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Properties of Extracts from Defatted Rice Bran by Its Subcritical Water Treatment

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Defatted rice bran was extracted with water and subcritical water at 50–250 °C for 5 min. The highest extract yield was achieved at 200 °C, at which the maximum amounts of protein and carbohydrate were also obtained. The total phenolic and furfural contents, radical scavenging activity, and antioxidative activity for the autoxidation of linoleic acid increased with increasing treatment temperature. The bran extracts exhibited emulsifying activity except for the extract prepared at 250 °C, which was concomitant with the disappearance of its high-molecular-mass substances. The extract prepared at 200 °C also had the highest emulsion-stabilizing activity.

KEYWORDS: Antioxidative activity; radical scavenging activity; rice bran; subcritical water; total phenolic content

INTRODUCTION

Rice bran is a major by-product obtained from the polishing process that produces white rice. After extraction of the edible oil, defatted rice bran is used to reduce the final cost in animal feeds or is discarded as agricultural waste. However, it still contains significant amounts of protein, carbohydrate, dietary fiber, and phenolic substances, which are beneficial as health-promoting and functional substances in foods (*1, 2*). The substances possess properties such as radical scavenging, antioxidative, and emulsifying activities (*3, 4*). Recently, these substances could be recovered by organic solvents, such as methanol and ethanol, and by supercritical carbon dioxide extraction (*3, 5*). Although these methods are convenient, they are time-consuming and sometimes produce toxic wastes after use.

Subcritical water extraction is a technique using hot water between 100 and 374 °C under high pressure to maintain its liquid state (critical point of water, 22.4 MPa and 374 °C). It is environmentally friendly because it does not use organic solvent and alleviates the problems accompanied with the conventional methods. As the temperature of the water increases from ambient temperature to 250 °C, its relative dielectric constant decreases from around 80 to near 27, which is similar to that of acetone at ambient temperature (*6, 7*). Consequently, subcritical water has the ability to dissolve hydrophobic substances. Furthermore, because the dissociation constant of water to hydrogen and hydroxyl ions is orders of magnitude greater than that in ambient water, the water acts as an acid or base catalyst in chemical reactions.

Biomaterials, such as proteins and carbohydrates, could be hydrolyzed in subcritical water without additional catalysts (*8, 9*). The cellulose and hemicellulose of brans, leaves, and grass could be degraded by subcritical water treatment at 160–260 °C (*10, 11*).

The purpose of this study is to demonstrate the potentiality of the subcritical water as an extractant to recover beneficial substances from defatted rice bran by its subcritical water treatment and to characterize the substances in the extracts prepared at different temperatures. The results would bring about the effective utilization of defatted rice bran, most of which is discarded as agro-waste, using an environmentally friendly and safe extractant.

MATERIALS AND METHODS

Materials. The bran of rice (*Oryza sativa*) was defatted according to the AOCS official method Ba 3-38 by Soxhlet extraction using petroleum ether as the extractant (*12*). The defatted rice bran contains moisture (13%), crude fat (1.3%), crude fiber (9.6%), crude ash (11.8%), crude protein (16.8%), and nitrogen-free soluble substances (47.5%). 1,1-Diphenyl-1-picrylhydrazyl hydrate (DPPH) and bovine serum albumin (99% purity) were purchased from Wako Pure Chemical Industries (Osaka, Japan). L-Ascorbic acid (>99.5%) was purchased from Nacalai Tesque (Kyoto, Japan). Folin–Ciocalteu reagent was purchased from ICN Biochemicals, Inc. (Aurora, OH, USA). Linoleic acid (>95%) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Gallic acid, furfural and trimethylsilyl diazomethane in hexane (2 mol/L) were purchased from Sigma-Aldrich Japan (Tokyo). Dextrans (10–500 kDa), polyethylene glycols, glucose, sucrose, and maltotriose, which were used as standards for the gel permeation chromatography,

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were purchased from Wako or Amersham Biochemicals (Uppsala, Sweden). The other chemicals of analytical grade were purchased from Wako or Nacalai Tesque.

Apparatus. A vessel, which was made from SUS-316 stainless steel with a volume of 117 mL (30 mm i.d.; 42 mm o.d.; 321 mm outside height) and was resistant to high pressure and temperature, was assembled by the Taiatsu Techno Corporation, Osaka, Japan. The temperature of defatted rice bran and water mixture in the vessel was measured using a thermocouple inserted into a tube, which was assembled with the vessel. The treatment temperature was regulated at a desired value using a temperature regulator and a mantle heater (Heater Engineer Co., Tokyo, Japan) as the heat source.

