Type of article: Original research paper

Title: Two-step hydrolysis of rice (*Oryza sativa*) husk as treated by semi-flow hot-compressed water

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Abstract

Two-step hydrolysis of husk obtained from rice (*Oryza sativa*) was investigated as one of the monocotyledonous angiosperms under the semi-flow hot-compressed water treatment at 230°C/10MPa/15min (1st stage) and 270°C/10MPa/30min (2nd stage). Prior to the hot-compressed water treatment, cold-water extraction at 20°C/10MPa/30min was performed. It was found that some inorganic constituents and free neutral sugars not being chemically bonded with the plant cell wall were recovered in the cold-water extracts. In the 1st stage, hemicelluloses and pectin were selectively hydrolyzed, as well as lignin being partially decomposed. In addition, protein was found to some extent to be hydrolyzed by the hot-compressed water treatment and various amino acids to form the protein of rice husk were identified. Hydrolysis of cellulose was, however, observed in the 2nd stage. Some additional decomposition of lignin and protein was revealed at this stage as well. In total, 96.1% of oven-dried extractives-free rice husk sample could be solubilized into cold and hot-compressed water. Various products in the water-soluble portion were primarily recovered as saccharides, which were partially isomerized and then dehydrated and fragmented. The 3.9% of residue after the treatment was composed mainly of lignin and a trace of silica.

Keywords:

Cellulose; Hemicellulose; Hot-compressed water; Hydrolysis; Lignin; Rice husk

Abbreviations:

CE, capillary electrophoresis; DP, degree of polymerization; EDX, energy-dispersive X-ray; GC-MS, gas chromatography-mass spectrometry; HPAEC, high-performance anion-exchange chromatography; HPLC, high-performance liquid chromatography.

1. Introduction

Rice (*Oryza sativa*) is the third most important grain crop in the world behind sugarcane and maize in terms of their total production (FAO, 2013). According to the FAO statistics, world annual production of paddy rice (husk + bran + starchy endosperm) in 2011 was about 720×10^6 million tons. It gives an estimation of about 140×10^6 million tons of rice husk produced per year globally (International Rice Research Institute, 2013). Removed in the rice refining process, the husk is undeniably considered to be a problem as an agricultural waste, even though some extent of husks is used, mainly as agricultural materials such as cattle feed.

From a taxonomic viewpoint, rice belongs to the grasses (Gramineae), monocotyledonous angiosperms (monocots). It is considered as a non-woody plant because of its difference in anatomy and lack of vascular cambium. Rice husk, however, contains a high percentage of organic substances, as do other lignocelluloses. It predominantly contains cellulose, hemicelluloses and lignin with some amounts of proteins, starch, extractives and inorganics (Rabemanolontsoa et al., 2011). Therefore, it is recognized as a potential source of bioenergy and organic biochemicals. In an attempt to utilize it, recently, several studies on hydrolysis of rice husk under hot-compressed water conditions have been done (Chareonlimkun et al., 2010; Mochidzuki et al., 2003; Vegas et al., 2008; 2004; Yu et al., 2008; Zhang et al., 2010). In addition, the high content of silicon, approximately 15 – 20% as SiO_2, is considered as a potential feature of rice husk (Chandrasekhar et al., 2003; Liou, 2004; Mochidzuki et al., 2001).

The treatment of biomass with hot-compressed water has a long tradition, mostly as a pretreatment method to improve dissolved pulp production (Al-Dajani and Tschirner, 2010), separation of hemicelluloses and lignin from biomass (Hasegawa et al., 2004), or enzymatic hydrolysis of biomass (Cara et al., 2008; Liu and Wyman, 2005; Mosier et al., 2005), and also aiming at the production of chemicals (biorefinery) with water under subcritical and supercritical conditions (Ando et al., 2000; Kabyemela et al., 1997a; Liu, 2010; Mok and Antal, 1992; Sasaki et al., 2002; Yu et al., 2008). The process is termed hydrothermolysis, if

high temperatures (and necessarily high pressures) are applied. Early efforts with this regards are summarized by Bonn et al. (1983).

In our previous works (Lu et al., 2009; Ogura et al., 2013; Phaiboonsilpa et al., 2011, 2010), a two-step hydrolysis of various lignocelluloses has been reported, in the course of which the samples are treated in a semi-flow system with hot-compressed water. Four lignocellulose samples from taxonomically different plant species have been studied, i.e., (1) Japanese cedar (*Cryptomeria japonica*) as one of the softwoods, gymnosperms, (2) Japanese beech (*Fagus crenata*) as one of the hardwoods, dicotyledonous angiosperms, (3) frond of nipa (*Nypa fruticans*) as one of the palms (Arecaceae), monocotyledonous angiosperms, and (4) straw of rice, as one of the grasses (Gramineae), monocotyledonous angiosperms. It was elucidated that hemicelluloses and cellulose were separately hydrolyzed in the 1st and 2nd stages of the treatment, respectively, while lignin was partially decomposed, mainly in the 1st stage. Various hydrolyzed (only mildly changed) and more or less heavily decomposed substances from the plant cell wall were obtained and identified.

In the present study, the two-step hydrolysis procedure was applied for rice husk to gain insights into its decomposition behavior in hot-compressed water. Qualitative and quantitative analyses were performed on the various products including amino acids liberated by the hydrolysis of protein in rice husk. Chemical conversion of rice husk under the treatment conditions was, thus, discussed.

2. Material and methods

2.1 Sample preparation

Husk was obtained from rice (*Oryza sativa*) collected from Aichi Prefecture, Japan. Detailed information about age, sampling location and time, storage condition before and during delivery to the laboratory was described in the previous study done by Rabemanolontsoa et al. (2011). The husk was pulverized with a Wiley mill (1029-C, Yoshida Seikakusho Co., Ltd.) and sieved to the size < 1 mm. The fines (< 150 μm) were rejected.

