RESEARCH ARTICLE

Improvement of bioethanol production from pomegranate peels via acidic pretreatment and enzymatic hydrolysis

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Abstract

The aim of this study was to improve the ethanol production from pomegranate peels (PPs). Therefore, the effect of enzymatic hydrolysis and different pretreatments on ethanol production by yeasts was examined. There were three different enzyme concentrations (3.6, 7.2, 14.4 FPU/g substrate) tested for enzymatic hydrolysis, and four different PP media, such as WSPP (whole slurry of PP), LFPP (liquid fraction of PP), WSFPP (washed solid fraction of PP) and N-WSFPP (non-washed solid fraction of PP), were prepared. Bioethanol production was monitored for 96 h. Maximum ethanol concentrations were obtained at WSPP medium as 12.69 g/L, 14.35 g/L and 4.23 g/L in *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Pichia stipitis*, respectively. On the other hand, the washing step of biomass increased the kinetic parameters dramatically and the highest theoretical ethanol yields and $Y_{P/S}$ values were obtained from WSFPP medium in all tested yeasts. Theoretical ethanol yields were 97.8%, 98.7% and 35.5% for *S. cerevisiae*, *K. marxianus* and *P. stipitis*, respectively. Q_p values were observed as 0.98 g/L h, 0.99 g/L h and 0.04 g/L h for the same yeasts. The highest $Y_{P/S}$ values were detected as 0.50 g/g for *S. cerevisiae*, 0.50 g/g for *K. marxianus* and 0.30 g/g for *P. stipitis* in the washed pomegranate peel biomass.

Introduction

In today's world, global energy demand is met by non-renewable energy sources, such as fossil fuels. However, fossil fuels have some major disadvantages, such as a negative impact on the environment. Growing population and increasing industrialisation cause the depletion of fossil fuels, and these fuels are also one of the main causes of greenhouse gas emissions and climate change. By this context, it is necessary to explore alternative energy sources such as biodiesel, bioethanol and hydrogen (Zhang et al. 2016; Zabed et al. 2017; Anwar Saeed et al. 2018). Renewable energy sources which are derived from lignocellulosic biomass can help avoid climate change or greenhouse gas emissions (Kharytonov et al. 2019). Ethanol can be produced from different feedstocks such

as edible crops (wheat, corn), lignocellulosic biomass (wheat straw, rice straw) or photosynthetic microorganisms such as microalgae or cyanobacteria. Edible crops are the source of first-generation ethanol. Although it is easier to produce ethanol from edible crops, the cost of raw material and some ethical and environmental concerns are major barriers for first-generation ethanol production. Therefore, many types of research have been focused on ethanol production from lignocellulosic biomass. Lignocellulose represents the most underutilised and cheap resource on Earth. It also has a low price and rich carbohydrate content and does not compete with edible crops (Naik et al. 2010; Djelal et al. 2017). Lignocellulose is composed of cellulose, hemicellulose and lignin which is resistant to degradation. Therefore, pretreatment is a crucial factor for the decomposition of lignocellulose. For this reason to obtain fermentable sugars, lignocellulosic biomass should be pretreated (Van Dyk and Pletschke 2012; Tsegaye et al. 2018). Pretreatment with dilute acid is generally considered as one of the most effective methods. In dilute acid pretreatment, the majority of the hemicellulose is degraded, and the cell wall matrix is damaged. By this reason, accessibility to the cellulosic structure by cellulase enzymes increases efficiently and this cellulose can be hydrolysed by the cellulase enzymes (Vohra

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et al. 2014). Enzymatic hydrolysis of lignocellulose after the dilute acid pretreatment is a very effective way to obtain fermentable sugars due to its high productivity rates. Moreover, hydrolysis happens in mild conditions and it does not have corrosion problems (Balat 2011). Furthermore, application of the appropriate enzyme concentration reduces the overall cost of the process and gives optimal results (Pessani et al. 2011).

In dilute acid pretreatment, some inhibitory compounds can be released to the medium (Palmqvist and Hahn-Hägerdal 2000). To remove these inhibitory compounds, pretreated biomass might be washed with water in order to eliminate the toxic materials; however, during the washing step, the concentration of soluble sugars decreases due to washing with water (Kumar et al. 2009a; Shi et al. 2009; Fernandes et al. 2015). On the other hand, during the acid pretreatment, a significant amount of fermentable sugars from cellulose and hemicellulose are generally released to the liquid fraction from the pretreated biomass slurry. In order to reduce the cost operation and utilise all of the sugars, hydrolysis of whole slurry (solid and liquid fractions together) is more desirable (Jung et al. 2013).

Pomegranates (*Punica granatum*) have gained much attention in recent years because of their antimicrobial, anticancer, antiviral and antioxidant properties. (Lansky and Newman 2007; Yasoubi et al. 2007; Johanningsmeier and Harris 2011; Rosas-Burgos et al. 2017). Besides these features, pomegranate peels have a considerable amount of fermentable sugars (Hasnaoui et al. 2014; Zhu et al. 2015). Therefore, pomegranate peels (PPs) were considered as the raw material for ethanol production in this study.

In our previous study, we showed that the PP is a suitable material for ethanol production and 5.58 g/L ethanol was obtained by *S. cerevisiae* (Demiray et al. 2018). In this study, we increased the ethanol production of *S. cerevisiae* to 12.69 g/L. Moreover, it was shown that our laboratory isolate *K. marxianus*, which is better than *S. cerevisiae* for bioethanol production, produced 14.35 g/L ethanol.

In this study, we have examined the effects of 4 differently pretreated PP media (washed solid PP, non-washed solid PP, whole slurry of PP and liquid fraction of PP) and 3 different cellulase loadings (3.6, 7.2 and 14.4 FPU/g substrate) on the ethanol production during a 96-h incubation period. *Saccharomyces cerevisiae*, *Kluyveromyces marxianus* and *Pichia stipitis* were used in these experiments. To our knowledge, this is the first paper which reports a high amount of bioethanol such as 14.35 g/L from PP via enzymatic hydrolysis.

Materials and methods

Raw material, microorganisms and media

PPs were supplied as stated in our previous study. *S. cerevisiae* and *K. marxianus* were obtained from Ankara University Culture Collection. *P. stipitis* was supplied from the NNRL Culture Collection. Fermentation media were prepared as in our previous study (Demiray et al. 2018). The yeast cells which have been pre-cultured 24 h were inoculated to the fermentation medium as 5% (v/v).

Pretreatment of PP

For pretreatment of PP, 1% H_2SO_4 (v/v) was added to 100 g/L PP (w/v) and autoclaved immediately at 121 °C for 15 min. To determine the effect of different pretreated media of PP to the enzymatic hydrolysis, the whole slurry of PP (WSPP/liquid and solid fractions together), the liquid fraction of PP (LFPP), washed solid fraction of PP (WSFPP) and a non-washed solid fraction (N-WSFPP) were prepared. The liquid fraction of PP (LFPP) was filtered through Whatman No. 1 paper and liquid filtrate was used for enzymatic hydrolysis. The washed solid fraction of PP (WSFPP) was washed with tap water until it reached a neutral pH value. The non-washed solid fraction of PP (N-WSFPP) was also prepared. N-WSFPP was not washed in tap water and it was directly used in enzymatic hydrolysis and fermentation experiments.