Preparation of Bran Extract by Subcritical Water Treatment. The defatted rice bran was treated in the subcritical water using the described vessel. The conditions were determined based on our previous research (*13*) in which black rice bran was treated with subcritical water using a small vessel (working volume of 10 mL). The mixture of the bran (1.5 g) and water (75 mL) in the vessel was heated to the determined temperatures and held for 5 min at each temperature. The times required to raise the temperature from the ambient to 50, 100, 150, 200, and 250 °C were 5, 11, 18, 28, and 39 min, respectively. The pressure in the vessel was 0.012–3.97 MPa according to the IAPWS formulation (*14*). The vessel was cooled to ambient temperature in an ice bath immediately after the treatment. The bran extract was clarified through a paper filter and centrifugation (RS-18II, Tomy Seiko Co., Tokyo, Japan) at 8500 rpm for 10 min. The bran extracts were kept in a refrigerator at 4 °C without any precipitation until they were used for analysis. The amounts and properties of the substances removed by the filtration and the centrifugation were not measured because their use was not considered in the practical process.

Total Carbohydrate Content. The total carbohydrate content in the bran extract was determined by the modified phenol–sulfuric acid method (*15*). A 25 μL aliquot of an 80% (w/w) aqueous phenol solution and 2.5 mL of H_2SO_4 were added to 1 mL of the diluted extract or glucose solution as a standard and then the sample was mixed well. The mixture was left for 10 min at ambient temperature and then cooled in the 25 °C water bath for 10 min. The total carbohydrate content was evaluated using a UV-1200 spectrophotometer (Shimadzu, Kyoto) at 490 nm versus water as the blank.

Protein Content. The protein content of the bran extract was evaluated according to the Lowry–Folin assay (*16*). Bovine serum albumin at 50–500 mg/L and water were used as the standards for the calibration curve and blank, respectively. Each bran extract was diluted serially before analysis. The absorbance at 500 nm was used to measure protein content in the bran extracts and protein standard.

Total Phenolic Content. The total phenolic content of the bran extract was evaluated using the Folin–Ciocalteu reagent (*3, 17*). The diluted bran extract (100 μL) was combined with the freshly prepared Folin–Ciocalteu reagent (400 μL) and 75 g/L sodium carbonate (1 mL). The mixture was filled to 5 mL with distilled water and then kept in the dark at ambient temperature for 2 h to complete the reaction. The total phenolic content was evaluated by measuring the absorbance at 765 nm. Gallic acid was used as the standard, and the results were calculated as the gallic acid equivalent (mg of gallic acid/g of bran).

Furfural Content. The furfural content of each bran extract was determined using an HPLC (LC-10AT, Shimadzu, Kyoto Japan) equipped with an YMC-Pack ODS-A column (4.6 mm i.d. × 100 mm; YMC, Kyoto, Japan) and a Shimadzu SPD-6A spectrophotometric detector (284 nm) according to our previous method (*18*). The mobile phase was 10% (v/v) methanol, and its flow rate was 0.5 mL/min. The determination was conducted in triplicate for each bran extract and the furfural content was expressed as mmol/g of bran.

Molecular Mass Distribution. The molecular mass distribution of the substances in the bran extract was estimated by HPLC (LC-6A Shimadzu, Kyoto) equipped with an YMC-pack Diol-60 column (500 × 8.0 mm ID, YMC, Kyoto) and an RIU-6A refractometer (Shimadzu) at ambient temperature. The mobile phase was 0.05 mol/L $NaNO_3$, and its flow rate was 1.0 mL/min. The injection volume of each bran extract was 20 μL, and the analysis was performed in triplicate. Glucose, sucrose, polyethylene glycols, and dextran (10–500 kDa) solutions were used as standards for determination of the molecular masses.

Absorption Spectrum. The UV and Vis spectra were measured using a UV-1600 spectrophotometer (Shimadzu, Kyoto) in the range of 200–400 nm. Each bran extract was serially diluted with water to obtain an absorbance less than unity for both the UV and Vis measurements.

Radical Scavenging Activity. The DPPH radical scavenging activity was evaluated by modifying the method of Fujinami et al. (*19*). A 1 mL aliquot of 0.5 mmol/L DPPH in ethanol was added to 4 mL of diluted bran extract or L-ascorbic acid (abbreviated VC) as the standard. The mixture was well shaken and placed in the dark for 20 min at ambient temperature. The remaining radical quantity was determined by the spectrophotometer at 516 nm. A 4 mL aliquot of 50% (v/v) aqueous ethanol was used as the blank. The radical scavenging activity of the diluted extract or VC solution was defined as the amount of the extract necessary to reduce the initial DPPH concentration by 50% and was calculated as follows:

$$\text{Radical scavenging activity} = (A - B + C)/A \times 100(\%) \quad (1)$$

where A is the initial absorbance of the blank, B is the absorbance of the mixture of the diluted extract or VC solution and DPPH solution at 20 min, and C is the absorbance of the diluted extract without the DPPH solution. The activity was expressed as mmol of VC equivalent/g of bran.

