JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Detoxification of Rice Straw and Olive Tree Pruning Hemicellulosic Hydrolysates Employing *Saccharomyces cerevisiae* and Its Effect on the Ethanol Production by *Pichia stipitis*

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ABSTRACT: The aim of this work was to study the ability of *Saccharomyces cerevisiae* (baker's yeast) to metabolize a variety of aromatic compounds found in rice straw (RSHH) and olive tree pruning (OTHH) hemicellulosic hydrolysates, obtained by acid hydrolysis at different sugar and toxic compound concentrations. Initially, the hydrolysates were inoculated with *S. cerevisiae* (10 g L^{-1}) and incubated at 30 °C under agitation at 200 rpm for 6 h. The results showed that this yeast was able to utilize phenolic and furan compounds in both hemicellulose hydrolysates. Next, the treated hydrolysates were inoculated with *Pichia stipitis* NRRL Y-7124 to evaluate the effect of biotransformation of aromatic compounds on ethanol production, and better fermentation results were obtained in this case compared to untreated ones. The untreated hemicellulose hydrolysates were not able to be fermented when they were incubated with *Pichia stipitis*. However, in RSHH treated hydrolysates, ethanol ($Y_{P/S}$) and biomass ($Y_{X/S}$) yields and volumetric ethanol productivity (Q_P) were 0.17 g g^{-1}, 0.15 g g^{-1} and 0.09 g L^{-1} h^{-1}, respectively. The OTHH-treated hydrolysates showed less favorable results compared to RSHH, but the fermentation process was favored with regard to untreated hydrolysate. These results showed that the fermentation by *P. stipitis* in untreated hydrolysates was strongly inhibited by toxic compounds present in the media and that treatment with *S. cerevisiae* promoted a significant reduction in their toxicities.

KEYWORDS: *biological detoxification, biotransformation of the aromatic compounds, rice straw, olive tree pruning, hemicellulosic hydrolysates, Saccharomyces cerevisiae*

INTRODUCTION

Lignocellulosic biomasses such as rice straw and olive tree pruning are inexpensive sources of polysaccharides that can be degraded in monomeric sugars.[1,2] One of the most abundant lignocellulosic feedstocks in developing countries, including Brazil, is the straw of various cereals, and the rice straw is being reported as one of the greatest byproducts of agricultural activities.[3] The world production of rice crop for 2012 has reached a record of 488 million tons of grain, generating 680 million tons of straw distributed around the world, with a potential production of energy of 205 billion liters per year.[4] Debris from olive tree pruning represents a great volume of biomass in Spain, especially in Andalucia, where annual production reaches 3.9 million tons, varying between 1700 and 3000 kg/(ha year), depending on culture conditions.[2,5] Due to the large volumes of this residue and the environmental damage associated with this biomass, the possibility of valuing these byproducts from the agricultural industry has been suggested.[1,2]

The major organic constituents of lignocellulosic materials are cellulose (30−50%), hemicellulose (20−40%) and lignin (15−30%).[6] The hemicellulosic fraction of the residues can be recovered by dilute acid hydrolysis, generating mainly D-xylose monomers in the rice straw hemicellulosic hydrolysate[1] and a combination of D-xylose and D-glucose monomers in olive tree pruning hydrolysate as well.[2] Besides these monosaccharides, several inhibitory compounds are also formed during hydrolysis processes, including some products of sugar degradation (furfural and 5-hydroxymethylfurfural-5-HMF), phenolic compounds derived from lignin degradation, and acetic acid, derived from hemicellulosic structure.[1,2,7] The main phenolic compounds present in rice straw and olive tree pruning hemicellulosic hydrolysates are vanillin, vanillyl alcohol, vanillic acid, ferulic acid and coumaric acid.[2,8,9] In addition, the olive tree hemicellulosic hydrolysate may also contain others phenolic compounds such as oleuropein, tyrosol, hydroxytyrosol and elenolic acid, known to present antioxidant properties, and antimicrobial properties by denaturing proteins and inactivating enzymes.[9−12] The amount and type of toxic compounds generated depends on both severity pretreatment and biomass source, and their effects on *Pichia stipitis* fermentation have been studied.[8,13,14] For example, Díaz et al.[13] reported a decrease of 35% in ethanol volumetric production when *P. stipitis* CECT 1922 was cultivated in synthetic medium containing D-glucose (20.0 g L^{-1}) and D-xylose (15.0 g L^{-1}), and 2.0 g L^{-1} of furfural. Evaluating the ethanol production by *P. stipitis* CBS 5773 in sugar cane bagasse hemicellulosic hydrolysate, Roberto et al.[14] observed that furfural concentrations above 2.0 g L^{-1} inhibited cell growth. However, at concentrations below 0.5 g L^{-1}, the

Received: June 10, 2013
Revised: August 29, 2013
Accepted: August 30, 2013
Published: August 30, 2013

aldehyde acted as carbon source, with a positive effect on the cell growth.