Enzymatic hydrolysis

After the pretreatment step, the pH of the pretreated PP medium was adjusted to 4.8 in the presence of 50 mM citrate buffer (Chen et al. 2012). There were 4 different PP media (WSPP, LFPP, WSFPP and N-WSFPP) used on enzymatic hydrolysis. All enzymatic hydrolysis was performed in 250-mL Erlenmeyer flasks with a working volume of 100 mL. CellicCTec2 is a commercial cellulase and was supplied from Novozymes, Denmark. There were 3 different enzyme concentrations (3.6, 7.2, 14.4 FPU/g substrate) tested. The experiments were carried out in a 50 °C water bath with a shaker apparatus at 100 rpm for 72 h. In order to stop the enzymatic hydrolysis, all PP media were boiled in a 100 °C water bath for 10 min. Filter paperase unit (FPU) activity of the enzyme was found to be 121 FPU/mL.

Fermentation assays

Fermentation media were incubated at 30 °C for 96 h for all yeasts at pH 4.8 with a 100-rpm agitation speed. Sugar consumption and ethanol production were monitored during the incubation.

Analytical methods

Samples were centrifuged at 10,000 rpm for 10 min. The supernatant was filtered with a 0.45-μm pore size filter. Ethanol concentration was detected with a Shimadzu/GC-2010 gas chromatography system equipped with a flame

ionisation detector (FID) and 0.25 mm i.d. RTX-Wax column. GC analysis was performed according to Wistara et al. (2016) with modifications. These modifications were as follows: The injection port and flame ionisation detector temperatures were held at 140 and 160 °C, the initial column temperature was 50 °C and the column temperature was increased to 150 °C within 19 min. Column flow was 1.86 mL/min.

For determining the sugar profile of PP, an HPLC analysis Shimadzu system with a BioRad Aminex HPX-87P column and refractive index detector (RID) was used. Before the analysis, 2 mL PP hydrolysate was centrifuged at 10,000 rpm for 10 min. All samples and standards were filtered through 0.45-μm pore size filter. Column oven temperature was held at 80 °C and filtered deionised water was used as a mobile phase. Total flow was adjusted to 0.6 mL/min. Samples and standards were analysed for 30 min (Saha et al. 2019).

Total reducing sugar content was determined according to the DNS method (Miller 1959). The FPU/mL of the cellulose was determined according to Adney and Baker (2008).

The theoretical ethanol yield was calculated according to Eq. (1) presented below (Kim and Lee 2005):

Theoretical Ethanol Yield (%):

$$\frac{\text{ethanol (g)}}{\text{initial sugar (g)} \times 0.511} \times 100 \qquad (1)$$

Ethanol productivity (Q_p) was determined according to Eq. (2), as described elsewhere (Roca and Olsson 2003).

$$Qp : \frac{\text{g/L ethanol}}{\text{h}} \qquad (2)$$

In this equation "g/L ethanol" is the maximum ethanol concentrations obtained from each microorganism at the specific time point. On the other hand, "h" refers to the time point where the highest ethanol is present.

The ethanol yield ($Y_{P/S}$) was calculated according to Eq. (3) (Günan Yücel and Aksu 2015) below:

$$Y_{P/S} \frac{\text{g max ethanol}}{\text{g consumed reducing sugar}} \qquad (3)$$

In Eq. (3) "g max ethanol" is the maximum ethanol amount obtained from microorganisms and "g consumed reducing sugar" shows the amount of consumed sugar concentration, when the highest amount of ethanol is detected in microorganisms.

Statistical analysis

Statistical analysis was performed at the level of the p value (< 0.05) and on SPSS 20.0 program to determine the significance of the difference of tested groups. Univariate analysis was performed in order to determine the effect of enzyme dosage and PP medium to the ethanol and sugar concentration. Standard errors and error bars that are presented in the tables and figures were calculated respectively and all experiments were performed as triplicate.

Results and discussion

The effect of enzymatic hydrolysis

Before the enzymatic hydrolysis, the PP was pretreated with 1% H_2SO_4 (v/v). Different kinds of fermentable sugars were determined after acid pretreatment. The data in Table 1 depicts that the most abundant sugar of the PP was glucose as 37.9%. Xylose, cellobiose, arabinose and fructose percentages were found as 17.6%, 16.7%, 14.9% and 12.9%, respectively. Sugar profiles of PP were determined in previous reports. For instance, Talekar et al. (2018) found the glucose, xylose and arabinose concentration of the pomegranate peels as 18.66, 4.17 and 1.65 g/L, respectively. To determine the efficiency of different pretreatment methods, 4 different fermentation media were prepared and 3 different enzyme concentrations were applied to these media. In all groups, initial PP loading was 100 g/L, and 1% H_2SO_4 was used for pretreatment. The data in Table 2 compares the initial sugar concentrations of the media which were hydrolysed with different enzyme concentrations from 0 to 14.4 FPU/g substrate. It can be concluded that higher enzyme loading caused higher reducing sugar concentrations. In the literature, there are similar studies which report that the whole slurry hydrolysis is favourable for sugar releasing from the biomass. For instance, Jung et al. (2014) pretreated the whole slurry of empty fruit bunches with maleic acid and enzyme. Researchers found that usage of the whole slurry increased the sugar concentration in the fermentation medium significantly. In our current study, the sugar concentrations of LFPP, WSFPP and N-WSFPP were lower than those of WSPP as expected.

The lowest initial reducing sugar concentrations before the enzymatic hydrolysis of the WSPP, LFPP and N-WSFPP were found as 19.71, 14.42 and 6.53 g/L hexose and 15.82, 11.63 and 5.37 g/L pentose, respectively. Furthermore, the lowest sugar concentrations were obtained from WSFPP for all the

Table 1 Sugar composition of PP (1% H_2SO_4, 121 °C at 15 min, 100 g/L PP, pH 5)

Type of sugar	(%)
Fructose	12.9
Glucose	37.9
Arabinose	14.9
Xylose	17.6
Cellobiose	16.7

Table 2 Initial sugar concentrations before and after 72-h enzymatic hydrolysis (100 g/L PP loading, pH 4.8, 100 rpm, 50 °C, hydrolysis time 72 h)