Emulsifying and Emulsion-Stabilizing Activities. An oil-in-water (O/W) emulsion was prepared by homogenization of the bran extract (3 mL) and soybean oil (0.15 g) at ambient temperature using a rotor/stator homogenizer (Physcotron NS-50, Nichion Irika Kiki, Tokyo) for 3 min at a power setting of 70 (ca. 10 000 rpm). The emulsifying activity of the bran extract was evaluated by measuring the particle-size distribution of the oil droplets in the emulsion using a SALD-2100 laser diffraction particle size analyzer (Shimadzu, Kyoto, Japan). The emulsion-stabilizing activity was also evaluated by measuring the particle-size distribution after a 3-h storage at ambient temperature.

Antioxidative Activity for Autoxidation of Linoleic Acid. A bran extract was concentrated by a rotary evaporator (Tokyo Rikakikai Co., Tokyo, Japan) at 65 °C and lyophilized to obtain its powder. The powder was kept in glass bottles in a refrigerator at 4 °C until used. Evaluation of the antioxidative activity was conducted according to our previous method (*20*). The extract powders (224 mg) prepared at 50–150 °C and at 200 and 250 °C were dissolved in water and a water–methanol mixture (60/40), respectively. The same amount (224 mg) of linoleic acid was separately dissolved in methanol. The extract and linoleic acid solutions were mixed to obtain a specific weight ratio of the extract to linoleic acid that ranged from 0.05 to 0.7. After distribution of 300 μL of the mixture into amber glass vials (1.5 × 4.5 mm) and removal of the methanol and water under reduced pressure, oxidation of the linoleic acid was measured at 65 °C and ca. 0% relative humidity by phosphorus (V) oxide. Methyl palmitate and trimethylsilyldiazomethane in hexane were used as the internal standard and methylating agent, respectively. The unoxidized linoleic acid was determined by a GC-14A gas chromatograph (Shimadzu, Kyoto) equipped with a separation column (3.2 mm i.d. × 3.1 m) packed with Advance-DS on Shinchrom A and a hydrogen flame-ionization detector. The column, injection, and detector temperatures were 200, 230, and 230 °C, respectively. N_2 gas was used as the carrier gas at a flow rate of 50 mL/min. The fraction of the unoxidized linoleic acid was determined from the ratio of the peak area of the unoxidized linoleic to that of methyl palmitate.

Water Sorption Isotherm. The lyophilized bran extract powders were used to determine their water sorption isotherms. The isotherms at 30 °C were determined according to the isopiestic method (*21*) using saturated lithium chloride (water activity 0.11), potassium acetate (0.22), magnesium chloride (0.32), potassium carbonate (0.43), magnesium nitrate (0.51), sodium bromide (0.59), potassium iodide (0.68), sodium chloride (0.75), and potassium chloride (0.84) solutions. Each bran extract (0.5 g) was placed in airtight stainless steel containers in the presence of the specified saturated salt solution to regulate the water activity and crystalline thymol to prevent microbial spoilage of the extract powders at high water activities ($a_w > 0.7$). The containers were maintained at 30 °C in an oven until the extracts reached equilibrium weight (the difference in weight within ± 0.0005 g), which took about

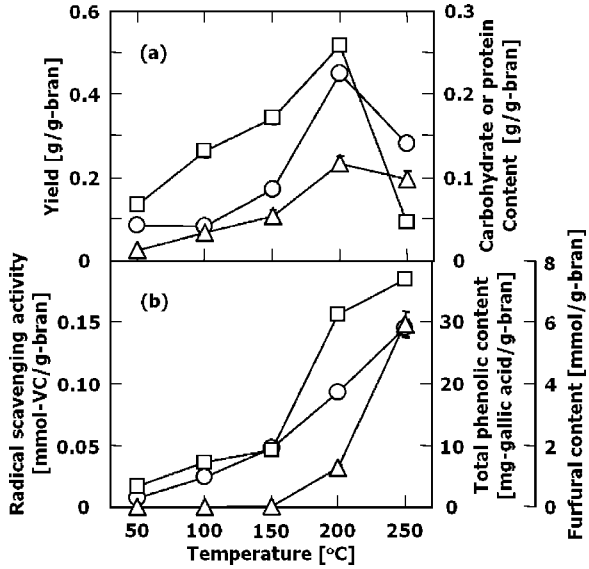

Figure 1. (**a**) Yield (○), the carbohydrate (□) and protein (△) contents, (**b**) radical scavenging activity (○), and total phenolic (□) and furfural (△) contents of the extracts from defatted rice brans using water or subcritical water at various temperatures.