It is important to identify the potential inhibitors present in the hemicellulose hydrolysates before proposing a method or a sequence of methods for detoxification. This knowledge not only helps to choose an efficient and low-cost treatment but also let to establish conditions for hydrolysis that minimize its formation.[7,8,15−17] Such strategies include physical, chemical and/or biological methods. Regarding to physical methods, the adsorption of inhibitors using activated carbon is effective in removing acetic acid and phenolic compounds, allowing better fermentation efficiency. However, these methods can remove substantial quantities of sugars in the medium by degradation.[7,15] Chemical detoxification methods, which are based on pH change, have also been extensively studied. In these methods, the reduction of the toxicity of the hydrolysates is related to precipitation of metal ions and/or decomposition of furans, which are instable under certain pH conditions. These methods alone do not provide satisfactory results and are often employed in combination with physical methods.[7,15,16]

The use of microorganisms and/or enzymes for detoxification of hydrolysates has been explored as a method able to overcome the toxicity of the hemicellulosic hydrolysates. In fact, studies using laccase to detoxify different hydrolysates, by oxidative polymerization of phenolic compounds, showed an increase in fermentation parameters compared with the untreated media.[15,17] Jonsson et al.,[17] evaluating fermentation of wood cellulosic hydrolysate by *S. cerevisiae*, and Chandel et al.,[15] fermenting hemicellulosic hydrolysate from sugar cane bagasse with *Candida shehatae* NCIM 3501, observed an increase on ethanol volumetric productivity (0.8−2.7 g L^{-1} h^{-1}) and xylitol (0.14−0.27 g L^{-1} h^{-1}), respectively. Using *Issatchenkia occidentalis* CCTCC M 206097 as detoxifying-agent and *Candida tropicalis* CCTCC M 205067 as fermentation-agent of hydrolysate from sugar cane bagasse hemicellulosic, Hou-Rui et al.[18] verified an increase of 50% xylitol volumetric productivity (1.03 for 2.01 g L^{-1} h^{-1}). However, *I. occidentalis* specie consumed D-xylose, the main sugar present in the hemicellulosic hydrolysate.[18,19]

In the present study, we propose the use of baker's yeast *Saccharomyces cerevisiae* to detoxify hemicellulosic hydrolysates from rice straw and olive tree pruning, since this microorganism is capable of converting furans and phenolic aldehydes into their corresponding alcohols, which are considered less toxic to the metabolism of various fermentative microorganisms, include *P. stipitis*.[20,21] In addition, *S. cerevisiae* does not consume D-xylose, the main sugar present in most plant biomass hemicellulose hydrolysates, which makes the advantageous process. The aim of this study is to contribute to the development of a biological detoxification procedure decreasing the toxicity of the hemicellulosic hydrolysates and to establish suitable conditions for the bioconversion of this hydrolysate fraction in ethanol.

MATERIALS AND METHODS

Raw Material. Rice straw was collected in Lorena, São Paulo state, Brazil. The material was sun-dried to approximately 10% moisture and milled to attain particles of about 1.0 cm in length and 1.0 mm in thickness. The olive tree pruning biomass consisted of leaves and branches (1:2 ratio) from trunks of olive trees and was collected in an olive groove situated in Jaén, Spain. The material was air-dried to 5% moisture, milled, screened to select the fraction of particles with diameter from 0.425 to 0.60 mm and homogenized in a single lot. The average chemical compositions of rice straw and olive tree pruning, determined according to the methodology previously described by Browning,[22] are shown in Table 1.

Table 1. Chemical Characterization of Rice Straw and Olive Tree Pruning Used in the Present Study

	content (% on dry matter)[a]	
component	rice straw	olive tree pruning
Cellulose	35.1 ± 0.6	24.9 ± 0.5
Hemicellulose	25.9 ± 0.5	15.3 ± 0.1
Acid-insoluble lignin	13.3 ± 1.9	17.6 ± 0.1
Acid-soluble lignin	5.1 ± 0.1	1.7 ± 0.1
Acetyl Groups	1.5 ± 0.1	2.0 ± 0.1
Ash	5.5 ± 0.1	3.4 ± 0.1

[a]Average of at least three determinations.

Hydrolysis Procedure. Rice straw hydrolysis experiments were carried out in a 350-L stainless steel reactor, at 120 °C, 50 rpm, during 30 min, and hydrolytic reaction of the olive tree pruning residue was carried out in autoclave, at 120 °C for 90 min. Both biomasses were impregnated with sulfuric acid solution at 1.0 and 2.0% w v^{-1} at solid/acid solution ration of 1:10, according to Roberto et al.[1] and Mateo et al.,[23] respectively. The slurries obtained after hydrolysis were separated by centrifugation (2000× *g*, 20 min) and the liquid phases (hemicellulosic hydrolysates) were concentrated under vacuum at 70 °C in order to obtain approximate concentrations of furans and phenolics compounds (6- and 3-fold increase of the rice straw and olive tree pruning hemicellulosic hydrolysates, respectively). The volume of the hemicellulosic hydrolysates obtained after hydrolysis-reaction was divided into 3 equal parts and concentrated under vacuum separately.

Hemicellulosic Hydrolysates Biodetoxification. The assays of biodetoxification were realized in triplicate. The pH of hemicellulosic hydrolysates was adjusted to 5.5 with NaOH (pellets), followed by centrifugation at 1000× *g* for 15 min to remove the insoluble materials. First, the liquors were treated with 10 g L^{-1} of lyophilized commercial *Saccharomyces cerevisiae* (Dr. Oetker). The yeast was previously hydrated in distilled water for 10 min at room temperature, with cellular viability greater than 85%. The biodetoxification experiments were performed in shaker at 30 °C, 200 rpm for 6 h, using 250 mL Erlenmeyer flasks filled with 50 mL of hemicellulosic hydrolysates.