Enzyme loading (FPU/g substrate)	WSPP	LFPP	WSFPP	N-WSFPP
	Initial reducing hexose concentration (g/L)			
0	19.71 ± 3.4^a	14.42 ± 2.6^b	1.49 ± 0.2^c	6.53 ± 1.0^d
3.6	27.96 ± 2.5^e	17.42 ± 0.1^f	9.72 ± 1.3^g	19.1 ± 2.4^h
7.2	31.55 ± 1.1^i	21.35 ± 1.5^j	14.92 ± 2.1^k	21.58 ± 1.3^j
14.4	36.34 ± 0.2^m	27.61 ± 2.7^n	23.45 ± 0.2^o	23.46 ± 0.4^o
	Initial reducing pentose concentration (g/L)			
0	15.82 ± 2.7^a	11.63 ± 2.1^b	1.37 ± 0.2^c	5.37 ± 0.8^d
3.6	22.37 ± 2.0^e	14.01 ± 0.1^f	7.9 ± 1.1^g	15.34 ± 1.9^f
7.2	25.22 ± 0.8^h	17.12 ± 1.2^i	12.02 ± 1.7^j	17.3 ± 1.0^i
14.4	29.01 ± 0.2^k	22.08 ± 2.1^l	18.59 ± 0.1^m	18.6 ± 0.3^m

Different letters in superscript within the same row indicate the significant differences with respect to different sugar concentrations statistically ($p \leq 0.05$)

tested enzyme concentrations. These lower sugar concentrations may be related to the washing steps of the biomass. However, initial sugar concentrations were increased from 1.49 to 23.45 g/L with the 14.4 FPU/g substrate and this value is nearly the same when N-WSFPP was used (Table 2). Similarly, in a study, Pocan et al. (2018) found the reducing sugar concentrations of the pomegranate peel about 20 g/L when they hydrolysed the pomegranate peels with 13.5 FPU/g biogazyme cellulase.

The highest reducing sugar concentrations were detected when WSPP was hydrolysed with 14.4 FPU/g substrate as 36.34 g/L hexose and 29.01 g/L pentose at the end of 72 h. It is because WSPP contains both fermentable sugars from liquid and solid residues of PP. Therefore, higher sugar concentrations were obtained in all tested enzyme amounts for WSPP. These results are coherent with the literature about enzymatic hydrolysis of the lignocellulosic biomass (Josefsson 2013).

Some similar reports about pretreatment of the lignocellulosic wastes showed that washed and unwashed biomass give similar sugar profiles. For example, Lu et al. (2010) investigated ethanol production from corn stover on high solid concentrations. Researchers found that the glucose amounts of washed and unwashed corn stovers were nearly the same (about 30 g/L) at the end of the enzymatic hydrolysis when they used 100 g/L corn stover in the presence of 20 FPU/g substrate cellulase enzyme. Furthermore, Tutt et al. (2012) found that the glucose concentrations of the washed and unwashed wheat straw biomass pretreated with sulfuric acid were

Table 3 Bioethanol concentrations of *S. cerevisiae*, *K. marxianus* and *P. stipitis* in different pretreated PP media (100 g/L PP loading, pH 4.8, 100 rpm)

Pretreatment conditions	Enzyme loading (FPU/g substrate)	*S. cerevisiae*	*K. marxianus*	*P. stipitis*
WSPP	3.6	$9.85 \pm 0.0*$	$9.55 \pm 0.5*$	$1.6 \pm 0.0**$
	7.2	$11.08 \pm 0.0*$	$10.89 \pm 0.2*$	$2.46 \pm 0.1**$
	14.4	$12.69 \pm 0.06*$	$14.35 \pm 0.72**$	$4.23 \pm 0.18***$
LFPP	3.6	$7.79 \pm 0.3*$	$8.08 \pm 0.4*$	$1.01 \pm 0.1**$
	7.2	$9.48 \pm 0.0*$	$9.89 \pm 0.0*$	$1.3 \pm 0.0**$
	14.4	$11.37 \pm 0.35*$	$12.35 \pm 0.18**$	$2.78 \pm 0.0***$
WSFPP	3.6	$5.78 \pm 0.1*$	$5.13 \pm 0.1*$	$1.52 \pm 0.0**$
	7.2	$6.53 \pm 0.2*$	$7.08 \pm 0.0**$	$2.35 \pm 0.1***$
	14.4	$11.72 \pm 0.38*$	$11.83 \pm 0.18*$	$3.37 \pm 0.1**$
N-WSFPP	3.6	$4.67 \pm 0.4*$	$4.47 \pm 0.0*$	$1.04 \pm 0.0**$
	7.2	$5.07 \pm 0.0*$	$6.26 \pm 0.0**$	$1.28 \pm 0.2***$
	14.4	$7.28 \pm 0.0*$	$7.14 \pm 0.1*$	$1.48 \pm 0.1**$

For all conditions, the maximum ethanol detection time was shown in the table. Different asterisks symbols (*, ** and ***) in superscript within the same row indicate the significant differences with respect to different ethanol concentrations statistically ($p \leq 0.05$)