The size-screened samples were then Soxhlet-extracted with acetone until the solvent was clear of any color according to Tappi Standard T204 om-88 (1988). Prior to use in all experiments, the extractives-free sample of rice husk was dried at 105°C for 6 h and kept in a desiccator. All chemicals were of reagent grade and used without purification.

Chemical composition of the rice husk used in this study is 36.5, 17.5, 24.4, 1.7, 0.2, and 17.8 wt% on an extractives-free basis for cellulose, hemicelluloses, lignin, protein, starch, and inorganic constituents, respectively. The methods of quantitative chemical analysis are described by Rabemanolontsoa et al. (2011). Protein content was quantified by the Kjeldahl method using a nitrogen factor of 6.25 (AOAC Official Method, 2001; Thiex et al., 2002), and starch content through colorimetry according to Humphreys and Kelley (1961). Its inorganic constituents were quantified by incinerating the sample into ash in a muffle furnace at 600ºC for 4 h.

2.2 Hot-compressed water treatment and its fractionation

The semi-flow system and its operational procedures were described by Lu et al. (2009). In brief, approximately 0.5 g of oven-dried rice husk sample was placed into the reaction vessel and treated with cold water (20°C/10MPa/30min) in the semi-flow manner, followed by two-step semi-flow hot-compressed water at 1^{st} stage, 230°C/10MPa/15min, and 2^{nd} stage, 270°C/10MPa/30min. The same fractionation process as our previous works was applied to the rice husk (Phaiboonsilpa et al., 2011). Solubles in cold and hot-compressed water were collected by the fraction collector every 1 min. Soluble portion in hot-compressed water was left at the ambient temperature and under atmospheric pressure for 12 h; the liquid was then filtrated over a 0.2-μm membrane prior to subsequent analyses. The solid residue was oven-dried and analyzed.

2.3 Analysis of products

The water-soluble portion was analyzed by high-performance anion-exchange chromatography (HPAEC), high-performance liquid chromatography (HPLC), gas

chromatography-mass spectrometry (GC-MS), and capillary electrophoresis (CE) as described in detail by Lu et al. (2009) and Phaiboonsilpa et al. (2010). Post-hydrolysis by dilute sulfuric acid followed by HPAEC analysis was performed to estimate all recovered oligosaccharides in the water-soluble portion in terms of acid-hydrolyzed monosaccharides (Yang and Wyman, 2008). The product percentages shown in Fig. 1 through Fig. 6, presented on oven-dried weight basis of the extractives-free sample, are calculated from the chromatogram peak areas of the HPAEC, HPLC, GC-MS, and CE.

As for amino acid analysis, the water-soluble portion was first derivatized with phenylisothiocyanate according to the described method (Rutherfurd and Gilani, 2009), followed by HPLC analysis. Analytical conditions are Wakosil-PTC (4mmX200mm) column, binary buffers (purchased from Wako Pure Chemical Industries, Ltd.) with a linear-gradient flow, total flow-rate 1.0 ml/min, oven temp 40ºC, and UV detector at 254 nm. Acid hydrolysis of the water-soluble portion by 6M HCl acid at 110°C for 24 h under N_2 atmosphere in a closed ampule was performed to estimate all recovered amino acids, existing in polypeptides and/or oligomeric form associated with saccharides, in terms of acid-hydrolyzed monomeric amino acids. This acid hydrolysis method was also applied to the solid residue left after the treatment to know its amino acid composition.

Ashes (obtained as described above) were characterized by means of energy-dispersive X-ray (EDX) spectroscopy. Scanning electron microscope (SEM, JSM-5800, JEOL Ltd.) equipped with an EDX spectroscopic instrument (EDAX Corp., Pheonix) was employed at an accelerating voltage of 15 kV (Fig. 7).

3. Results and discussion

3.1 Free sugars and inorganics in cold-water extracts

As treated by cold water in the semi-flow system at 20°C/10MPa/30min, free neutral sugars and inorganic constituents were recovered from rice husk as the cold-water extracts. Figure 1 shows the temperature profile of the treatment and the yields of the free sugars − glucose, arabinose, and xylose − in cold water (-30 min to -10 min). Obviously, glucose was

the dominant free sugar, followed by arabinose and xylose. In total, 0.01% of these sugars were obtained.

Around 0.5% of inorganic contents in rice husk could be recovered in the cold-water extracts (Table 1), while 12.5% was dissolved in the 1st stage and 4.6% in the 2nd stage hot-compressed water treatment. The rest of 0.2% was left in the solid residue. Accordingly, the balance of total inorganic components (0.5 + 12.5 + 4.6 + 0.2 = 17.8%) was satisfactory with 100% recovery rate.

In rice straw (Ogura et al., 2013), however, larger amounts of free sugars (0.03%) and inorganic constituents (2.8%) were found in the cold-water extracts. This recovery was more pronounced in the study of nipa frond (Phaiboonsilpa et al., 2011). It was reported that 1.5% of free sugars and 7.4% of inorganic constituents could be achieved. These results clearly reflect the effects of differences in morphological parts and characteristics of lignocelluloses on their chemical compositions and changes under the treatment conditions applied.

3.2 Hydrolysis of major cell wall components

The two-step hot-compressed water treatment (1st stage, 230°C/10MPa/15min and 2nd stage, 270°C/10MPa/30min) in a semi-flow system, liquefied 96.1% of the rice husk. The solid residue left (3.9%) consists mainly of 2.9% lignin with 0.8% incompletely-hydrolyzed cellulose and 0.2% inorganics.

