments prepared from corn hemicellulose, which is also a Gramineae plant, have antioxidative activity. This fact suggested

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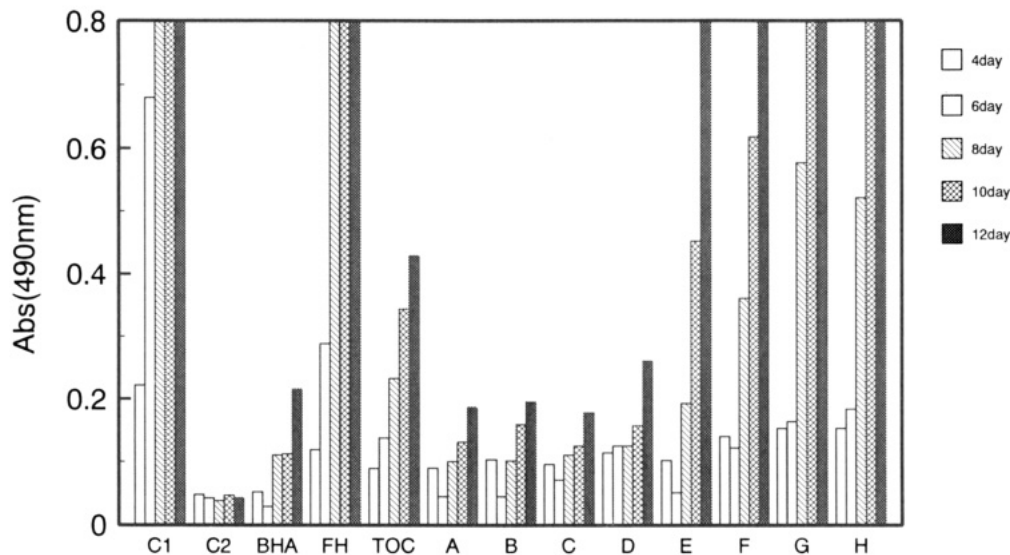
Figure 3. Antioxidative activity of RCB hydrolysate. Symbols: C1, control; C2, control (−20 °C); FH, ferulic acid; TOC, α-tocopherol.

Table 1. Relative Amount of Phenolic Acids in Enzymatic Hydrolysate of RCB

fraction	relative amount of phenolic acid		
	p-coumaric	ferulic	diferulic
fraction B	9.93	87.41	2.66
fraction D	12.02	85.82	2.16
fraction F	7.73	92.08	1.19
fraction H	2.02	97.77	0.21
total	15.65	81.77	2.57

**Table 2. Antioxidative Activity of RCB Hydrolysate in Lipid Peroxidation of Rat Liver Microsome Induced by CCl_4**

additive[a]	TBARS (%)	additive[a]	TBARS (%)
control	100	fraction C	44.3
blank	2.0	fraction D	24.4
ferulic acid	96.9	fraction E	29.0
BHA	14.5	fraction F	29.8
α-tocopherol	45.8	fraction G	42.7
fraction A	41.2	fraction H	18.3
fraction B	32.8		

[a] Test compounds were added as described under Materials and Methods.
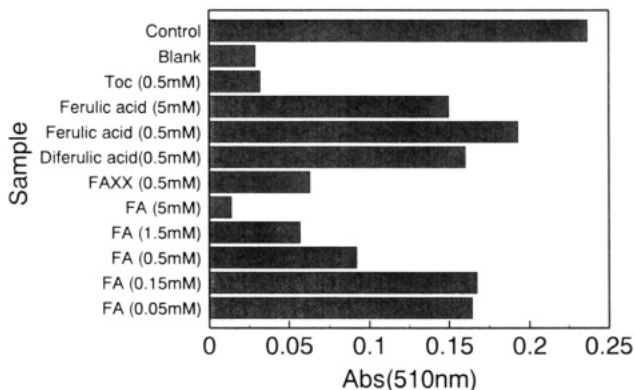
Figure 4. Antioxidative activity of RCB hydrolysate in lipid peroxidation of rat liver microsome induced by CCl_4.