7–20 days for each water activity (*22*). After the equilibrium had been reached, the samples were dried in an oven at 105 °C for 24 h (*12*).

The Guggenheim–Anderson–de Boer (GAB) equation [eq 2] was used to fit the experimental data:

$$\frac{q}{q_m} = \frac{CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \quad (2)$$

where q is the amount of water sorbed at equilibrium on a dry basis, a_w is the water activity, q_m is the q value for a monolayer coverage, C is a constant related to the first layer heat of sorption, and K is a factor related to the heat of sorption of the multilayer (*23*). The three parameters, q_m, C, and K, of the GAB equation were estimated to best-fit the calculated results to the experimental ones using the Solver in Microsoft Excel.

Statistical Analysis. All the experiments except the antioxidative activity and water sorption isotherm were conducted twice, and each analysis was conducted in triplicate. The experimental data of the bran extracts were evaluated for mean comparisons using statistical software (SAS Institute, NC) with an analysis of variance (ANOVA). The mean difference among each bran extract at a significant level of $P < 0.05$ was compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Composition of Bran Extracts Prepared at Various Temperatures. The bran extracts, which were prepared at various temperatures and clarified by filtration, were lyophilized to measure the yield (**Figure 1a**). The yield increased with increasing temperature up to 200 °C, at which the maximum yield of ca. 50% was observed ($P < 0.05$), while it sharply decreased over 200 °C.

Figure 1a also shows the dependencies of the carbohydrate and protein contents on the treatment temperature. The carbohydrate and protein contents of the extracts increased with increasing temperature from 50 to 200 °C. In constrast, the carbohydrate content substantially decreased and the protein content was subjected to a slight decrease at 250 °C ($P < 0.05$). The solubility of the rice bran protein in water was generally low due to strong aggregation through a hydrophobic interaction and extensive association with the cell wall (*24*). An increase in its solubility at higher temperatures would result from hydrolysis of the protein and cell wall. A sharp decrease in the carbohydrate content at 250 °C was attributed to decomposition of the saccharides into degraded products, which could not respond to the phenol–sulfuric method.

Figure 1b shows the total phenolic contents of the bran extracts prepared at various temperatures. The total phenolic content increased with increasing treatment temperature ($P < 0.05$). A gradual increase in the total phenolic content was observed when the bran was treated from 50 to 150 °C, and a drastic increase was observed when treated over 150 °C. The phenolic components of the rice bran are mainly classified as free and bound phenolic substances. Most phenolic substances in the rice bran are cross-linked to polysaccharides containing glucose, arabinose, xylose, galactose, rhamnose, and mannose residues in the cell wall and difficult to be recovered by an organic solvent unless the rice bran is treated under an acidic or basic condition (*25*, *26*). Increase in the solubility of the phenolic substances at high treatment temperatures could be due to disruption of the bran during subcritical water treatment and the subsequent release of the phenolic substances from the cell wall into the extract. In addition, the sharp increase in the phenolic content at 200 °C would be explained by the significant decomposition of the cell wall due to severe heat treatment which promoted the solubilization of those substances.

It has been reported that the phenolic substances in the bran extract predominantly contain ferulic, *p*-coumaric, *p*-hydroxybenzoic, and vanillic acids and a minority of caffeic, gentisic, protocatechuic, and syringic acids (*1*, *27*, *28*). The present results indicated that subcritical water was one of suitable methods to recover the phenolic substances from the rice bran.

As the bran extract contains proteins and carbohydrates, the browning reaction is one of dominant reactions occurring during the subcritical water treatment. Furfural is a well-known intermediate product formed during the early stage of the nonenzymatic browning reaction and used as an indicator for the progress of either the Maillard browning reaction or caramelization (*29*, *30*). The furfural content increased with increasing temperature. The formation of furfural scarcely occurred at 50–150 °C. In contrast, it was significantly generated during the treatment at 200 and 250 °C. A significant decrease in the carbohydrate content at 250 °C did not affect the formation of furfural. These results indicated that the browning reaction significantly occurred when the bran was treated at over 150 °C.