The following xylo-saccharides were obtained in the 1st stage (Fig. 2): xylose and xylo-oligosaccharides, such as xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose, and the molecules with higher degree of polymerization (DP). Moreover, arabinose, acetic acid, glucuronic acid, methanol and galacturonic acid were detected. These products are possibly from acetyl-methylglucuronoarabinoxylan, which is the major hemicellulose found in monocotyledonous angiosperms (Scheller and Ulvskov, 2010; Suzuki et al., 1998), while galacturonic acid is from pectin (O'neill et al., 1990). In addition, hydrolyzed monomeric guaiacyl, syringyl and *p*-hydroxyphenyl units of lignin − such as coniferyl, sinapyl and *p*-coumaryl alcohols − were obtained in this stage. It was elucidated that ferulic acid, which is

known as a characteristic component covalently cross-linked between hemicelluloses and lignin in monocotyledonous plant cell wall through ester and ether linkages, respectively, (Buranov and Mazza, 2008; Higuchi et al., 1967a; Iiyama and Lam, 2001) was also detected. These similar hydrolyzed products were observed in our previous study on rice straw (Ogura et al., 2013).

As for glucose and cello-oligosaccharides − such as cellobiose, cellotriose, etc. including the fragments with higher DP − were produced throughout the whole 60 min of the two-step treatment (Fig. 2). Products from the 1st stage (1 − 25 min) were derived perhaps from glucomannan in hemicelluloses and para-crystalline cellulose (with disordered crystallinity), while the rest in the 2nd stage (25 − 60 min) from crystalline cellulose.

The production trends of mono-saccharides are displayed in Fig. 1. All hemicellulose-derived mono-saccharides (xylose, arabinose, galactose, rhamnose, and glucose) arise in the 1st stage, while glucose set free in the 2nd stage is from the hydrolysis of cellulose. It should be noted that a small peak of glucose after 0 − 5 min is also observed. This might be attributed to the hydrolysis of starch to glucose at the beginning of this stage, where the corresponding temperatures are 180 – 210°C. Even though this temperature range is relatively low for the starch hydrolysis as reported by Miyazawa et al. (2008), differences in starting materials, reactor characteristics, and treatment conditions might possibly cause a variation. Other oligosaccharides from starch such as maltose were, however, not detected.

Arabinan units are hydrolyzed and recovered as arabinose obviously faster than other mono-saccharides. Similar results were also observed in our previous studies (Lu et al., 2009; Ogura et al., 2013; Phaiboonsilpa et al., 2011, 2010). The relatively high susceptibility of arabinose in wood hemicelluloses to acid hydrolysis is well known (Fengel and Wegener, 1984; Rydholm, 1965; Sano et al., 1989). Fructose and mannose were formed as isomerized compounds of glucose in the 2nd stage (25 − 60 min) as reported previously (Lu et al., 2009; Ogura et al., 2013; Phaiboonsilpa et al., 2011, 2010).

3.3 Degradation products from hemicelluloses and cellulose

The yields of levoglucosan, 5-HMF, and furfural (Fig. 3) as a function of treatment time can be clearly interpreted. As hemicelluloses in rice husk are mainly composed of pentoses, furfural was predominantly produced from xylose by elimination of 3 mol water in the 1st stage. A certain production of 5-HMF at this stage can be explained by the decomposition of glucose obtained from hydrolysis of glucomannan and para-crystalline cellulose. In the 2nd stage, on the other hand, the yields of furfural and 5-HMF increased due to more severe conditions. Levoglucosan as a mono-dehydrated glucose was detected exclusively in the 2nd stage. Similar findings were reported by Lu et al. (2009), Ogura et al. (2013), and Phaiboonsilpa et al. (2011).

As for the heavily fragmented compounds, Fig. 4 shows that methylglyoxal and glycolaldehyde were produced in both 1st and 2nd stages, while erythrose was formed in the 2nd stage only. In the 1st stage, it is likely that pentoses such as xylose and arabinose from hemicelluloses would be decomposed to glycolaldehyde and glyceraldehyde, and then glyceraldehyde would be dehydrated to methylglyoxal as observed in glyceraldehyde pathway of hexose fragmentation (Kabyemela et al. 1997b).

In the 2nd stage, glycolaldehyde and erythrose were formed via retro-aldol condensation in the glycolaldehyde/erythrose pathway (Kabyemela et al., 1999, 1997c), while methylglyoxal arises probably via the glyceraldehyde/dihydroxyacetone pathway of hexose fragmentation (Kabyemela et al., 1999; Watanabe et al., 2005). However, under the conditions applied, glyceraldehyde and its isomerized dihydroxyacetone as part of the glyceraldehyde pathway were not detected. This is probably due to the fast dehydration reaction of glyceraldehyde to methylglyoxal and/or organic acids (Kabyemela et al., 1997b). Similar decomposition and fragmented compounds were observed in our previous works (Lu et al., 2009; Ogura et al., 2013; Phaiboonsilpa et al., 2011).

3.4 Production of organic acids

As shown in Fig. 5, the produced organic acids are acetic, lactic, glycolic, and formic acids. The origin of acetic acid in the 1st stage is the acetyl groups of hemicelluloses. On the other hand, acetic acid from the 2nd stage must be a result of decomposition of cellulose and/or lignin (Lu et al., 2009; Yoshida et al., 2005). In addition, the production of lactic, glycolic, and formic acids, observed in both stages of the treatment, indicates that decomposition of dehydrated and fragmented compounds took place. Acrylic acid and levulinic acid were not detected.

3.5 Production of amino acids from protein

It was remarkably found that protein in rice husk was hydrolyzed and formed into various amino acids. Figure 6 depicts production of 5 amino acids such as – glutamic acid, aspartic acid, glycine, proline and alanine. As seen, they were recovered mainly in the 1st stage, whereas some extents of the amino acids were additionally produced in the 2nd stage. After the hot-compressed water treatment, traces of glycine, histidine, arginine, alanine and proline were found to remain in the residue of rice husk (Table 2).

These results are in good agreement with a structure of plant cell wall protein. It was reported that in addition to the protein-protein (or protein-phenolic-protein) cross-links, the cell wall protein appears to be crossed-linked with pectic substances, and may have sites for lignification (Higuchi et al., 1967b; Qi et al, 1995; Whitmore, 1982). Thus, amino acids were mainly liberated in the 1st stage, where hemicelluloses, pectin and lignin being hydrolyzed. Higher treatment temperature in the 2nd stage allowed some additional protein which resides in relatively high resistant location to hydrolyze. The remaining protein might be encrusted by lignin with a number of condensed-type linkages so that it would not be fractionated and eventually left over in the residue.