***Pichia stipitis* Inoculum Preparation.** Cultures of the *Pichia stipitis* NRRLY-7124 were maintained on malt extract agar slants at 4 °C. The inoculum was prepared by transfer of yeast cells in the maintenance medium to 250 mL Erlenmeyer flasks containing 50 mL of the medium composed by (g L^{-1}): xylose (30.0), $MgSO_4 \cdot 7H_2O$ (1.0), $(NH_4)_2HPO_4$ (3.0), KH_2PO_4 (19.0) and yeast extract (3.0). The inoculated flasks were incubated in rotary shaker at 30 °C under stirring at 200 rpm for 24 h. Next, the cells were recovered by centrifugation (1100× *g* for 20 min) and resuspended in sterile distilled water in order to obtain the cell concentrated suspension which was used as inoculum.

Medium and Fermentation Conditions. Fermentations assays were realized in triplicate carried out in untreated and treated hemicellulosic hydrolysates. First, the pH of hemicellulosic hydrolysates was adjusted to 5.5 with NaOH (pellets). Next, the hydrolysates were centrifuged to remove solid residue. The hemicellulosic hydrolysate from rice straw was supplemented with yeast extract (3.0 g L^{-1}),[24] and the olive tree hydrolysate with (g L^{-1}): $MgSO_4 \cdot 7H_2O$ (1.0), $(NH_4)_2SO_4$ (3.0), KH_2PO_4 (2.0), peptone (3.6), and yeast extract (4.0).[23] The experiments were carried out in 125 mL Erlenmeyer flasks containing 50 mL of hemicellulosic hydrolysates and inoculated with 1 g L^{-1} of cells. The flasks were incubated in a rotator shaker at 30 °C, 200 rpm for 96 h.

Analytical Methods. Sugars, acetic acid, and ethanol concentrations were determined by high performance liquid chromatography (HPLC) in Waters (Milford, MA) equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column (300 × 7.8 mm)

Table 2. Concentration of Sugars and Toxic Compounds of the Rice Straw and Olive Tree Pruning Hemicellulosic Hydrolysates in Their Original and Concentrated Forms

	RSHH		OTHH	
concentration (g L^{-1})[a]	original	concentrated	original	concentrated
D-glucose	2.5 ± 0.1	14.4 ± 0.5	10.6 ± 0.2	33.8 ± 0.2
D-xylose	14.0 ± 0.6	87.2 ± 1.4	9.3 ± 0.2	28.8 ± 0.7
L-arabinose	2.4 ± 0.1	15.9 ± 0.8	3.8 ± 0.1	15.7 ± 0.2
Acetic acid	1.4 ± 0.1	1.8 ± 0.3	2.2 ± 0.1	4.0 ± 0.1
Furans[b]	0.21 ± 0.01	0.87 ± 0.03	0.74 ± 0.04	0.83 ± 0.03
Phenolics[c]	2.8 ± 0.1	10.5 ± 0.3	3.6 ± 0.2	9.3 ± 0.3

[a]Average of at least three determinations. [b]Martinez et al.[25] [c]Singleton et al.[26]

(Hercules, CA). Operation conditions included: temperature of 45 °C, 0.005 M sulfuric acid as eluent in a flow of 0.6 mL min^{-1} and sample volume of 20 μL. Dry-weight (g L^{-1}) calibration was determined by the absorbance of the cell suspension at a wavelength of 600 nm. Total furans were determined by spectrophotometric method described by Martinez et al.[25] The total content of phenolic compounds in hemicellulosic hydrolysates was estimated by the Folin-Ciocalteu modified method[26] using gallic or caffeic acids as standard. The ultraviolet-spectra (UV) of the hydrolysates were carried out in the range of 450−200 nm, with a pitch of 5 nm using quartz cuvettes. The spectrum determination was made with hydrolysate diluted with alkaline water (pH around 12) and distilled water used as blank (pH 12).

RESULTS AND DISCUSSION

Raw Materials Composition. The chemical composition and the percentage contents of rice straw and olive tree pruning employed in this study are presented in Table 1. It is found that among the structural components, cellulose corresponded to a greater portion of the material, and hemicellulose present in rice straw was 41% higher than that found for the olive tree pruning. It is also observed in Table 1 that the ash rice straw content is 40% higher than the olive tree pruning. This behavior was expected, since rice straw when compared with other lignocellulosic materials, straw ash has above. For example, the ash content present in malt bagasse and olive pruning, reported by Roberto et al.[1] and Mateo et al.,[27] were 11.4 and 3.8%, respectively. This peculiarity of rice straw can be explained by the fact that the material contains a high content of silicon dioxide (SiO_2), which confers high-level resistance against pathogenic microorganisms, however, complicates the process of degradation.[3,5]

When comparing the results of rice straw characterization in the present study with other works reported in the literature, it appears that the cellulose, hemicellulose and lignin contents are within the range of variation 35−43%, 23−26% and 13−25%, respectively. As for the ash content, the material of the present study showed a value much lower than other values reported in the literature (approximately 12%).[1,3,5,6,8] For the olive tree pruning, it is found that the levels of cellulose (25−31%), hemicellulose (13−23%) and lignin (18−24%) are also within the variation range reported in the literature.[2,13,23,27] The differences in the composition observed for this type of residue may be associated with the ratio of wood:sheet generated after the pruning process.[23]

Hemicellulosic Hydrolysates Composition. The composition of the rice straw (RSHH) and olive tree pruning (OTHH) hemicellulosic hydrolysates obtained by acid catalysis is showed in Table 2. As can be seen, the sugars and inhibitor compounds concentrations in hydrolysates varied with raw materials employed as well as the conditions that have been employed in the hydrolysis process.