Figure 1b also shows the effect of the treatment temperature on the DPPH radical scavenging activity. The activity was higher for the extract prepared at the higher temperatures ($P < 0.05$). The radical scavenging activity would result from several substances depending upon their water solubility and heat stability. The strong radical scavenging activity against the DPPH radical mainly resulted from the phenolic substances in accordance with a linear correlation between the total phenolic content and the radical scavenging activity with a slight variation. At low treatment temperatures, free phenolic substances and proteins may be responsible for the radical scavenging activity (*2*, *31*, *32*). At high treatment temperatures, in particular, at 200 and 250 °C, the substances in the bran extracts were exposed to hydrolysis and simultaneously underwent the browning reaction. The bound phenolic substances, which were released into the bran extract, and the browning reaction products, such as protein–carbohydrate conjugates, melanoidins, and heterocyclic compounds, would also contribute to the radical scavenging activity (*27*, *33*, *34*).

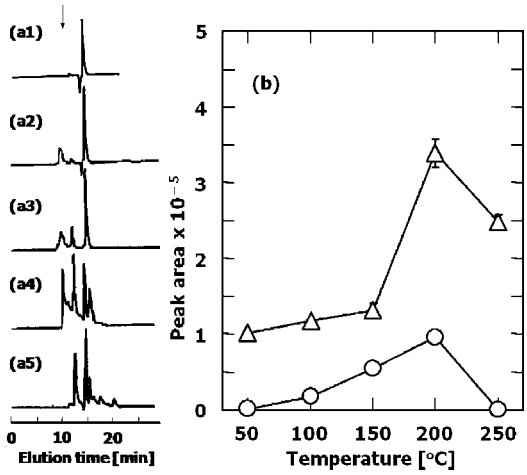

Figure 2. (**a1**–**a5**) Gel permeation chromatograms of the extracts prepared at 50, 100, 150, 200, and 250 °C, respectively, and (**b**) the contents of the low- (△) and high-molecular-mass (○) substances of the extracts. The arrow in (**a**) indicates the elution time corresponding to the molecular mass of 10^4.

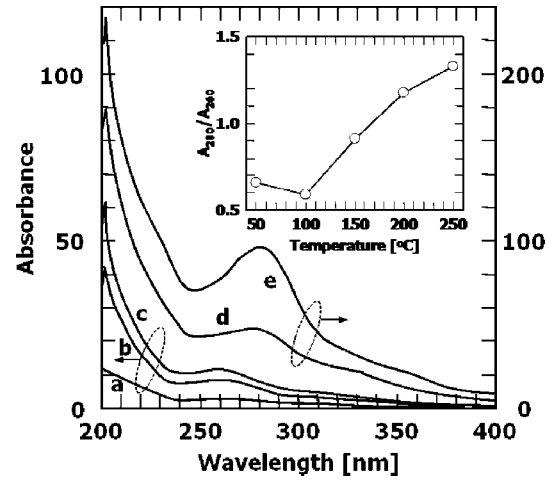

Figure 3. UV–vis spectra for the extracts prepared at 50 °C (**a**), 100 °C (**b**), 150 °C (**c**), 200 °C (**d**) and 250 °C (**e**). Inset: Temperature dependence of the ratio at the absorbance of 280 to 260 nm for the extracts.

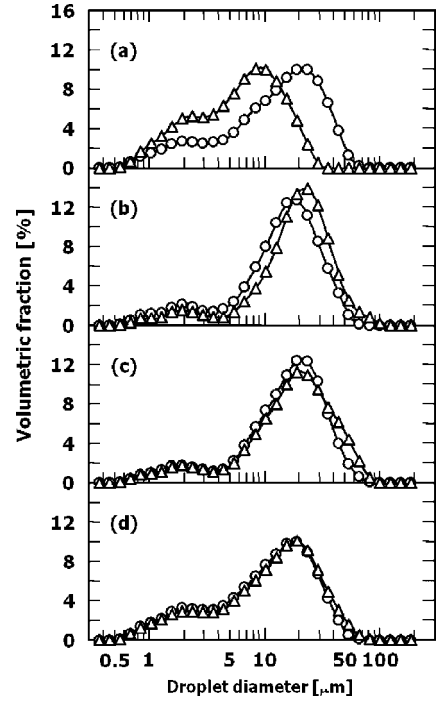

Figure 4. Distribution in diameter of oil droplets in O/W emulsions prepared using the extracts at (**a**) 50 °C, (**b**) 100 °C, (**c**) 150 °C, and (**d**) 200 °C immediately after preparation (○) and after a 3-h storage at ambient temperature (△).

Among the phenolic substances in the rice bran, ferulic acid was considered to be in a significant quantity and had a relatively strong radical scavenging activity (*1*, *27*, *28*).