Fig. 1 Ethanol and glucose
concentrations in fermentation
system with (a) *S. cerevisiae*, (b)
K. marxianus and (c) *P. stipitis* in
the presence of 14.4 FPU/g
substrate in the WSPP. Rectilineal
triangles, squares and circles are
sugar concentrations and dotted
lines with triangles, squares and
circles are the ethanol
concentrations. ▲ *S. cerevisiae*.
■ *K. marxianus*. ● *P. stipitis* (100
g/L initial PP loading, pH 4.8, 30
°C, 100-rpm agitation speed)
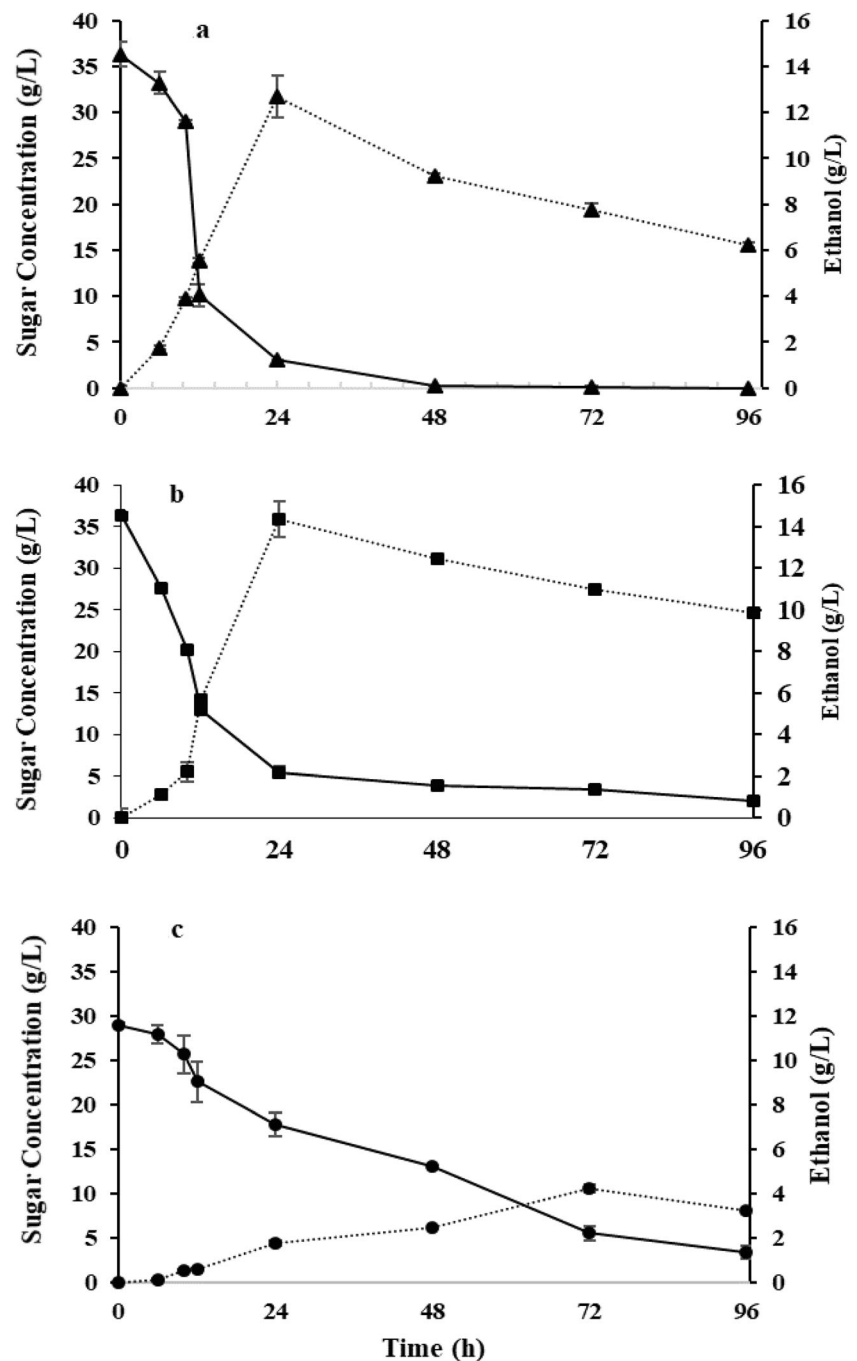
27.6 g/100 g (unwashed samples) and 26.7 g/100 g
(washed samples), respectively.

In our current study, the highest sugar amounts were ob-
tained at 14.4 FPU/g substrate which is the highest enzyme
concentration in all groups. Sugar and ethanol concentrations
increased with increasing enzyme loading. Similar
observations were reported previously. For instance, Narra
et al. (2015) investigated ethanol production from *K.
marxianus*. The authors tested 3 different enzyme loadings
(6, 9, 12 FPU/g substrate) at different time intervals. The

increase in enzyme amount did not cause a significant increase
in ethanol concentration in their study. In our study, the
highest sugar amounts were obtained at 14.4 FPU/g sub-
strate, which is the highest enzyme concentration in all
groups. Sugar and ethanol concentrations increased with
increasing enzyme loading. Jung et al. (2013) also found
that enzymatic digestibility of empty fruit bunches was as
maximum as 87.5% when they used maximum enzyme
concentrations such as 60 FPU + 30 CBU cellulase and
β-glucosidase, respectively.

Fig. 2 Ethanol and glucose concentrations in fermentation system with (a) *S. cerevisiae*, (b) *K. marxianus* and (c) *P. stipitis* in the presence of 14.4 FPU/g substrate in the LFPP. Rectilineal triangles, squares and circles are sugar concentrations and dotted lines with triangles, squares and circles are the ethanol concentrations. ▲ *S. cerevisiae*. ■ *K. marxianus*. ● *P. stipitis* (100 g/L initial PP loading, pH 4.8, 30 °C, 100-rpm agitation speed)
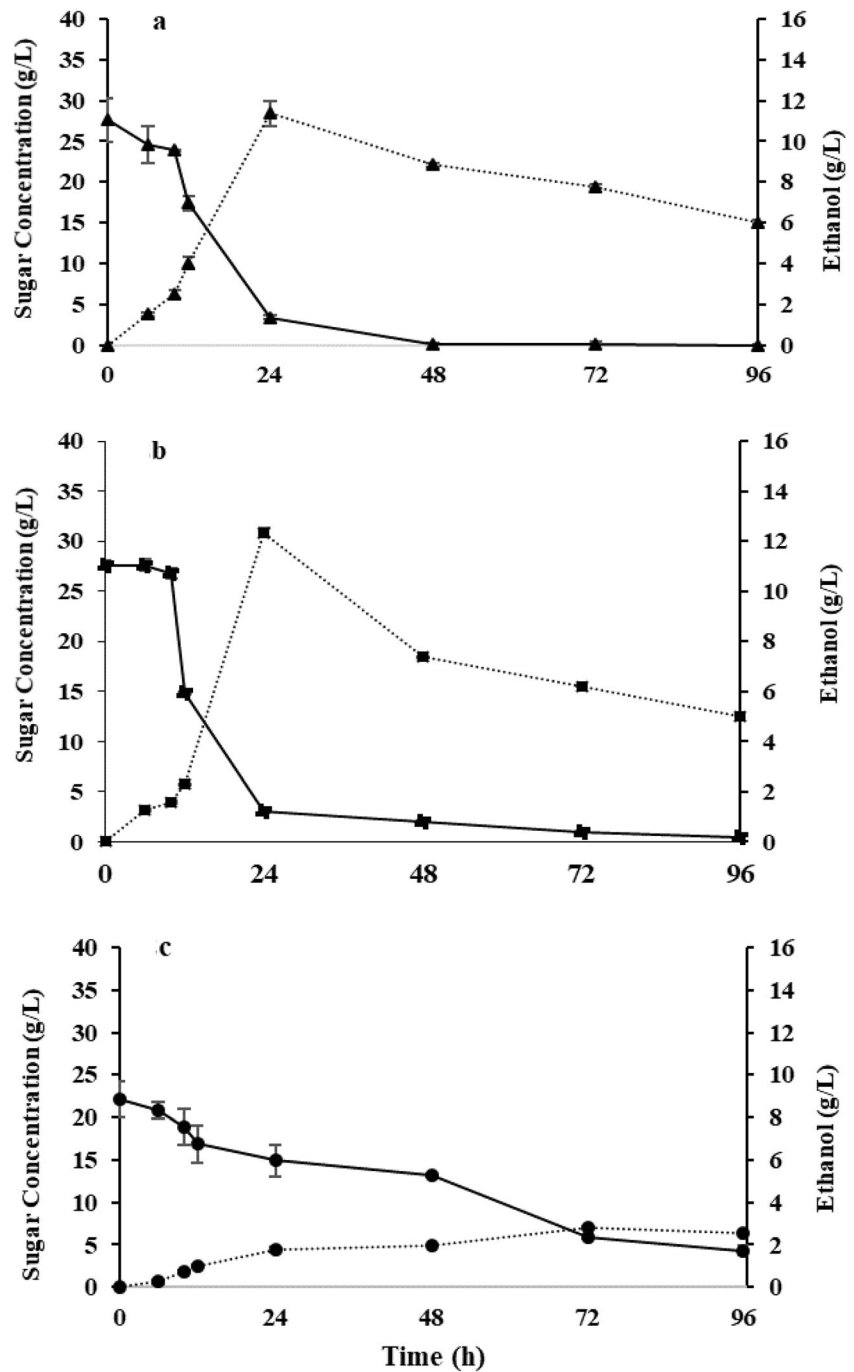