Total sugar concentration presented in RSHH and OTHH after dilute acid treatment were of 18.9 and 23.7g L^{-1}, respectively. It is verified that D-xylose (14.0 g L^{-1}) as the highest concentration monomer in the RSHH. On the other hand, in OTHH both D-xylose (9.3 g L^{-1}) and D-glucose (10.6 g L^{-1}) were the major products obtained. In this hydrolysate, the D-glucose:D-xylose ratio may depend on the leaves and branches proportion present in the olive tree pruning. In fact, Mateo et al.[23] showed that the leaves and branches mixture of olive tree pruning contain a 1.3/1.0 hemicellulose/cellulose ration, while this fraction goes to 1.0/1.0 when olive tree pruning leaves free is used. The authors also found that in leaves of the olive tree pruning hemicellulosic fraction is twice than cellulose. In other work, Mateo et al.[27] show that higher D-glucose and D-xylose yields in olive tree pruning hemicellulosic acid hydrolysate, with leaves/branches proportion near to one, were 10.0 and 9.4% respectively, and obtained hydrolysis conditions were 190 °C, 0 min and 1% of H_2SO_4. These results are very similar to those obtained in present work.

Table 2 also shows that D-glucose concentration obtained in OTHH is 4.2 times greater than in RSHH. While D-xylose was the main monosaccharide in RSHH, and the pentoses proportion in this hydrolysate was 1.3 times higher than in OTHH. This can be justified because of the different nature of these biomasses. Besides sugar concentrations, Table 2 shows that the hydrolysates contain compounds potentially harmful to the fermentation process, such as acetic acid, furans and phenolic compounds. It is noticed that OTHH has higher concentrations of these toxic/inhibitory compounds. In both hemicellulosic hydrolysates, the total phenolic compound represents the main problem as its concentration is highest.

The concentration under vacuum process promoted proportional variations to the concentration factor only in the D-glucose and D-xylose levels, in both hemicellulosic hydrolysates (6.0 and 3.0 times for RSHH and OTHH, respectively), according to Table 2. The total fermentable sugars (D-glucose + D-xylose) concentrations were 101.6 and 62.6 g L^{-1} for RSHH and OTHH, respectively, while L-arabinose concentration in both hydrolysates was approximately 15.8 g L^{-1}. Regarding inhibitor compounds, it was observed that the concentration of acetic acid increased too much in OTHH, corresponding 81%. The increase in total furans was 76 and 11%, with about 0.85 g L^{-1} for both hydrolysates, and phenolic compounds levels, after the process of concentration, reached near 10.0 g L^{-1} in both concentrated hydrolysates. The nonproportional increase of these inhibitor compounds after concentration process can be explained by their different boiling points under vacuum. So

during concentration step, acetic acid and furfural get more easily volatilized.[28]

The presence of these toxic compounds in hemicellulosic hydrolysates pose problems for the bioconversion of sugars to ethanol by *Pichia stipitis*.[2,7,8] For this reason, the RSHH and OTHH were submitted to biodetoxification procedures employing *S. cerevisiae*, aiming towards a reduction of these compound concentrations, taking advantage of the very similar concentrations of total furans and phenolics in both concentrated hemicellulosic hydrolysates.

Biodetoxification of the Hemicellulosic Hydrolysates. The reduction percentages in sugars and inhibitors concentration present in hemicellulosic hydrolysates from rice straw and olive tree treated with *S. cerevisiae* are showed in Figure 1.

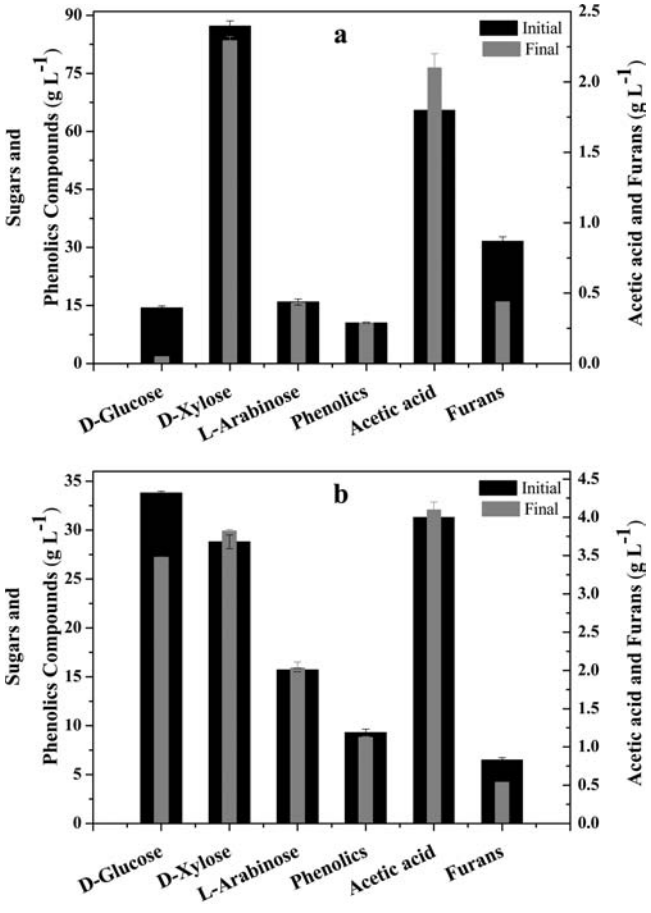

Figure 1. Sugars and inhibitor compounds concentrations in (a) RSSH and (b) OTHH, before (black) and after (gray) biotransformation treatment by *Saccharomyces cerevisiae*.