Molecular Mass Distribution. Figure 2a1–**a5** show the chromatograms for the extracts at 50 to 250 °C. The substances having molecular masses of less and more than 10 kDa were designated as the low- and high-molecular-mass ones, respectively. **Figure 2b** shows the temperature dependences of the peak areas for the high- and low-molecular-mass substances. Both the low- and the high-molecular-mass contents increased with increasing temperature from 50 to 200 °C. However, the low-molecular-mass content began to decrease at 250 °C, and the high-molecular-mass completely disappeared at that temperature. The fraction of the low-molecular-mass substances was the highest in the bran extract prepared at 200 °C. Increases in both the low- and the high-molecular-mass substances in the extracts prepared at 50–200 °C contributed to increase the yield.

A decrease in the low-molecular-mass substances in the extract prepared at 250 °C would be attributed to their further degradation by the subcritical water. The decrease responded to the decrease in the yield and the protein and carbohydrate contents at that temperature. While a drastic decrease in the high-molecular-mass substances was observed, the protein content only slightly decreased. This fact would be explained by hydrolysis of the protein to peptides or amino acids, which were still positive in the Lowry–Folin method (*16*). These results would suggest that the high-molecular-mass substances would consist of mainly proteins and carbohydrates.

Absorption Spectra. The UV–vis spectra were measured for the extracts prepared at the different temperatures (**Figure 3**). The extracts prepared at 50–150 °C showed absorption maxima at ca. 260 nm, while those at 200 and 250 °C had maxima at ca. 280 nm. The two absorption maxima both suggested the presence of various phenolic substances (*35*, *36*). The absorption maxima around 280 nm also indicated proteins, browning reaction products such as furfural and pyrazine compounds, and soluble lignin (*37*–*39*). The UV spectra of the extracts prepared at 50–150 °C showed similar characteristics, and those of the extracts at 200 and 250 °C were also similar to each other. This mutual similarity would indicate that their constituents would likely be identical substances but at different concentrations.

The inset of **Figure 3** shows the ratio of the absorbance at 280 to 260 nm for the extracts obtained at different temperatures. The ratios were almost the same for the extracts at 50 and 100 °C, and they were higher for the extract prepared at the higher temperature. This fact might relate to the higher phenolic content for the extract obtained at the higher temperature.

Emulsifying and Emulsion-Stabilizing Abilities. Figure 4a–**d** shows the distributions of oil droplets in the emulsions prepared with soybean oil and the bran extracts at 50 to 200 °C. The distributions were also measured after a 3-h storage at ambient temperature to estimate the emulsion-stabilizing ability of the extract. The extract at 50 °C showed an increase in the small oil droplets after storage. This would be interpreted as follows: the oil droplets in the emulsion prepared with the extract at 50 °C were large. Because the large oil droplets were more unstable than the small ones, they coalesced to produce larger droplets and oiled-off. The small droplets retained their size,

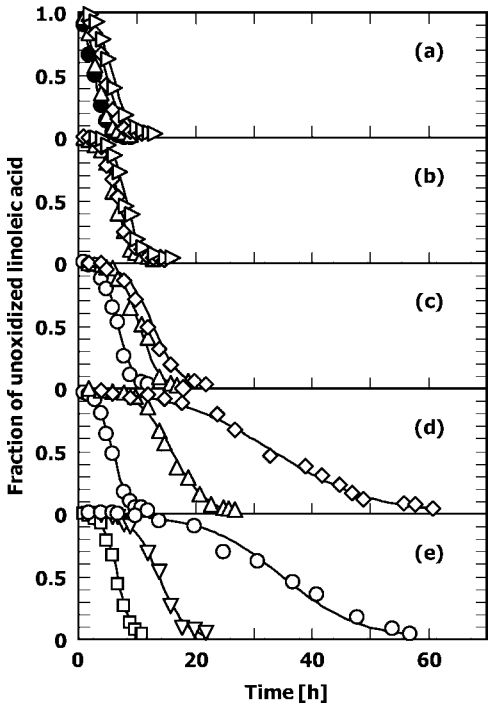

Figure 5. Oxidation processes at 65 °C and 0% relative humidity of linoeic acid mixed with the extracts prepared at (**a**) 50 °C, (**b**) 100 °C, (**c**) 150 °C, (**d**) 200 °C, and (**e**) 250 °C. The weight ratios of the extract to linoleic acid were 0 (●), 0.05 (□), 0.08 (▽), 0.1 (○), 0.3 (△), 0.5 (◇), and 0.7 (▲).