that Gramineae cell wall was hydrolyzed by fungal cellulase and/or hemicellulase. In the case of sake brewing, cellulase, and/or hemicellulase produced by *Aspergillus oryzae* may be responsible for the solubilization of AOA. Occurrences of phenolic acids arabinoxylane esters, whose AOA are proved in this paper, are already reported in the *L. multiflorum* (Hartley, 1976), in the rice cell wall (Shibuya,
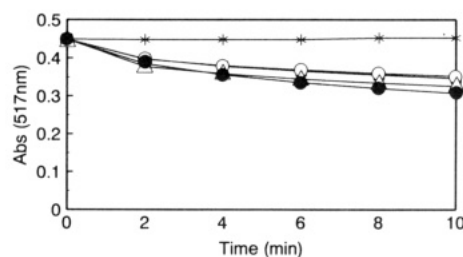
Figure 5. Radical scavenging activity of RCB hydrolysate. Symbols: control (∗), ferulic acid (●), fraction F (□), fraction G (△), fraction H (○).

1984), in the corn cell wall (Kato et al., 1985), and in the bamboo shoot (Ishii et al., 1990; Ishii and Hori, 1990), respectively.

The amounts of these phenolic acids calculated using their relative amounts and the total amount (32.59 mg/g of RCB) of phenolic acids present in the cell wall were 26.7, 5.1, and 0.84 mg/g of RCB for ferulic, *p*-coumaric, and diferulic acids, respectively. These values are larger than those obtained for the cell walls of *Oriza sativa* endosperm cell wall (9.1, 2.5, and 0.56 mg of each compound/g of cell wall, respectively) by Shibuya (1984) and those of *L. multiflorum* (6.5, 0.9, and 0.2 mg of each compound/g of cell wall, respectively) by Hartley and Jones (1976).

In the autoxidation system, AOAs of high molecular fractions (HMF) were stronger than ferulic acid, FA, and FAXX. Antioxidative activities (AOAs) of HMF may depend on the DFA concentration, because HMF contained much more DFA than FA and FAXX. Diferulic acid is formed from ferulic acid by oxidative coupling (Markwalder, 1976). Radical scavenging effect and/or chelating effect of diferulic acid formed from ferulic acid by oxidative coupling may be responsible for AOA in the HMF. Contrary to the autoxidation system, free ferulic and diferulic acids have weak AOA, and whether they are ester forms or not was responsible for the AOA in the microsomal oxidation system. If the surface of microsomal membrane is negatively charged, like the cell surface, this phenomena is easily described in repulsion between negative charge of ferulic acid and that of membrane surface.

In the present paper, refined corn bran was used as hemicellulose sample. Using other gramineae plants such as rice, barley, and wheat, or using other parts such as hull

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and endosperm cell wall, similar results will be obtained in proportion to phenolic acid concentration.

According to *Standard Tables of Food Composition in Japan* (Resources Council, 1982) and Ayano et al. (1993), a human takes about 20 g of dietary fiber, which includes 10 g of cereal dietary fiber, in 1 day. In addition, a human takes about 500 mg of ferulic acid and 20 mg of diferulic acid as sugar esters in a day, but without enteric bacteria this compound is useless.

We are now trying to solve the question of whether these phenolic acids which are present as arabinoxylane esters in the cell wall are available for humans or not. Refined corn bran (RCB) is not digested by human digestive enzymes. Enteric bacteria may play an important role on solubilities of the phenolic acid esters. Hemicellulose fermentation to short chain fatty acids by enteric bacteria (Takeuchi et al., 1991) indicate that insoluble cell walls (phenolic acids arabinoxylane esters) are digested to soluble oligoxylosaccharide. The AOA of DFA itself and that of DFA esters should be compared.

In conclusion, antioxidative compounds were solubilized from an insoluble dietary fiber, refined corn bran (RCB), by enzymatic hydrolysis, and the presented results shows that low molecular and high molecular fragments of corn bran hemicellulose, one of cereal dietary fibers, have antioxidative activities in the several peroxidation systems, suggesting that AOA of cereal hemicellulose fragments may be responsible for some physiological functions of cereal dietary fiber.

ACKNOWLEDGMENT

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