Effect of different pretreatment methods on ethanol production

Ethanol production in WSPP medium

Both liquid and solid fractions of PP were used in the WSPP fermentation medium. In the presence of 3.6 FPU/g substrate enzyme loading, *S. cerevisiae*, *K. marxianus* and *P. stipitis* produced 9.85, 9.55 and 1.6 g/L ethanol, respectively (Table 3). In 7.2 FPU/g substrate enzyme loading, these

amounts were 11.08, 10.89 and 2.46 g/L, respectively, for the same yeasts. In all tested yeasts, the highest concentrations of ethanol were detected when the enzyme loading was 14.4 FPU/g substrate. In these experiments, using the whole slurry (solid and liquid residues together) resulted in the highest sugar concentrations. As expected, higher sugar yields led to higher ethanol concentrations. Ethanol concentrations were 12.69 and 14.35 g/L for *S. cerevisiae* and *K. marxianus* at the end of the 24 h and 4.23 g/L for *P. stipitis* at the end of the 72 h. It was also detected that after the 96-h fermentation

Fig. 3 Ethanol and glucose concentrations in fermentation system with (a) *S. cerevisiae*, (b) *K. marxianus* and (c) *P. stipitis* in the presence of 14.4 FPU/g substrate in the WSFPP. Rectilineal triangles, squares and circles are sugar concentrations and dotted lines with triangles, squares and circles are the ethanol concentrations. ▲ *S. cerevisiae*. ■ *K. marxianus*. ● *P. stipitis* (100 g/L initial PP loading, pH 4.8, 30 °C, 100-rpm agitation speed)
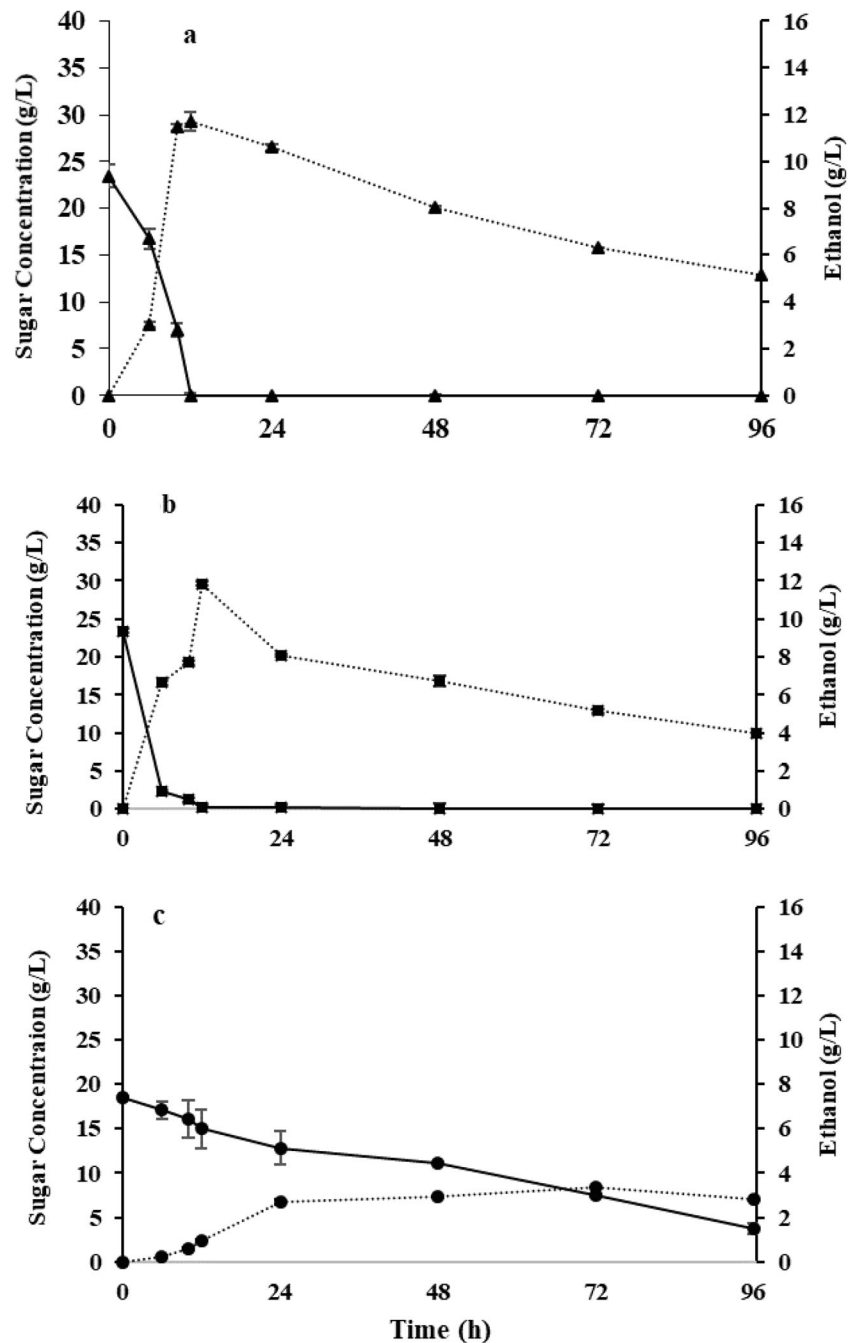

time, reducing sugars of the WSPP were almost depleted. The results and literature comparison are summarised in Figs. 1 and 5 and Tables 2 and 5.

It was also monitored that 3.6 and 7.2 FPU/g substrate enzyme loadings resulted in the highest ethanol yields at the end of 12 h in *K. marxianus* and *S. cerevisiae*. However, 14.4 FPU/g substrate enzyme loading gave the highest results at the end of 24 h for *K. marxianus* and *S. cerevisiae* and 72 h for *P. stipitis*. This may be due to the fact that yeasts have longer log phase because there were higher sugar amounts than when the

WSPP was hydrolysed with 14.4 FPU/g substrate enzyme rather than 3.6 and 7.2 FPU/g substrate. Our results are comparable with some other studies (Kumar et al. 2009b; Kim and Ho 2014).