Although relatively low yields of amino acids were observed, after acid hydrolysis of the water-soluble portion by 6M HCl acid at 110°C for 24 h under N_2 atmosphere in a closed ampule followed by amino acid analysis, recovery of additional 13 amino acids and increase in all amino acid yields could be obtained, as shown in Table 2. Those are hydroxyproline,

serine, histidine, arginine, threonine, tyrosine, valine, methionine, cysteine, isoleucine, leucine, phenylalanine and lysine. In total, 0.40% (= 0.3992% + 0.0015%) of 18 amino acids were recovered in hot-compressed water-soluble portion and residue of rice husk.

These results suggest that not only ether (R^1-O-R^2) and ester ($R^1-CO-O-R^2$) linkages in plant cell wall, but also the peptide bond ($R^1-CO-NH_2-R^2$) in protein could be hydrolyzed by water under the hot-compressed conditions applied. Nevertheless, its degree of hydrolysis was not enough to convert protein into monomeric amino acids. Therefore, the further acid hydrolysis was required after the hot-compressed water treatment to evaluate protein components.

As indicated by the mol% of amino acids found in acid-hydrolyzed sample (as depicted in parentheses, Table 2), the major amino acids are found to be proline (5.1×10^{-4} %), glycine (4.7×10^{-4} %), glutamic acid (3.8×10^{-4} %), aspartic acid (3.4×10^{-4} %) and alanine (3.3×10^{-4} %). The greater mole numbers of proline and glycine than the other amino acids can be ascribed to the structure of plant cell wall protein which is typically present in 5 forms including (1) proline-rich protein, (2) glycine-rich protein, (3) hydroxyproline-rich protein, (4) solanaceous lectin, and (5) arabinogalactan protein (Showalter, 1993; Sommer-Knudsen et al., 1998). The yield of glutamic acid, on the other hand, is also found to be relatively high. This might be due to an additional number from glutamine which was converted to glutamic acid during the HCl acid hydrolysis. It is the case for aspartic acid as well, which was interfered by the yield of asparagine. Similar findings of high recovery of glutamic acid and aspartic acid as well as alanine were observed in the study on maize silage by Phipps and Oldham (1979).

3.6 Inorganic constituents in the fractions

The EDX spectra of inorganic constituents dissolved in the cold-water extracts and two-step hot-compressed water-soluble portions, and that of the solid residue are presented in Fig. 7. In rice husk, the elements Na, Si, Cl, and K were detected. These elements are present as parts of salts in oxalates and carbonates, but they can also be bound to cell wall components such as carboxyl groups of hemicelluloses or pectic materials (Saka 2001). The

chlorine (Cl) is exclusively present in salts, as they can be simply removed by dissolving in cold water, while Na and K probably occur in both forms. On the other hand, Si is certainly part of silica. It was partially removed by hot-compressed water in the 1st and 2nd stage treatment and the rest remained in the solid residue.

3.7 Overall products from hemicelluloses, cellulose, lignin, and others

In Table 1, the results of this study are summarized. It is elucidated that the water-soluble portion contains 37.0% hydrolyzed products as various saccharides, uronic acids, methanol, and acetic acid. The quantification in detail is: 14.5% (= 13.8% + 0.7%) are from hemicelluloses and 22.5% (= 2.9% + 19.6%) are from cellulose. In addition, 21.5% lignin-derived products are obtained, mainly in oligomeric forms. As for the decomposed compounds, 5.9% including 2.1% from dehydrated compounds, 2.5% from fragmented compounds, and 1.3% from organic acids are realized. Moreover, 0.01% free sugars, 0.4% amino acids and 17.6% inorganic constituents were recovered. The rest 13.7% are unidentified products in the water-soluble portion.

Rice husk consists of 17.5% hemicelluloses, 36.5% cellulose, and 24.4% lignin. Hemicelluloses were hydrolyzed approximately to an extent of 82.9% (= (13.8 + 0.7) / 17.5 × 100%), cellulose to 61.6% (= (2.9 + 19.6) / 36.5 × 100%), and lignin to 88.1% (= 21.5 / 24.4 × 100%) in the two-step hot-compressed water treatment.

Although the percentages of hydrolyzed products from hemicelluloses and cellulose in rice husk were basically the same as the ones of rice straw reported previously by Ogura et al. (2013), the decomposed products from lignin were slightly higher in case of rice straw. On the other hand, more residue and larger lignin proportion in the residue of rice husk were obtained as treated under the same hot-compressed water conditions. A clear reason for this is not known. However, this might be due to the differences in its morphological parts used, and the lignin content which is somewhat greater in husk compared to the straw.

4. Conclusions

Hemicelluloses and crystalline cellulose of rice husk were hydrolyzed separately by a two-step hot-compressed water treatment in a semi-flow system. Lignin was partly decomposed into hot-compressed water. Galacturonic acid was detected from the hydrolysis of pectin. The production of *p*-coumaryl alcohol and ferulic acid evidently clarified the characteristics of lignin in monocotyledonous angiosperm plant species. Various amino acids, moreover, revealed the hydrolysis of rice husk protein and possibility to obtain additional products from lignocelluloses. Free neutral sugars soluble in cold-water extracts and inorganic constituents recovered in different fractions were also observed under the studied treatment conditions. The yields of products as a function of elution time permit the interpretation of the locations and connectivity of the molecules associated in the plant cell wall. A comparison of this present study with the previous works on Japanese cedar, Japanese beech, nipa frond, and rice straw emphasizes the inherent effects of native chemical compositions of plant cell wall on their chemical conversion behaviors. These lines of study would provide very useful information for a novel technology to efficiently utilize various kinds of lignocellulosics for biochemicals and biofuels.

