Properties of Rice Stem Extracts Obtained by Using Subcritical Fluids

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Rice stems were subjected to a subcritical fluid treatment at 230 °C, using ethanol or acetone at a dilution of 0–100% in water. The obtained extracts were determined for their yield, carbohydrate content, phenolic content, DPPH radical scavenging ability, and color. The highest yield and carbohydrate content were achieved with the subcritical 20% (v/v) organic solvent, while the highest phenolic content was obtained with subcritical 80% (v/v) acetone. The highest radical scavenging ability was achieved with subcritical 60% (v/v) ethanol and 80% (v/v) acetone. The lightness of the extracts obtained with subcritical ethanol and acetone was negatively correlated with their radical scavenging ability (R = −0.85). The relationship between the lightness and phenolic content of the extracts was not significant, suggesting that other substances in the extract could also possess radical scavenging ability.

Key words: rice straw; subcritical fluid treatment; subcritical water

As one of the major cultivated plants, rice generates one million dry tons of rice straw. Rice straw is regarded as agricultural waste and is disposed of by open-field burning or soil incorporation.1,2 Rice straw has many potential uses, its three major constituents of cellulose, hemicellulose, and lignin3 offering potential use as sources for extracting carbohydrates and phenolic compounds. Our previous study has shown that these carbohydrate and phenolic compounds can be retrieved in a high yield from rice straw that has undergone a subcritical fluid treatment.4

Subcritical fluid extraction, also known as pressurized liquid extraction or accelerated solvent extraction, is a method employing a pressurized liquid extractant at a temperature above its boiling point at ambient temperature. The penetration of the extractant into the sample matrix can be enhanced by increasing the treatment temperature, resulting in a decrease in the extractant surface tension and viscosity.5 The process is called a subcritical water treatment or hot compressed water extraction when water is applied as the sole extractant. A subcritical water treatment takes advantage of its high ion product which is over 1 × 10−13 fold greater than that of water at room temperature.6 The high concentrations of hydronium and hydroxide ions endow subcritical water with the ability to hydrolyze hemicellulose, lignin, and the amorphous part of cellulose. The dielectric constant of subcritical water decreases to match that of a polar organic solvent by increasing the temperature,7 and a further decrease is achieved by adding an organic solvent, i.e., ethanol or acetone.8 It has been reported that the lignocellulosic materials submitted to a subcritical organic solvent produced a black liquor which had radical scavenging ability.9–11

Chiou et al. have reported that subcritical aqueous ethanol and acetone were effective for extracting carbohydrates and phenolic compounds from rice bran.12 We therefore investigated the effects of the ethanol or acetone content in the extractant on the carbohydrate and phenolic contents of a rice stem extract. The phenolic compounds in the rice stem extract were concluded in our previous study to be one of the sources of its radical scavenging ability.4 We investigated in this present study the other potential sources of radical scavenging ability in the extract by relating the color to the radical scavenging ability of the extract.

Materials and Methods

Materials. A cultivated rice straw (Oryza sativa) sample from Hyogo Prefecture, Japan, was sun-dried and then kept at 4 °C in a storage room. The stems were separated from the leaves and cut into 1-cm-long pieces. L-Ascorbic acid (>99.5% purity) was purchased from Nacalai Tesque (Kyoto, Japan), and gallic acid was from Sigma-Aldrich Japan (Tokyo, Japan). The Folin-Ciocalteu reagent was from ICN Biochemicals (Aurora, OH, USA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and all other chemicals of reagent grade were from Wako Pure Chemical Industries (Osaka, Japan). Distilled water was used in all the experiments.

Preparation of extract. The rice stems were subjected to a treatment using subcritical water, ethanol, acetone, or their mixtures. A 5-g sample of the rice stems and 55 mL of an extractant were added to a 117-mL SUS-316 stainless steel vessel (30 mm i.d. × 165.5 mm) made by Taiatsu Techno (Osaka, Japan), before the vessel was tightly closed. Although the pressure inside the vessel was not measured, it was estimated to range from 0.33 to 3.6 MPa at 120 °C to 260 °C from a calculation of the vapour pressure and the expansion of air in the head space. This range of pressure produced from the gas phase of the extractant during the treatment was enough for ethanol and acetone to maintain their liquid state at 230 °C which is below the respective critical temperatures of 241 °C and 235 °C for ethanol and acetone.13,14 The vessel was heated to 230 °C at a heating rate of 7.2 °C/min by using a 200 W mantle heater (Sogo Laboratory Glass Works, Kyoto, Japan). The extraction was stopped by immediately cooling down the vessel to room temperature by putting the vessel in an ice bath after reaching the desired temperature. Each crude extract was passed through a No. 2 Advantec filter paper (Toyo Roshi, Tokyo, Japan) to obtain a clarified extract. The extracts were stored in a refrigerator at 4 °C until being used for the analysis.