There are other reports that stated that the whole slurry fermentation is beneficial for ethanol production. Tomás-Pejó et al. (2008) compared the whole slurry ethanol fermentation of wheat straw versus water insoluble solids (WIS) of wheat straw. Authors detected that final sugar concentrations of the WIS were 23.9 g/L glucose and 6.6 g/L xylose and in

Fig. 4 Ethanol and glucose concentrations in fermentation system with (a) *S. cerevisiae*, (b) *K. marxianus* and (c) *P. stipitis* in the presence of 14.4 FPU/g substrate in the N-WSFPP. Rectilineal triangles, squares and circles are sugar concentrations and dotted lines with triangles, squares and circles are the ethanol concentrations. ▲ *S. cerevisiae*. ■ *K. marxianus*. ● *P. stipitis* (100 g/L initial PP loading, pH 4.8, 30 °C, 100-rpm agitation speed)
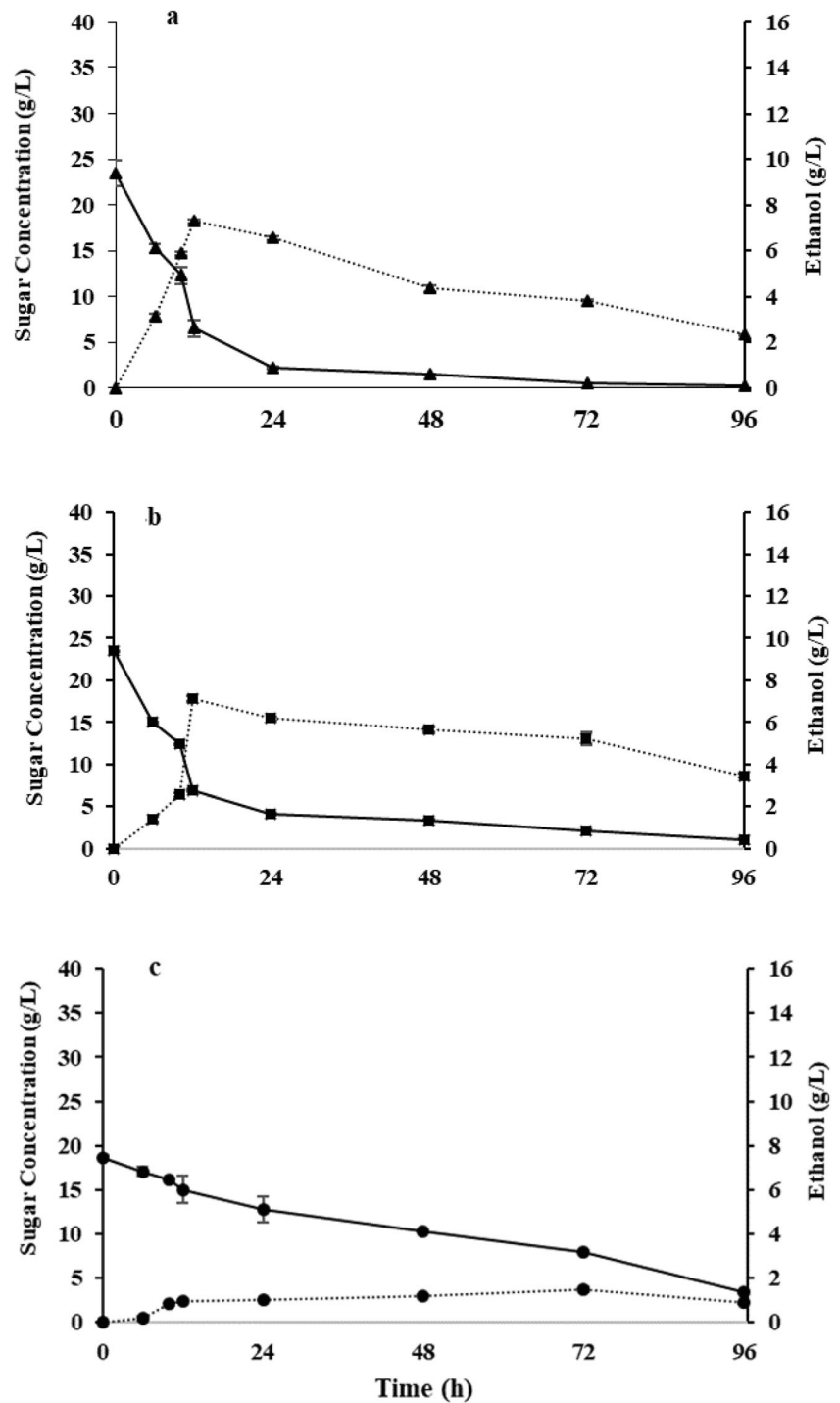
the case of the whole slurry, these amounts were 37.5 g/L glucose and 20.7 g/L xylose, respectively.

Because WSPP medium contains both solid and liquid fractions of PP, higher concentrations of fermentable sugars were obtained. Higher sugar concentrations lead to higher ethanol yields. Some previous works also indicated that whole slurry fermentation caused higher ethanol yields. For instance, in a study, bioethanol yield of the whole slurry of the dilute acid–pretreated spruce was found

around 300 g/kg. On the other hand, when spruce biomass was washed, it was detected that 36% of total sugars of the slurry were excluded and ethanol yield was found as 210 g/kg at 30 °C (Xiros and Olsson 2014).