Acknowledgements

Financial support from the Grant-in-Aid for Scientific Research (KAKENHI), Grant Number 23・01099, Japan Society for the Promotion of Science: JSPS (FYs 2011-12) and the Advanced Low Carbon Technology Research and Development Program (ALCA), Japan Science and Technology Agency: JST (FYs 2011-12) is greatly appreciated.

List of figure captions

Fig. 1 Mono-saccharides from rice husk as treated in a semi-flow reaction cell with cold water at 20°C/10MPa/30min followed by two-step hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min. Arrows indicate recovery of xylose, arabinose, and glucose in cold-water extracts. Inserted figure depicts the enlarged peaks of xylose, arabinose and glucose.

Fig. 2 Hydrolyzed products from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min. The inserted figure is the enlargements of glucuronic acid, methanol, galacturonic acid in the 1st stage.

Fig. 3 Dehydrated compounds from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

Fig. 4 Fragmented compounds from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

Fig. 5 Organic acids from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

Fig. 6 Amino acids from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

Fig. 7 Comparison of EDX spectra of inorganic constituents in ashes of rice husk, obtained in cold-water extraction, the 1st and 2nd stage hot-compressed water-soluble portions, as well as residue.

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Table 1 Summarized yields of products from rice husk as treated by semi-flow cold-water (20°C/10MPa/30min) followed by two-step semi-flow hot-compressed water treatments at 230°C/10MPa/15min and 270°C/10MPa/30min [a-e].

Products	Yield (wt% on oven-dried extractives-free basis)							Total
	Cold-water	1st Stage			2nd Stage			
	Extracts	Hemicelluloses	Cellulose	Lignin	Hemicelluloses	Cellulose	Lignin	
Free sugars	**0.01**	-	-	-	-	-	-	**0.01**
From hemicellulose and cellulose								
Σ	-	**13.8**	**2.9**	-	**0.7**	**19.6**	-	**37.0**
- Xylo-saccharides	-	12.20	-	-	0.70 [c]	-	-	12.90
- Arabinose	-	0.70	-	-	0.01 [c]	-	-	0.71
- Acetic acid	-	0.60	-	-	-	-	-	0.60
- Glucuronic acid	-	0.02	-	-	-	-	-	0.02
- Methanol	-	0.01	-	-	-	-	-	0.01
- Galactose	-	0.20	-	-	-	-	-	0.20
- Rhamnose	-	0.03	-	-	-	-	-	0.03
- Mannose	-	0.01	-	-	-	0.05 [d]	-	0.01
- Galacturonic acid	-	0.01 [a]	-	-	-	-	-	0.01
- Cello-saccharides	-	-	2.90 [b]	-	-	19.50	-	22.40
- Fructose	-	-	-	-	-	0.03 [d]	-	0.03
From lignin								
Σ	**-**	**-**	**-**	**17.7**	**-**	**-**	**3.8**	**21.5**
- Coniferyl alcohol	-	-	-	2.03	-	-	0.24	2.27
- Sinapyl alcohol	-	-	-	0.18	-	-	0.01	0.19
- *p*-Coumaryl alcohol	-	-	-	0.06	-	-	0.01	0.07
- Ferulic acid	-	-	-	0.17	-	-	0.01	0.18
- Dimeric, trimeric and oligomeric products	-	-	-	15.26	-	-	3.53	18.79
Dehydrated compounds								
Σ	**-**	**0.2**	**-**	**-**	**-**	**1.9**	**-**	**2.1**
- Levoglucosan	-	-	-	-	-	0.50	-	0.50
- 5-HMF	-	0.06	-	-	-	1.30	-	1.36
- Furfural	-	0.10	-	-	-	0.10	-	0.20
Fragmented compounds								
Σ	**-**	**1.6**	**-**	**-**	**-**	**0.9**	**-**	**2.5**
- Methylglyoxal	-	0.90	-	-	-	0.20	-	1.10
- Glycolaldehyde	-	0.70	-	-	-	0.20	-	0.90
- Erythrose	-	-	-	-	-	0.50	-	0.50
Organic acids								
Σ	**-**	**0.4**	**-**	**-**	**-**	**0.9**	**-**	**1.3**
- Acetic acid	-	-	-	-	-	0.20	-	0.20
- Lactic acid	-	0.10	-	-	-	0.20	-	0.30
- Glycolic acid	-	0.10	-	-	-	0.20	-	0.30
- Formic acid	-	0.20	-	-	-	0.30	-	0.50
Amino acids								
Σ	-	**0.4**	**-**	**-**	**-**	**0.0**	**-**	**0.4**
- Glutamic acid	-	0.02	-	-	-	0.00	-	0.02
- Aspartic acid	-	0.01	-	-	-	0.00	-	0.01
- Glycine	-	0.00	-	-	-	0.00	-	0.00
- Proline	-	0.00	-	-	-	0.00	-	0.00
- Alanine	-	0.00	-	-	-	0.00	-	0.00
- Oligomeric amino [e]	-	0.37	-	-	-	0.00	-	0.37
Inorganics	**0.5**	**12.5**	**-**	**-**	**-**	**4.6**	**-**	**17.6**
Total	**0.5**	**28.9**	**2.9**	**17.7**	**0.7**	**27.9**	**3.8**	**82.4**
Unknown								**13.7**
Residue								**3.9**

[a] Galacturonic acid from pectin; [b] Cello-saccharides from glucomannan and para-crystalline cellulose; [c] Xylo-saccharides and arabinose from hemicelluloses incompletely hydrolyzed in the 1st stage; [d] Mannose and fructose are considered as hydrolyzed products from cellulose; [e] Oligomeric amino acids are quantified from the yields of monomeric amino acids after HCl acid hydrolysis of hot-compressed water-soluble portion.

Table 2 Yields (wt% on oven-dried extractives-free basis) of amino acids in hot-compressed water-soluble portion of rice husk and its residue as treated by two-step semi-flow hot-compressed water treatments at 230°C/10MPa/15min and 270°C/10MPa/30min [a-c].