As can be seen, *S. cerevisiae* consumed 88 and 20% of the D-glucose, with ethanol production of 5.5 and 1.5 g L^{-1} in RSHH and OTHH, respectively. As expected, the pentoses were nonassimilated, in both hemicellulosic hydrolysates, due to a lack of the enzyme responsible for D-xylose and L-arabinose assimilation.[29]

It is verified also in Figure 1 that the acetic acid concentration increased slightly after detoxification process. This increase can be attributed to the reduction of the furans and phenols aldehydes to their respective alcohols by yeast *S. cerevisiae* These reactions are catalyzed by nonspecific reductase enzymes, such as alcohol dehydrogenase, which leads to an increase in intracellular acetaldehyde levels and subsequent oxidation of the aldehyde to acetic acid.[13,20,21]

Baker's yeast was able to reduce the total furans in RSSH and OTHH by 50 and 35%, with residual concentrations of 0.44 and 0.54 g L^{-1}, respectively. These results were expected, since yeast has metabolic capacity to reduce furan aldehydes to their corresponding alcohols.[30−35] However, it was observed no reduction in total phenolic compounds concentration in the RSHH (Figure 1a), and only 5% in OTHH (Figure 1b). This fact may be associated with the reaction of Folin-reagent and phenolic compounds. The reaction occurs between the hydroxyl groups present in these compounds and *S. cerevisiae* shows capability of reducing phenolic aldehydes and acids groups to their respective alcohols, which can compromise the analysis.[36] In order to confirm the reduction in the furans and phenolic compounds concentration, ultraviolet spectra were performed before and after the hydrolysates biotransformation. This technique can provide information regarding the presence of phenolic compounds derived from lignin and furans resulting from the degradation of carbohydrates and permits evaluation of the changes in concentration or even changes in the chemical structure of these molecules.[8,25,37,38]

Figure 2 shows ultraviolet spectrum of the hemicellulosic hydrolysates before and after biological detoxification by *S. cerevisiae*, and it was verified a decrease of peaks in the 250−400 nm regions in both hydrolysates employed. In the present study, it was observed an absorptive reduction of 36 and 17% at 280 nm for RSHH and OTHH, respectively. According to Martinez et al.,[25] most of the reduction in 280 nm is attributable to furfural and/or 5-HMF, since this region is verified as the maximum absorption area of these inhibitors, and only a small part of the soluble lignin is quantified. It is also known that the reduction products of furans compounds have greater ultraviolet-absorptive in the 190−210 nm regions.[39] Some studies have reported a reduction of 5-HMF to alcohol-HMF,[30−32] as well as the furfural reduction to furfuryl-alcohol under anaerobic and aerobic conditions when *S. cerevisiae* is used.[30,33,34] Liu et al.[31] suggest that the aldehyde functional group present on the furan ring is the cause of toxicity of these compounds; however, their reduced forms are less inhibitory to fermentative yeasts, such as *P. stipitis*.

Regarding the soluble products from acid-degradation of lignin, the spectra range among 320−400 nm is attributed to aromatic compounds having aromatic ring mediated conjugation between hydroxyl and carbonyl groups. The peak with a maximum at approximately 300 nm is characteristic of nonconjugated hydroxyl groups.[8] In the present study the UV-profiles show for RSHH and OTHH reductions of 36 and 13% respectively, for both regions (300−350 nm). These results suggest a reduction in the low molecular weight phenolic compounds concentrations present in the hemicellulosic hydrolysates, which was not observed for the Folin-reagent methodology. This reduction in UV absorptivity was expected, since *S. cerevisiae* has the metabolic capacity to convert aldehyde and phenolic acid in their corresponding alcohols. Mathew et al.[40] indicated that the ferulic acid decarboxylation to produce 4-vinyl-guaiacol leads to an increase in the maximum UV-absorptivity in the 210 nm region. The authors suggested that this change is due to phenolic alcohol formation. Karmakar et al.[41] analyzed the degradation product of the ferulic acid by employing the bacterium *Bacillus coagulans* and detected 4-vinyl-guaiacol as well as a decrease in UV absorptivity at the 290−310 nm regions.