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Yield of the extract. An FDU-1200 freeze-dryer (Tokyo Rikakikai, Tokyo, Japan) was used to freeze-dry a 15-mL portion of the rice stem extract. The freeze-dried sample was then placed in a DN 400 hot-air oven (Yamato Scientific, Tokyo, Japan) at 105 °C for 3 h to ensure its dryness. The yield of the extract was calculated by dividing the weight of the dry solid extract by that of the dry rice stems.

Total carbohydrate content. The modified phenol-sulfuric acid method was employed to measure the total carbohydrate content of the extract.15) A 1-mL amount of the diluted extract, a 25-μL aliquot of an 80% (w/v) aqueous phenol solution, and 2.5 mL of sulfuric acid were added to a test tube and mixed well. The test tube was placed at room temperature for 10 min, and then put in a 30 °C water bath for another 10 min. The absorbance at 490 nm was measured with a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). The total carbohydrate content was calculated by using glucose as the standard.

Total phenolic content. A test tube was filled with 100 μL of an appropriately diluted extract, 400 μL of a freshly prepared Folin-Ciocalteu reagent, and 1 mL of 75 g/L of sodium carbonate.16,17) After adjusting the volume of the mixture to 5 mL by filling with distilled water, the test tube was placed in the dark at room temperature for 2 h to complete the reaction. The absorbance at 765 nm was then measured. The amount of the phenolic compounds in the extract was compared to gallic acid and is expressed as the gallic acid equivalent (mg of gallic acid/g of stems).

Radical scavenging ability. A mixture of 4 mL of the diluted extract and 1 mL of 0.5 mmol/L of DPPH in ethanol was loaded into a light-blocking black tube and then well agitated. The tube was left for 20 min at room temperature. The absorbance at 516 nm was measured to determine the remaining radical quantity. The percentage of the radical scavenging ability was calculated as follows:18)

\[ \text{Radical scavengingability} \% = \left( 1 - \frac{A - B + C}{A} \right) \times 100 \]  

(1)

where \( A \) is the initial absorbance of the DPPH solution, \( B \) is the absorbance of the mixture of the sample and DPPH solution, and \( C \) is the absorbance of the diluted sample without the DPPH solution. The radical scavenging ability is defined as the amount of the extract necessary to reduce the DPPH concentration by 50%. This was compared to l-ascorbic acid (VC) and is expressed in mmol VC/g of stems.

Color measurement. The color of the rice stem extracts was measured with an NF 333 colorimeter (Nippon Denshoku Industries, Tokyo, Japan). A 0.5-mL amount of the stem extract was loaded into a quartz cuvette (10 × 10 × 43 mm). A white color screen was immersed in the extract to reach a height of 5 mm above the cuvette bottom to reflect light back to its source. The loaded cuvette was then placed in an opaque measurement chamber. The measured color is reported according to the CIE color system of \( L^* \) (lightness), \( a^* \) (redness), and \( b^* \) (yellowness).

Statistical analysis. All the experiments were conducted in triplicate. The obtained results were analyzed by using Microsoft Excel 2010 for a two-way analysis of variance (ANOVA).

Results and Discussion

Yield and total carbohydrate content

Figure 1 shows the yield and total carbohydrate content of the extracts obtained with different ethanol or acetone concentrations. The extracts obtained by using subcritical ethanol/water and acetone/water mixtures demonstrated an exponential correlation between the yield and total carbohydrate content \( (R^2 = 0.93 \) and 0.95 for the respective extracts with water/ethanol and water/acetone mixtures) as shown in the inset to Fig. 1. This strong correlation suggests that most of the extracted substances were carbohydrates. Kadam et al. have reported that the rice straw consisted of approximately 24% hemicellulose which was considered to be the major source of carbohydrates in the extracts.23) The other components of the extracts have been reported to be protein and ash,19,20) although their contents were low and neglected in this present study. The yield and total carbohydrate content of the extracts obtained by using ethanol or acetone were not statistically different \( (p < 0.05) \). This statistical indifference would indicate that the extraction of carbohydrates from rice stems may have been governed by the water fraction in the extractant. It has been reported that the subcritical water treatment provided an effective means to hydrolyze hemicellulose, one of the major rice stem components.21–23) The yield and total carbohydrate content of the extracts using 20% ethanol or acetone were higher than those of the extracts obtained by using water, and then decreased with increasing concentration above 20%. Decomposition of the carbohydrates also occurred in parallel with extraction under the subcritical conditions.24) The rates of extraction and decomposition of carbohydrate were therefore balanced when the extractant with 20% (v/v) ethanol or acetone was used under the subcritical conditions, resulting in the maximum yield and total carbohydrate content.