No.	Amino acid	Hot-compressed water-soluble			Residue
		As received sample [a]	Acid-hydrolyzed sample [b]		
1.	Glutamic acid	0.0154	0.0557	(3.4×10^{-4}) [c]	-
2.	Aspartic acid	0.0125	0.0449	(3.8×10^{-4})	-
3.	Hydroxyproline	-	0.0185	(1.4×10^{-4})	-
4.	Serine	-	0.0203	(1.9×10^{-4})	-
5.	Glycine	0.0003	0.0354	(4.7×10^{-4})	0.0004
6.	Histidine	-	0.0080	(0.5×10^{-4})	0.0001
7.	Arginine	-	0.0174	(1.0×10^{-4})	0.0001
8.	Threonine	-	0.0199	(1.7×10^{-4})	-
9.	Alanine	0.0002	0.0289	(3.3×10^{-4})	0.0004
10.	Proline	0.0003	0.0581	(5.1×10^{-4})	0.0005
11.	Tyrosine	-	0.0178	(1.0×10^{-4})	-
12.	Valine	-	0.0203	(1.7×10^{-4})	-
13.	Methionine	-	0.0051	(0.3×10^{-4})	-
14.	Cysteine	-	0.0010	(0.1×10^{-4})	-
15.	Isoleucine	-	0.0101	(0.8×10^{-4})	-
16.	Leucine	-	0.0150	(1.1×10^{-4})	-
17.	Phenylalanine	-	0.0193	(1.2×10^{-4})	-
18.	Lysine	-	0.0035	(0.2×10^{-4})	-
	Total	0.0287	0.3992		0.0015

[a] As received sample: the soluble portion was directly subjected to amino acid analysis; [b] Acid-hydrolyzed sample: the soluble portion was hydrolyzed by 6M HCl acid at 110°C for 24 h under N_2 atmosphere in a closed ampule prior to the amino acid analysis; [c] Numbers in parentheses indicate yields of amino acids in acid-hydrolyzed sample on mol% basis.

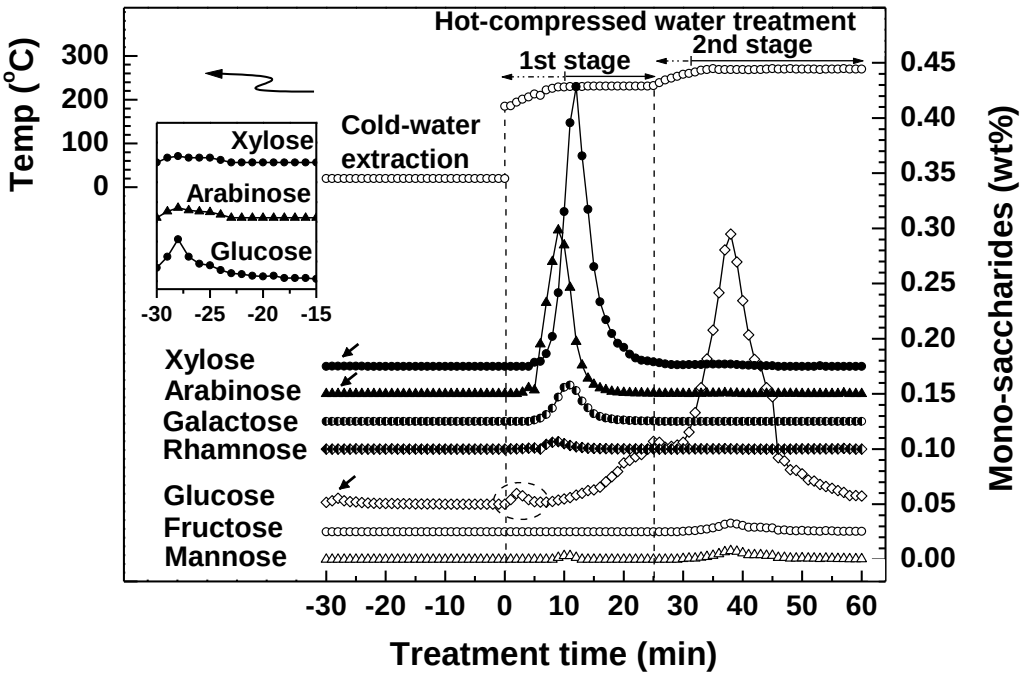

Fig. 1 Mono-saccharides from rice husk as treated in a semi-flow reaction vessel with cold water at 20°C/10MPa/30min followed by two-step hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min. Arrows indicate recovery of xylose, arabinose, and glucose in cold-water extracts. The inserted figure depicts the enlarged peaks of xylose, arabinose and glucose.