dx.doi.org/10.1021/jf402474s | J. Agric. Food Chem. 2013, 61, 9658−9665

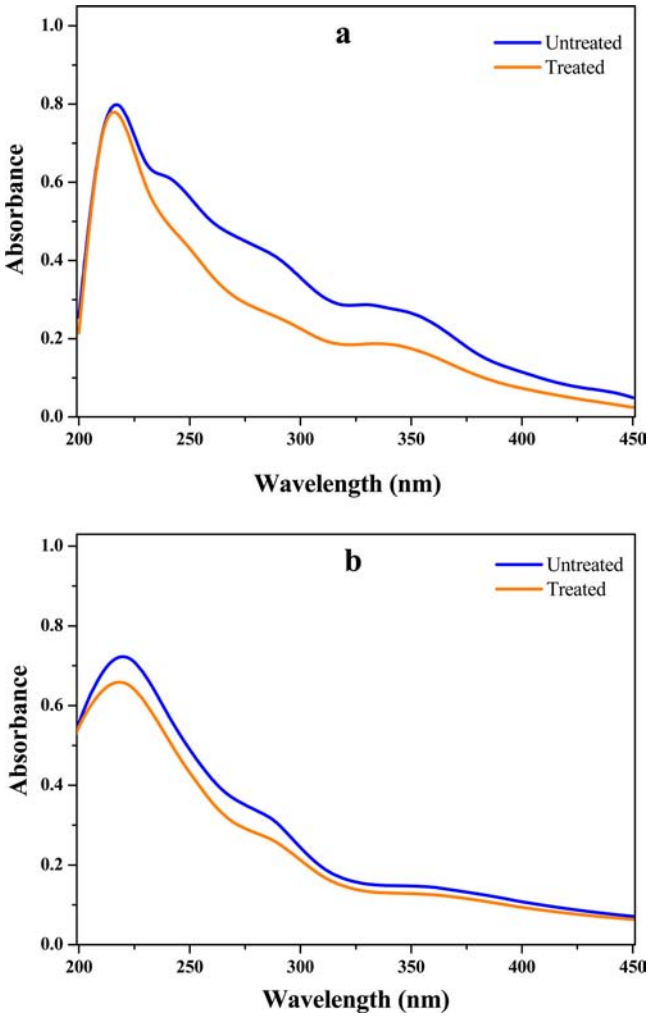

Figure 2. Ultraviolet-spectrum profile before and after the biotransformation treatment by *Saccharomyces cerevisiae* in (a) RSHH and (b) OTHH. (blue) Untreated and (orange) treated.

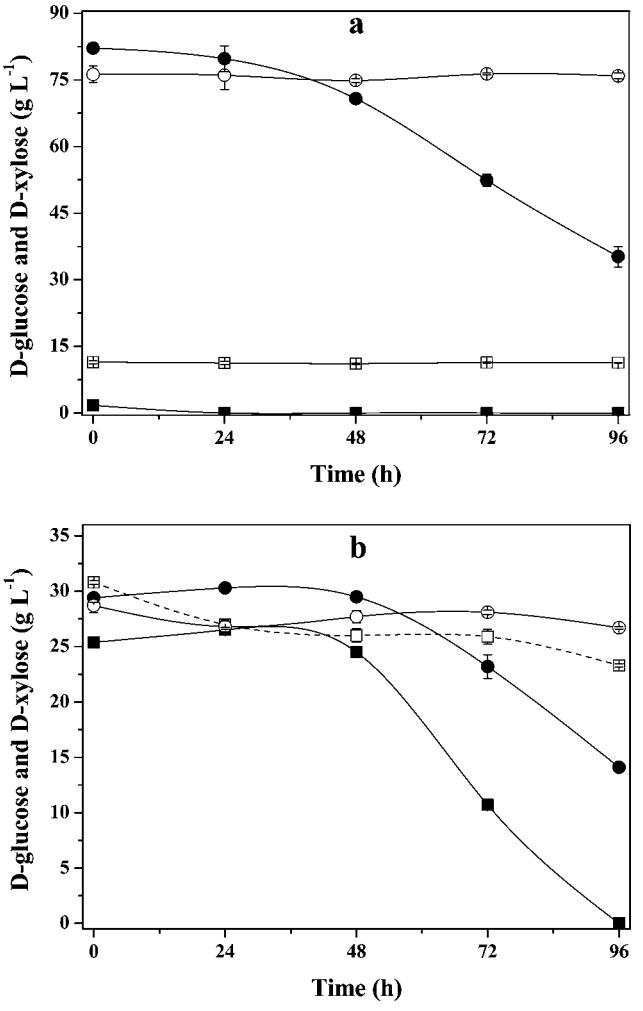

Figure 3. Sugars consumption by *Pichia stipitis* in (a) RSHH and (b) OTHH treated (solid) by *Saccharomyces cerevisiae* and untreated (hollow). D-Glucose (square) and D-xylose (circle).

On the other hand, the reduction percentages of phenol and furan compounds were higher at RSHH than OTHH. This may be explained attending to the different acetic acid concentrations in theses hydrolysates. It is significant that a double acetic acid concentration in OTHH causes a half reduction of aromatic compounds compared with RSHH.

Although reductions in furan and phenolic compounds in the present study have not been completed, one advantage to using *S. cerevisiae* as the detoxifying agent is that this yeast does not consume the main sugar present in hemicellulose hydrolysates (D-xylose). Some studies of biological detoxification have reported total reduction of these toxic compounds, but also with consumption of pentoses. For example, Zhang et al.[42] reported that *Amorphotheca resinae* ZN1 demonstrated the ability to degrade completely furans contained in corn stover hemicellulosic hydrolysate; however, the strain consumed 57% of D-xylose, making the method disadvantageous because the loss of this sugar compromises the subsequent fermentation step.

Effect of Biodetoxification on Ethanol Production. The treatment efficiency of hydrolysates with *S. cerevisiae* was evaluated by *Pichia stipitis* regarding the sugar consumption, cell growth and ethanol conversion (Figures 3 and 4). Figure 3 shows the sugars consumption by *P. stipitis* in RSHH and OTHH, respectively. D-Xylose consumption by *P. stipitis* was not observed in both untreated hemicellulose hydrolysates, and only 25% of the D-glucose was consumed in the OTHH, with no hexose consumption in the RSHH.