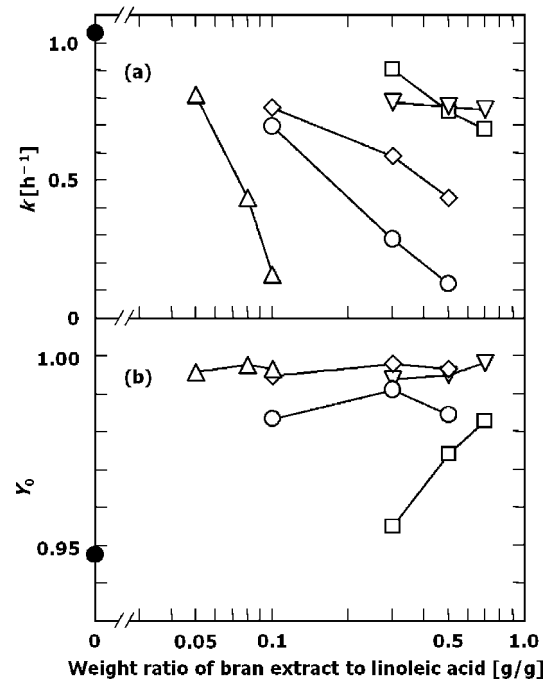

Figure 6. Dependences of the (**a**) k and (**b**) Y_0 values on the weight ratio of the extract to linoleic acid. The symbols, □, ▽, ◇, ○, and △, represent the extracts prepared at 50, 100, 150, 200, and 250 °C, respectively.

and hence, the distribution shifted to the smaller size after the 3-h storage. These facts would be ascribed to the low content of the high-molecular-mass substances in the extract at 50 °C. The extract at 200 °C, which had the highest content of high-molecular-mass substances among the tested extracts, produced the most stable emulsion, in which the distribution of oil droplets did not change during the storage. The emulsion prepared using the extract at 250 °C, which had no high-molecular-mass substances, was oiled-off within a few minutes after its preparation. These results indicated that the high-molecular-mass substances responded to both the emulsifying and the emulsion-stabilizing abilities of the bran extract.

Antioxidative Ability for the Oxidation of Linoleic Acid. **Figure 5** shows the oxidation processes of linoleic acid to which the bran extracts prepared at different temperatures were added at various weight ratios. Each bran extract obviously showed the ability to retard the oxidation of linoleic acid in comparison with the control with no extract addition. For every bran extract, the oxidation was retarded more effectively at the higher weight ratio of the extract to linoleic acid. Moreover, at the same weight ratio, the higher the treatment temperature, the slower the oxidation proceeded. The entire oxidation process of linoleic acid could be expressed by the autocatalytic-type equation 3 (*40*).

$$dY/dt = -kY(1 - Y) \quad (3)$$

where Y is the fraction of the unoxidized linoleic acid, t is the time, and k is the rate constant. Under the initial condition of $Y = Y_0$ at $t = 0$, the integration of eq 3 gives eq 4.

$$\ln[(1 - Y)/Y] = kt + \ln[(1 - Y_0)/Y_0] \quad (4)$$

Y_0 is a parameter, which is introduced to solve eq 3, and reflects the induction period of the oxidation. The parameters k and Y_0 for each oxidation process were estimated by plotting $\ln[(1 - Y)/Y]$ versus t. The solid curves in **Figure 5** were drawn using the estimated k and Y_0 values.

The relationship between the k or Y_0 value and the weight ratio of the bran extract to linoleic acid is illustrated in **Figure 6** for the extracts prepared at the various temperatures. The k value decreased with increasing weight ratio, and the k values for the linoleic acid to which the bran extract prepared at any temperature was added were lower than the k value for the linoleic acid with no extract. These results clearly implied that all the bran extracts could suppress the autoxidation of linoleic acid and that the bran extracts prepared at the higher temperatures were more effective in suppressing the autoxidation of the linoleic acid.

The Y_0 value increased with increasing weight ratio. All the Y_0 values for the oxidation of linoleic acid with the extracts were higher than the Y_0 value for the control experiment. Because the Y_0 value governed the oxidation induction period, an increase in the Y_0 value indicated the extension of the induction period of the oxidation. The induction period of linoleic acid to which the extract was added at the higher weight ratio was longer.

The higher antioxidative activity of the extract prepared at the higher treatment temperature had a similar inclination to increase in the total phenolic content, DPPH radical scavenging activity, UV absorbances at 260 and 280 nm for the extract. This fact would suggest that the active substances in the bran extract were the phenolic or aromatic substances.

Although the treatment of the bran at 250 °C would acquire the highest antioxidative activity, the treatment of the bran at 200 °C seemed to be more suitable for obtaining the extract having multiple properties such as emulsifying, emulsion-stabilizing, and antioxidative abilities.