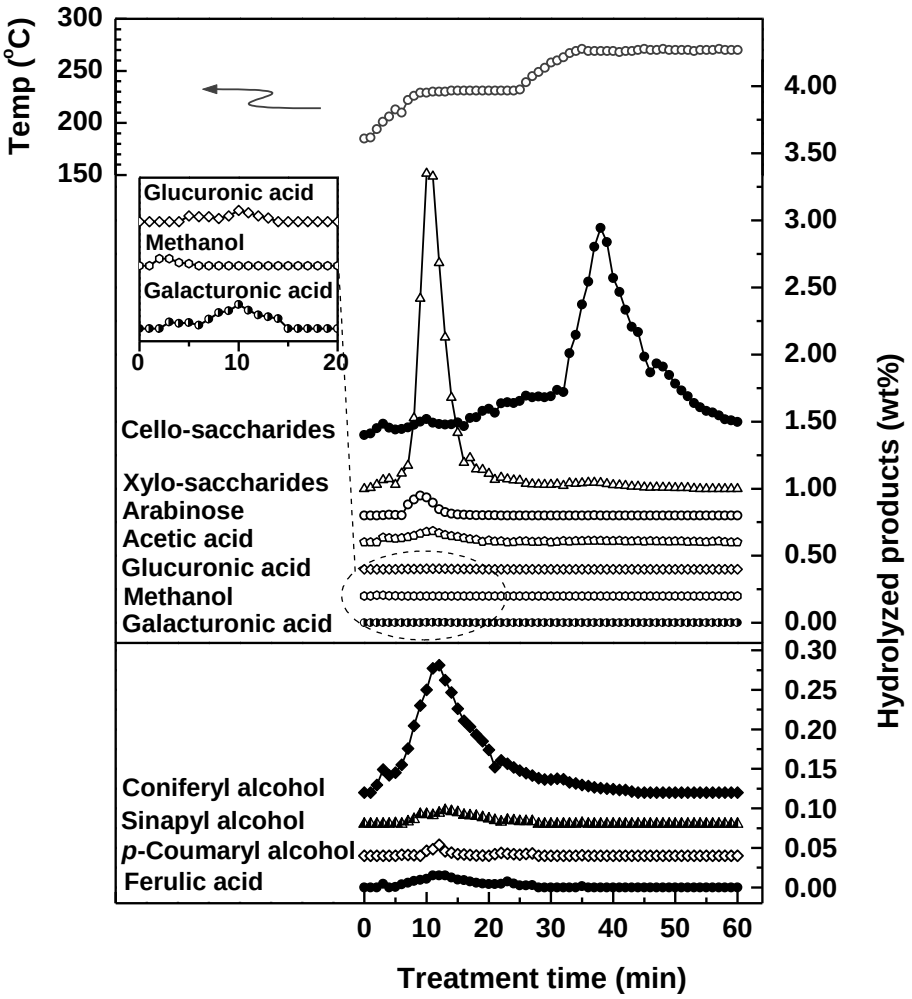

Fig. 2 Hydrolyzed products from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min. The inserted figure is the enlargements of glucuronic acid, methanol, galacturonic acid in the 1st stage.

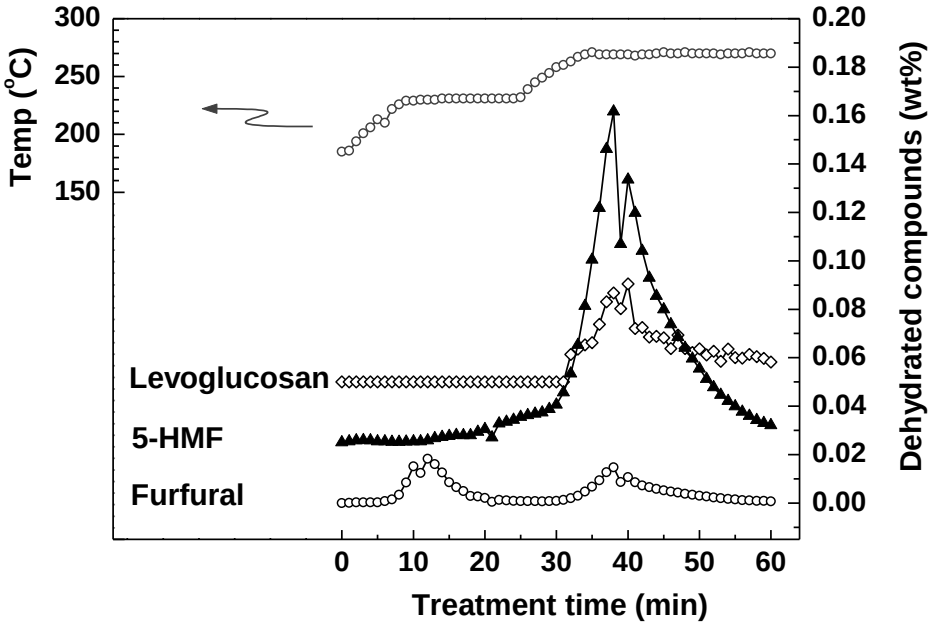

Fig. 3 Dehydrated compounds from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

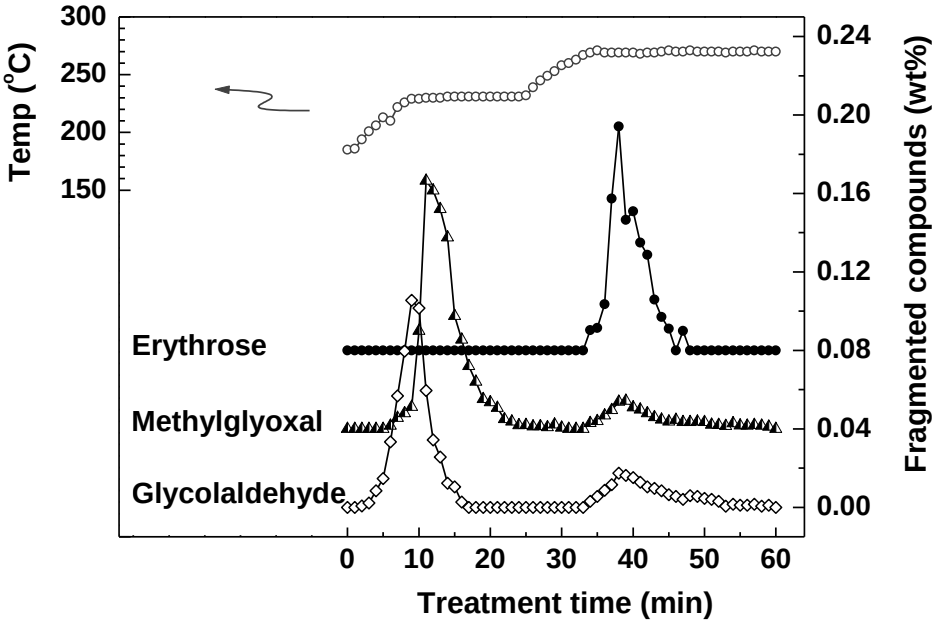

Fig. 4 Fragmented compounds from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