Once both hydrolysates were biodetoxified, D-glucose was completely consumed by *P. stipitis*. This consumption requires just less than 24 h for RSHH and 96 h for OTHH. The D-xylose consumption includes a lag phase of 24 h for RSHH and 48 h for OTHH. After this time, D-xylose consumption increased to approximately 55% after 96 h fermentation assays, with a consumption rate of approximately 0.49 and 0.16 g L^{-1} h^{-1} for RSHH and OTHH, respectively. In OTHH, it was observed that the D-glucose and D-xylose consumption occurred simultaneously, beginning after 48 h of the assay. This behavior was not observed by Agbogbo et al.[43] when growing *P. stipitis* CBS 6054 in media containing different proportions of these sugars (60.0 g L^{-1} of total sugars). The authors found that first the yeast consumed all the D-hexose present in the medium to finally start D-xylose consumption, and the D-glucose consumption rate was always higher than that of D-xylose, as observed in the present study. This delay in sugar consumption can be associated with yeast adaptation phase in medium containing high acetic acid concentration. In the present study, the acetic acid initial concentration in OTHH was 4.1 g L^{-1},

dx.doi.org/10.1021/jf402474s | J. Agric. Food Chem. 2013, 61, 9658−9665

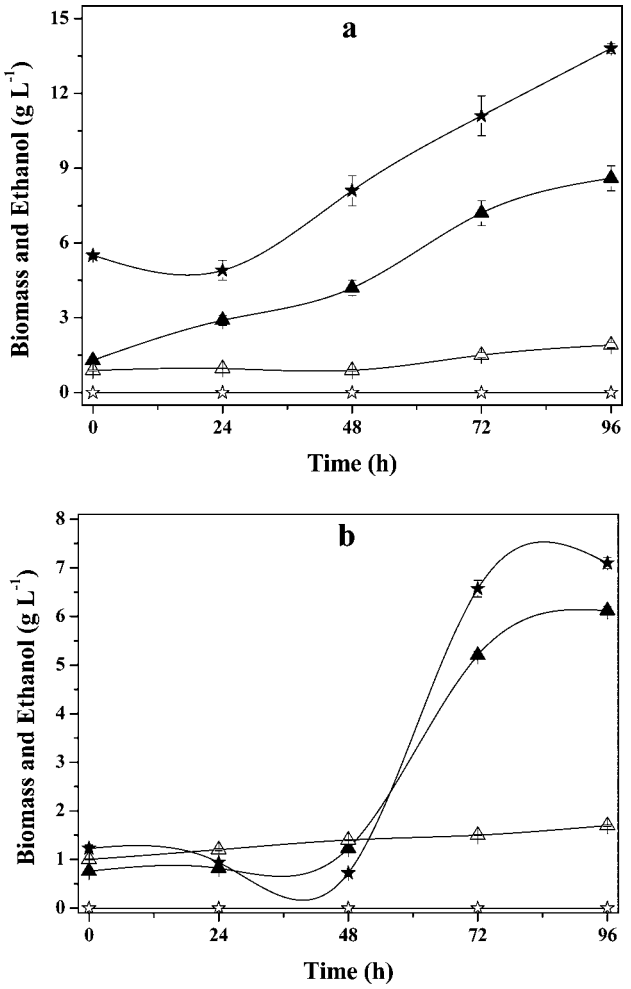

Figure 4. Biomass and ethanol production by *Pichia stipitis* in (a) RSHH and (b) OTHH treated (solid) by *Saccharomyces cerevisiae* and no-treated (hollow). Biomass (triangle) and ethanol (star).

which represents acid concentration of approximately 50% higher than in RSHH. Diaz et al.[13] observed that the presence of acetic acid in a fermentation medium with D-glucose (20.0 g L^{-1}) and D-xylose (15.0 g L^{-1}) affects *P. stipitis* metabolism, even at the lowest concentration tested (3.0 g L^{-1}). Another study demonstrates that acetic acid concentrations of 3.5 g L^{-1} completely inhibit the growth of *P. stipitis* and ethanol production.[44] According to Bellido et al.,[44] the D-xylose consumption was more affected by the acid presence than D-glucose, being more pronounced as the concentration of acetic acid increased. Another fact that would justify the low D-xylose consumption by *P. stipitis* in OTHH is the presence of D-glucose at higher concentrations which could lead to enzymes catabolic repression that utilize D-xylose.[29]

Figure 4 shows that the biotransformation favored *P. stipitis* growth and ethanol production in both hydrolysates. These results can be explained by reduction in the total furan and phenolic concentration. It can be seen that the biotransformation improved *P. stipitis* growth by 4.5 and 3.6 times in RSHH and OTHH, respectively, compared with untreated results. The growth rate in treated-RSHH was approximately 0.08 g L^{-1} h^{-1}, that is, 25% higher than observed for treated-OTHH, where we again observed an adaptation phase of near 48 h.