Ethanol production in LFPP medium

In these set of experiments, the only liquid fraction of acid hydrolysed PP was used in LFPP fermentation medium. In 3.6
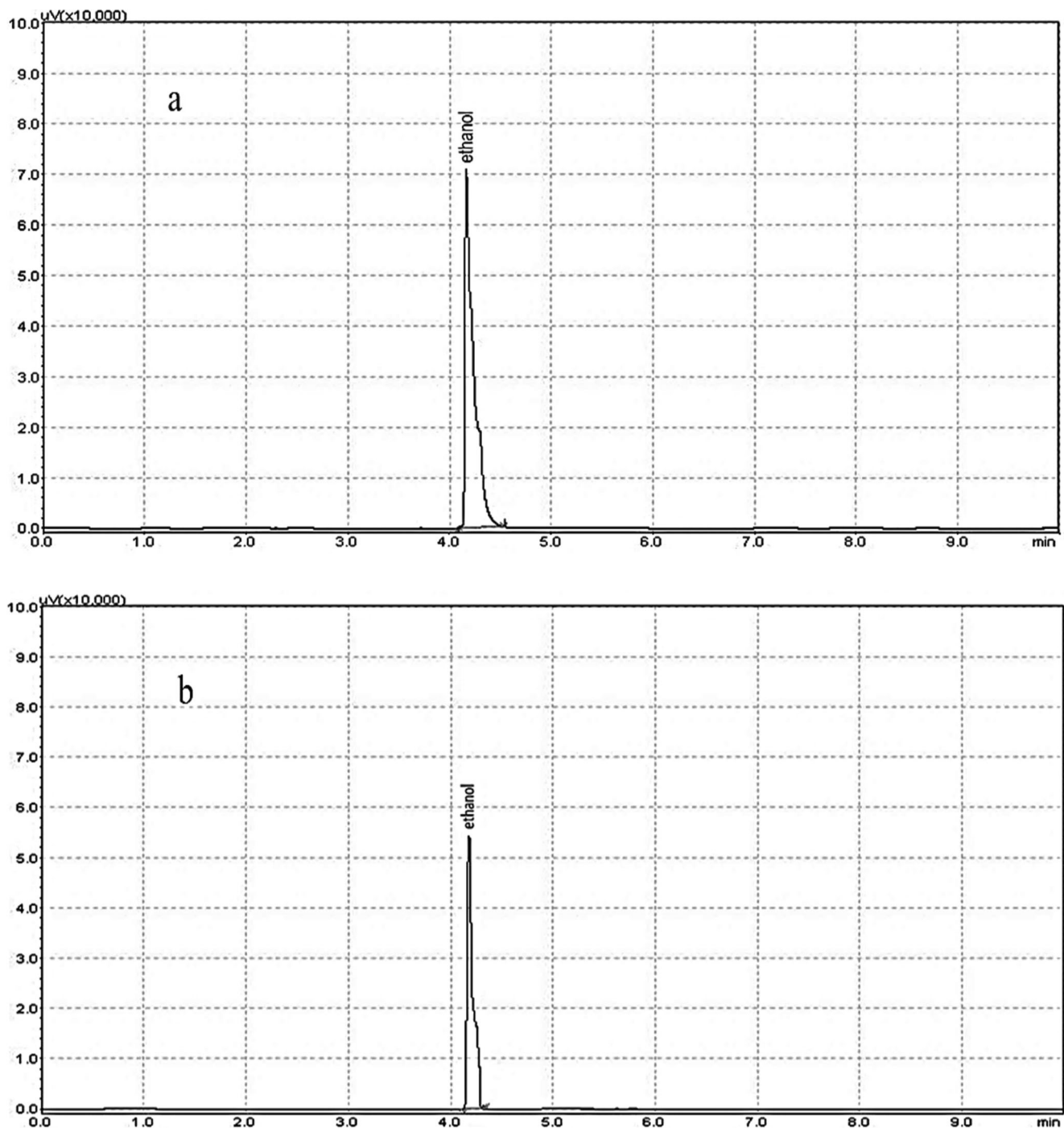
Fig. 5 Some GC spectra examples. (a) Ethanol chromatogram of *K. marxianus* in the presence of WSPP hydrolysed with 14.4 FPU/g substrate enzyme. (b) Ethanol chromatogram of 10 g/L ethanol standard

FPU/g substrate enzyme loading, ethanol contents were 7.79 g/L, 8.08 g/L and 1.01 g/L. In the presence of 7.2 FPU/g substrate enzyme loading, these amounts were 9.48 g/L, 9.89 g/L and 1.30 g/L. Similarly, the WSPP in 3.6 and 7.2 FPU/g substrate enzyme loadings gave the highest value at 12 h for *S. cerevisiae* and *K. marxianus* and 72 h for *P. stipitis*. In the LFPP medium, the highest ethanol concentrations were 11.37 g/L, 12.35 g/L and 2.78 g/L for *S. cerevisiae*, *K.*

marxianus and *P. stipitis* in the presence of the 14.4 FPU/g substrate enzyme. When the enzyme concentration increased to 14.4 FPU/g substrate, the highest ethanol was detected at 24 h for *S. cerevisiae* and *K. marxianus*. In *P. stipitis* maximum, ethanol was detected at the end of 72 h when enzyme loading was 14.4 FPU/g substrate (Table 3; Fig. 2). Petersen et al. (2009) showed that the liquid fraction of the wheat straw contains cellulose and hemicellulose after pretreatment.

Table 4 Kinetic parameters defined for fermentation process at different PP media (enzyme loading 14.4 FPU/g substrate, PP loading 100 g/L, pH 4.8, 100 rpm)

		PP media			
		WSPP	LFPP	WSFPP	N-WSFPP
S. cerevisiae	Q_P, g/L h	0.52	0.47	0.98	0.60
	$Y_{P/S}$, g/g	0.38	0.47	0.50	0.43
	Theoretical yield, %	68.3	80.6	97.8	60.7
K. marxianus	Q_P, g/L h	0.59	0.51	0.99	0.59
	$Y_{P/S}$, g/g	0.45	0.50	0.50	0.43
	Theoretical yield, %	77.3	87.5	98.7	59.5
P. stipitis	Q_P, g/L h	0.05	0.03	0.04	0.02
	$Y_{P/S}$, g/g	0.18	0.17	0.30	0.13
	Theoretical yield, %	28.5	24.7	35.5	15.5

$Y_{P/S}$ and theoretical ethanol yields were calculated according to hexose (glucose) concentration for *S. cerevisiae* and *K. marxianus*. For *P. stipitis* pentose (xylose), concentration was calculated for the determination of the kinetic parameters

Similar results have also been reported by Evcan and Tari (2015) who obtained 8.74 g/L bioethanol from the liquid fraction of the apple pomace.

Ethanol production in WSFPP and N-WSFPP media

In order to examine the effect of washing on ethanol production from PP, solid residues of dilute acid–pretreated PP were washed with water. It was observed that the washing step significantly increased the ethanol amounts for 3 yeasts when compared with non-washed PP. In these set of experiments, the highest ethanol concentrations were detected at the end of the 12-h fermentation time for *S. cerevisiae* and *K. marxianus*, respectively. When WSPP was used, the highest ethanol content was detected at the end of the 24 h. These results revealed that washing of the PP has accelerated the process efficiently.

According to the data stated in Table 3, ethanol concentrations of the washed solid fraction of PP were higher than those of non-washed PP samples as 60.9%, 66% and 127% for *S. cerevisiae*, *K. marxianus* and *P. stipitis*. It can be clearly seen that the washing of PP had a positive effect on ethanol production in all tested yeast strains. Figures 3 and 4 showed the ethanol production from WSFPP and N-WSFPP in 3 yeasts after acid pretreatment and enzymatic hydrolysis of PP. Although N-WSFPP had higher initial reducing sugar concentrations than WSFPP before enzymatic hydrolysis, ethanol concentrations obtained from N-WSFPP (unwashed samples) were lower than those from washed samples (WSFPP) in all cases. The ethanol content of the N-WSFPP was 7.28, 7.14 and 1.48 g/L in 14.4 FPU/g substrate enzyme loading for *S. cerevisiae*, *K. marxianus* and *P. stipitis*, respectively (Fig. 5). This

Table 5 Comparison of the bioethanol production from different lignocellulosic materials