A higher ethanol content in the extractant would require a longer treatment time in order to obtain a higher carbohydrate content. This was evident in our previous study, in which the highest carbohydrate content was obtained by a subcritical 50% (v/v) ethanol treatment at 230 °C for 5 min.25)

Total phenolic content and DPPH radical scavenging ability

Figure 2 shows a similar trend for the total phenolic content of the extracts obtained by using subcritical ethanol/water and acetone/water. The total phenolic content was linear up to 40% (v/v) ethanol or acetone in water. It then increased with increasing ethanol or acetone concentration up to 80%, but markedly
decreased thereafter. At a lower concentration of ethanol or acetone (0–40% (v/v)), the extracts obtained by using subcritical ethanol and acetone showed no difference in the total phenolic content. Differences in the total phenolic content between the extracts obtained by using subcritical ethanol and acetone were apparent at a higher concentration of ethanol or acetone (60–100% (v/v)). The extracts obtained by using subcritical acetone showed a higher total phenolic content than those obtained by using subcritical ethanol. The major phenolic compounds in rice stems were p-coumaric acid, p-hydroxybenzoic acid, ferulic acid, and vanillic acid, which possess moderate polarity. It has been reported that the less polar phenolic compounds were more readily dissolved in a less polar extractant. A higher total phenolic content could therefore be obtained in acetone which is less polar than ethanol.

The DPPH radical scavenging ability of the extracts is shown in Fig. 2. It slightly decreased with increasing addition of ethanol or acetone up to 20% (v/v). This decrease was followed by a progressive increase in the 40–60% (v/v) ethanol or acetone concentration range and then by a decrease in the 80–100% (v/v) ethanol or acetone concentration range.

The radical scavenging ability of a plant extract is related to its phenolic content. Figure 3 shows two curves obtained from the relationship between the total phenolic content and DPPH radical scavenging ability; one for the extracts obtained by the subcritical aqueous ethanol treatment, and the other for the extracts obtained by the subcritical aqueous acetone treatment. These data suggest that one of the sources for the antioxidative ability of the extracts obtained by using subcritical aqueous ethanol or acetone were phenolic compounds. Excluding the extracts obtained by using subcritical 20% and 80% (v/v) ethanol, there was a strong exponential correlation ($R^2 = 0.98$) between the total phenolic content and the DPPH radical scavenging ability of the extracts obtained by using subcritical ethanol/water. The positions below the curve of the subcritical 20% and 80% ethanol extracts may have been due to the precipitation of hydrophobic substances. A small portion of ethanol or water would have caused the hydrophobic substances to become supersaturated and be subsequently precipitated. This precipitation also occurred in the extracts obtained by using subcritical acetone. However, the total phenolic content of the extracts obtained by using subcritical aqueous acetone still showed a strong exponential correlation to the DPPH radical scavenging ability ($R^2 = 0.92$). Moreover, the curve for the extracts obtained by the subcritical aqueous ethanol treatment was positioned higher than that for the extracts obtained by the subcritical aqueous acetone treatment, indicating that ethanol was a better solvent to extract phenolic compounds with more effective antioxidative ability.

**Color of the extracts**

All the color parameters ($L^*$, $a^*$, and $b^*$) are in the positive region, indicating that the extracts were yellow-orange in color (Fig. 4). A single logarithmic curve ($R^2 = 0.93$) was empirically obtained from the relationship between the lightness and yellowness of the extracts obtained by using subcritical ethanol/water and acetone/water. This would mean that extracts with a similar color could be obtained from the rice stems by treating either with subcritical aqueous ethanol or acetone. The redness of the extracts, however, did not show any relation to the lightness of the extracts.

The relationship between the lightness of the extracts and the DPPH radical scavenging ability is shown in Fig. 5. The lightness of the extracts had a negative linear correlation with the DPPH radical scavenging ability ($R = -0.85$). This negative correlation would imply that the DPPH radical scavenging ability increased with darkening of the rice stem extract. The darkness of the extract was due to the degradation products of hemicellulose and lignin that had been rendered soluble in the extractant during the extraction process. The lightness did not show any correlation with the total phenolic
Conclusions

The use of different organic solvents, which were ethanol and acetone in this study, resulted in a non-statistical difference in the total carbohydrate content of the extract from rice stems by a subcritical fluid treatment. This would suggest that extracting the carbohydrate under subcritical conditions might not be related to the type of organic solvent. A higher total phenolic content and DPPH radical scavenging ability were achieved with the extracts obtained by the treating with subcritical 80% (v/v) acetone. The highest DPPH radical scavenging ability was found in the extracts obtained by using subcritical 60% (v/v) ethanol and 80% (v/v) acetone. The DPPH radical scavenging ability could be related to the darkness of the extracts. This may indicate that the source of the antioxidative ability could be the degradation products and browning reaction products of hemicellulose, in addition to the phenolic compounds. Measuring the color, which is relatively easy, could be used to quantify the DPPH ability of such an extract according to our study. Further study might be needed to understand the relationship involved.

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References