The ethanol production by *Pichia stipitis* during fermentation of the hemicellulosic hydrolysates also can be observed in Figure 4. Ethanol productions (final minus initial concentration) of 8.3 and 5.9 g L^{-1} in RSHH and OTHH, respectively, were observed. In the OTHH fermentation (Figure 4b) the ethanol concentration started with 1.2 g L^{-1}, and after 48 h decrease to 0.72 g L^{-1}, reaching 7.1 g L^{-1} at the fermentation end time (96 h). According to Figure 4a, this profile was not observed for RSHH, *P. stipitis* produced 13.8 g L^{-1} in the fermentative process. The ethanol effect in the D-glucose and D-xylose fermentability by *P. stipitis* was studied by Meyrial et al.[45] The authors demonstrated that the alcoholic fermentation of *P. stipitis* NRRL Y-7124 in a medium containing D-glucose or D-xylose (50.0 g L^{-1}), can be affected depending of the ethanol initial concentration. It was also observed that ethanol concentrations down to 10 g L^{-1} do not affect yeast growth and ethanol volumetric production.

The fermentation parameters such as ethanol ($Y_{P/S}$) and biomass ($Y_{X/S}$) yield factors, ethanol volumetric productivity (Q_P) and ethanol efficiency (η) are given in Table 3. Ethanol yield factor (grams per gram) was defined as the ratio between ethanol concentration (grams per liter) and total substrate consumed (grams per liter). Ethanol volumetric productivity (grams per liter per hour) was calculated as the ratio between the ethanol concentration (grams per liter) and the fermentation time (hour). Cell yield factor (grams per gram) was defined as the ratio between cell concentration and total substrate consumed (grams per liter). The efficiency of sugar conversion to ethanol (η, %) was determined as the ratio between $Y_{P/S}$ (g g^{-1}) and the theoretical value (0.51 g g^{-1}) of this parameter.

Table 3. Effect of Biodetoxification Method on Fermentation Parameters of the Rice Straw (RSHH) and Olive Tree (OTHH) Hemicellulosic Hydrolysates by *P. stipitis*

	RSHH		OTHH	
fermentative parameters[a]	untreated	treated	untreated	treated
$Y_{X/S}$ (g g^{-1})	0	0.15	0.07	0.13
$Y_{P/S}$ (g g^{-1})	0	0.17	0	0.14
Q_P (g L^{-1} h^{-1})	0	0.09	0	0.06
η (%)	0	33.3	0	27.0

[a]Average of at least three determinations. Ethanol yield ($Y_{P/S}$), Biomass yield ($Y_{X/S}$), Ethanol volumetric productivity (Q_P), Efficiency (η).

The fermentations of biotransformation hydrolysates are characterized by quick kinetics with larger yield and productivity. The biomass yield increased approximately 46% in OTHH when compared with untreated hydrolysate. It was also verified that biomass and ethanol yields, and ethanol production efficiency in RSHH were approximately 15% superior to in OTHH. On the other hand, the ethanol volumetric productivity was 33% greater at RSHH than at OTHH, 0.09 and 0.06 g L^{-1} h^{-1}, respectively. However, compared with the untreated hydrolysates, both had improved their fermentative parameters, since no ethanol production was observed for the untreated hydrolysates. As previously discussed, one explanation for the low ethanol productivity values obtained may be associated with the presence of acetic acid. According to Diaz et al.,[13] in D-glucose (20.0 g L^{-1}) and D-xylose (15.0 g L^{-1}) medium, the acid acts more negatively in ethanol volumetric productivity than in *P. stipitis* growth. The authors observed that in the presence of 3.0 g L^{-1} acetic acid the ethanol volumetric productivity was 22% lower than in the

dx.doi.org/10.1021/jf402474s | J. Agric. Food Chem. 2013, 61, 9658−9665

control without acetic acid (0.38 and 0.49 g L^{-1} h^{-1}, respectively). It was also observed that 6.0 g L^{-1} acetic acid completely inhibits the ethanol production in the 24 h fermentation process.

The results of this study showed that biological detoxification of RSHH and OTHH could be successfully performed by *S. cerevisiae*, thus removing and/or reducing the inhibitory compounds and allowing the ethanol production by *P. stipitis*.

In conclusion, sugars and toxic compound concentrations present in hemicellulose hydrolysates depend on the type of raw material as well as the conditions that have been employed in the hydrolysis. The method and the conditions of biotransformation process also should be specific for each hydrolysate. The biological detoxification of RSHH and OTHH by *S. cerevisiae* is a very interesting method because it reduces the inhibitory compounds without affecting the D-xylose levels in the medium. *S. cerevisiae* has a versatile and apparently inducible enzyme mechanism for biotransformation of inhibitory to less inhibitory compounds; thus, this yeast provides a new alternative for a biological treatment method. It is remarkable that to *P. stipitis* fermentation it is not necessary for the total removal of inhibitor compounds since this yeast allows little concentrations of those compounds and a right biotransformation must preserve a good quality of sugar hydrolysates. However, although this process improved the ethanol fermentation of RSHH and OTHH by *P. stipitis*, the optimization of detoxification conditions is necessary, so that it can reduce the concentrations of aromatic compounds and acetic acid.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of "Ciência Sem Fronteiras" of the "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq), "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior" (CAPES) and "Fundação de Amparo à Pesquisa do Estado de São Paulo" (FAPESP 12/51834-6) (Brazil) and the University of Jaén (Bioprocesses Research Group).

ABBREVIATIONS

RSHH, rice straw hemicellulosic hydrolysate; OTHH, olive tree hemicellulosic hydrolysate; $Y_{P/S}$, ethanol yield; $Y_{X/S}$, biomass yield; Q_P, volumetric ethanol productivity; 5-HMF, 5-hydroxymethylfurfural; UV, ultraviolet; η, efficiency

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