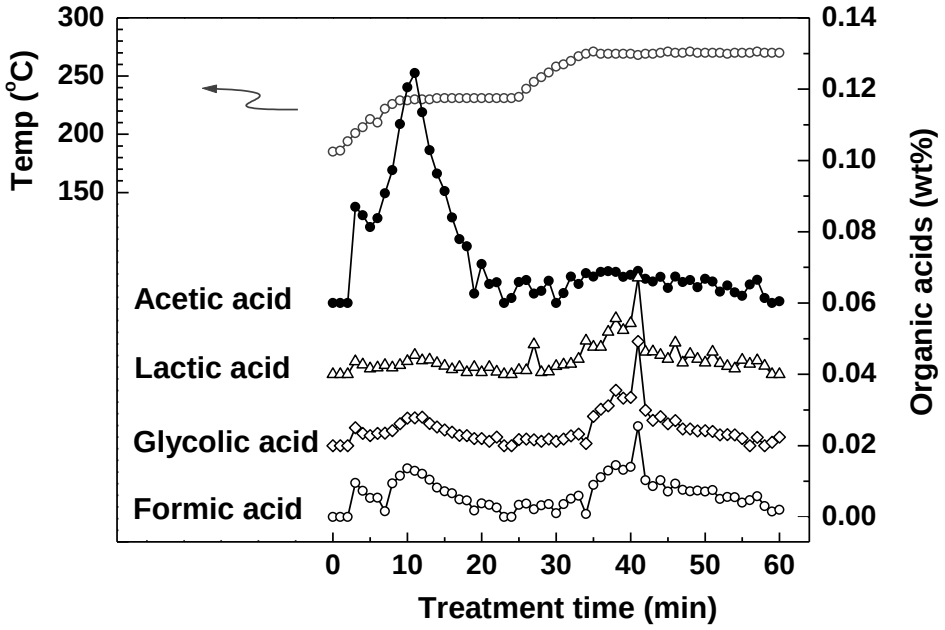

Fig. 5 Organic acids from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

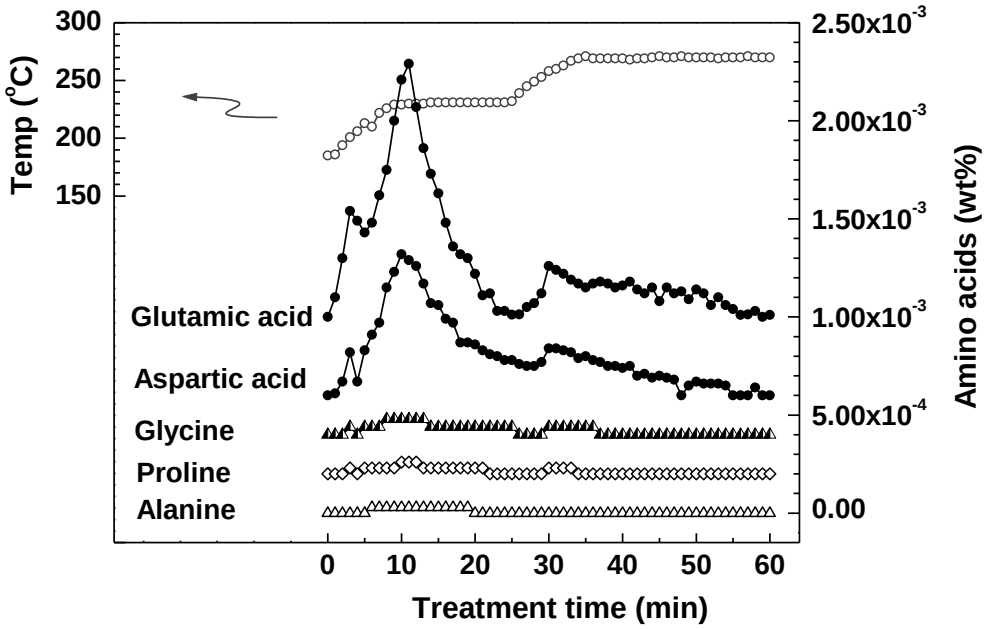

Fig. 6 Amino acids from rice husk as treated by two-step semi-flow hot-compressed water at 230°C/10MPa/15min and 270°C/10MPa/30min.

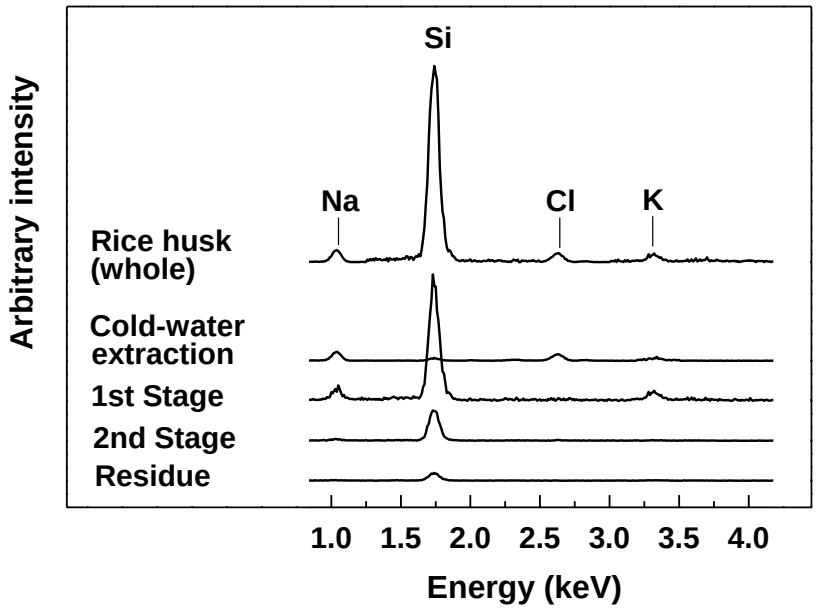

Fig. 7 Comparison of EDX spectra of inorganic constituents in ashes of rice husk, obtained in cold-water extraction, the 1st and 2nd stage hot-compressed water-soluble portions, as well as residue.