Water Sorption Isotherm. Because the bran extracts contain carbohydrates and proteins of various molecular masses, they would be considered as hygroscopic products. A profile of the water sorption isotherm is characteristic of a hygroscopic

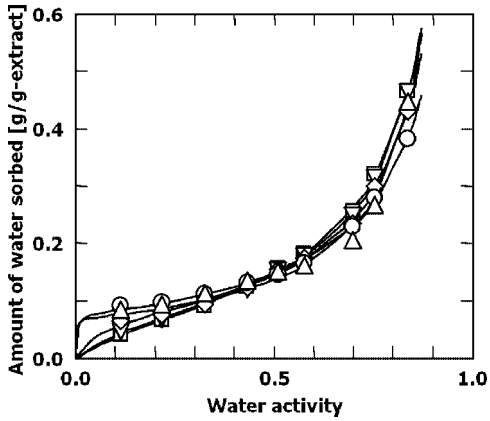

Figure 7. Water sorption isotherms at 30 °C for the extracts prepared at different temperatures. The symbols are the same as in Figure 6.

Table 1. Parameters Fitted to GAB Equation for the Bran Extracts Prepared at Various Treatment Temperatures

temperature for bran extract [°C]	$q_m \times 10^2$[g/g]	*C*	*K*
50	9.35	5.036	0.97
100	8.55	6.873	0.98
150	7.87	15.74	0.98
200	7.72	419.0	0.96
250	6.84	1095	1.01

product. The water content of a weakly hygroscopic product shows no or only a slight change in relation to the variation in the relative humidity, while a strong hygroscopic product shows a big difference in their water content (*41*).

Figure 7 shows the water sorption isotherms at 30 °C for the bran extracts prepared at various temperatures. The experimental data were fitted to the GAB equation. The estimated q_m, *C*, and *K* values for each sorption isotherm are listed in **Table 1**. The q_m values of the bran extracts prepared at 50 to 250 °C were close to each other and ranged from 0.0935 to 0.0684 g of H_2O/g of bran extract. The *C* values increased with increasing treatment temperature. The *K* values of all the bran extracts were close to 1, which were consistent with the typical value of hygroscopic products such as dried fruits and chocolate powder (*42*–*44*). As inferred from the *C* values, all the bran extracts would be classified as the type II sigmoid shape, which is common for dehydrated foods (*45*). However, the sorption isotherms of the bran extracts prepared at 50 and 100 °C were close to the type III J shape. The J shape profile represents the low content of the biopolymers leading to a low water sorption at low water activities and a relatively high content of low-molecular-mass sugars, which result in the prevailing effect of the solute–solvent interactions in association with the dissolution of low-molecular-mass sugars (*42*, *43*, *46*). The characteristics of the sorption isotherms of the bran extracts prepared at 50 and 100 °C implied that they contained a relatively low content of high-molecular-mass substances and a predominant amount of water soluble solutes such as sugars.

For the bran extracts prepared at 150 to 250 °C, the amount of water sorbed at low water activities gradually increased. Thus, the profiles of the sorption isotherms were closer to the sigmoid shape than the J shape. This result indicated an increase in the content of the high-molecular-mass substances in the bran extracts, which were also in accordance with the result from the gel permeation chromatography.

Although all the substances in the bran extracts had dissolved in the water after the subcritical water treatment, they could not completely rehydrate in the water after drying. The reasons for this may be explained by the fact that some of the substances were water-insoluble, such as phenolic substances, browning reaction products, and the high-molecular-mass substances of proteins and carbohydrates. After drying, the active points for water binding would likely be modified, which resulted in the inaccessibility of the water molecules during the dampening process (*46*, *47*). A notable aspect of heterogeneous systems was visually observed at the equilibrium moisture content for the sorption isotherms of the bran extracts prepared at 150–250 °C. The different phases of the water-soluble and -insoluble materials at equilibrium were observed at the same water activity. The bran extracts prepared at 50 and 100 °C exhibited only the water-soluble phase at the high water activities. However, those prepared at 150 to 250 °C exhibited the two different phases of water-soluble and -insoluble materials. Consequently, the amount of water retained and water-binding capacity of each phase can vary depending upon the constituents of each phase. This may lead to crossing of the isotherms.

Although the high-molecular-mass substances of the bran extract prepared at 250 °C were completely degraded, its sorption isotherm also showed a high water content at low water activities in comparison to the other bran extracts. The reason for this would be explained by the fact that the degraded products from the high-molecular-mass substances were still oligosaccharides according to its gel permeation chromatogram. Moreover, crossing of its isotherm to the other isotherms was also observed at water activities higher than 0.5. This result would imply that degraded products from the high temperature treatment could interact with the water, which then leads to their dissolution.

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