Lignocellulosic material	Pretreatment method	Microorganism	Ethanol yield	Reference
Pomegranate peel	Dilute acid + enzymatic hydrolysis	*K. marxianus*	14.3 g/L	This study
Pomegranate peel	Dilute acid	*S. cerevisiae*	5.5 g/L	(Demiray et al. 2018)
Pomegranate peel	Dilute acid	*K. marxianus*	7.2 g/L	(Demiray et al. 2019)
Eichhornia crassipes	Dilute acid + enzymatic hydrolysis	*S. cerevisiae* + *Z. mobilis*	13.6 g/L	(Das et al. 2016)
Prosopis juliflora wood	Acid + enzymatic hydrolysis	*P. stipitis*	7.1 g/L	(Gupta et al. 2009)
Oil palm frond	Biological + enzymatic hydrolysis	*S. cerevisiae*	13.7 g/L	(Farah Amani et al. 2018)
Cotton gin trash	Dilute acid + enzymatic hydrolysis	*S. cerevisiae*	14.1 g/L	(McIntosh et al. 2014)
Wheat straw	Steam explosion + enzymatic Hydrolysis	*S. cerevisiae*	10.5 g/L	(Horn et al. 2011)

situation may be related to the removal of inhibitory materials (Kumar et al. 2009a). Positive effects of the washing step were also reported in the literature. In a study, ethanol yields were found slightly higher in the two-step washed spruce biomass in comparison with those in the one-step non-washed biomass. It was also reported that hydroxymethylfurfural and furfural concentrations of the washed biomass were 0.88 and 2.53 g/L, respectively. However, in the non-washed biomass, these values were found as 3.71 and 4.23 g/L, respectively (Söderström et al. 2004). In another study, Tomás-Pejó et al. (2009) detected that yeasts were fully inhibited because of toxic compounds when they used whole slurry of wheat straw. On the other hand, 30.2 g/L ethanol was found from washed wheat straw of 14% (w/v) as a substrate in their study. Frederick et al. (2014) investigated the effect of washing pretreated raw material on bioethanol production. Researchers found the ethanol concentration of non-washed biomass as 0.14 and 0.19 g/L for 2 different yeasts. On the other hand, these values were observed as 1.36 and 1.43 g/L when washed biomass was used.

Comparison of the kinetic parameters

The highest Q_p and theoretical ethanol yield of *S. cerevisiae* were 0.98 g/L h and 97.8%, respectively, in the WSFPP medium hydrolysed with 14.4 FPU/g substrate enzyme concentration in this study (Table 4). Singh et al. (2014) reported the Q_p value and the theoretical ethanol yield of the rice husk hydrolysate were 0.36 g/L h and 76.5%, respectively, when they used *S. cerevisiae* for fermentation, which is lower than our results. Furthermore, in another study, the Q_p value of the enzymatically hydrolysed sugarcane bagasse was detected as 0.77 g/L h for *S. cerevisiae* by Santos et al. (2012). Moreover, Romaní et al. (2014) obtained 0.63 g/L h ethanol productivity from *S. cerevisiae* when they used whole slurry of *Eucalyptus globulus* wood.

For *K. marxianus*, maximum Q_p and theoretical ethanol yield values were also detected as 0.99 g/L h and 98.7% in the WSFPP medium (Table 4), which are higher than what is found in most of the reports in the literature. García-Aparicio et al. (2011) reported the Q_p of the enzymatically hydrolysed 10% barley straw as 0.63 g/L h at the end of the 24 h, in the presence of the standard enzyme mixture with the SHF process. Ballesteros et al. (2004) found the maximum theoretical ethanol yield as 71.2% from 100 g/L poplar hydrolysed with 15 FPU/g substrate enzyme.

In this study, lower yields were observed for *P. stipitis* due to its low ethanol production capacity and tolerance to various factors (Song et al. 2019). For *P. stipitis*, the maximum Q_p was detected as 0.05 g/L h from WSPP, and the highest theoretical ethanol yield was obtained as 35.5% WSFPP (Table 4). These results indicate that the washing of the biomass has positively

affected the kinetic parameters which is inconsistent with the literature. For instance, Toquero and Bolado (2014) showed that 45% theoretical ethanol yield is produced from washed dilute acid–pretreated wheat straw in *P. stipitis*, whereas unwashed samples' theoretical ethanol yield was 39.5%. Similarly, in this study, the washing step increased the theoretical ethanol yield of *P. stipitis* from 15.5 to 35.5% (Table 5).

Conclusions

In this study, we effectively hydrolysed pomegranate peels enzymatically and evaluated them for ethanol production. Using the whole slurry resulted in the highest reducing sugar and ethanol concentrations. In our former study, we showed that 5.58 g/L ethanol was obtained by *S. cerevisiae* in the presence of 100 g/L PP. On the other hand, in the current study, we have increased the ethanol production of *S. cerevisiae* to 12.69 g/L. Moreover, it was shown that our laboratory isolate *K. marxianus*, which is better than *S. cerevisiae* for bioethanol production, produced 14.35 g/L ethanol.

It was also observed that *S. cerevisiae* and *K. marxianus* have reached 97.8% and 98.7% of the theoretical ethanol yield when WSFPP was used. However, theoretical ethanol yields of N-WSFPP were lower such as 60.7% and 59.5% for the mentioned yeasts. Thus, these results demonstrate that enzymatic hydrolysis and washing of PP increased the fermentation efficiency significantly.

References

Adney B, Baker J (2008) Measurement of cellulase activities laboratory analytical procedure (LAP) Issue date: 08/12/1996 Measurement of cellulase activities laboratory analytical procedure (LAP). Renew Energy 8:296–301. https://doi.org/10.1016/j.biortech.2006.01.007

Anwar Saeed M, Ma H, Yue S, Qunhui W, Maobing T (2018) Concise review on ethanol production from food waste: development and sustainability. Environ Sci Pollut Res 25:28851–28863. https://doi.org/10.1007/s11356-018-2972-4

Balat M (2011) Production of bioethanol from lignocellulosic materials via the biochemical pathway: a review. Energy Convers Manag 52:858–875. https://doi.org/10.1016/j.enconman.2010.08.013

Ballesteros M, Oliva JM, Negro MJ, Manzanares P, Ballesteros I (2004) Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces marxianus CECT 10875. Process Biochem 39:1843–1848. https://doi.org/10.1016/j.procbio.2003.09.011

Chen X, Shekiro J, Franden MA, Wei W, Min Z, Kuhn E, Johnson DK, Tucker MP (2012) The impacts of deacetylation prior to dilute acid pretreatment on the bioethanol process. Biotechnol Biofuels 5:1–14

Das A, Ghosh P, Paul T, Ghosh U, Pati BR, Mondal KC (2016) Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (Eichhornia crassipes). 3. Biotech 6:1–9. https://doi.org/10.1007/s13205-016-0385-y

Demiray E, Karatay SE, Dönmez G (2018) Evaluation of pomegranate peel in ethanol production by Saccharomyces cerevisiae and Pichia stipitis. Energy 159:988–994. https://doi.org/10.1016/j.energy.2018.06.200

Demiray E, Ertuğrul Karatay S, Dönmez G (2019) Efficient bioethanol production from pomegranate peels by newly isolated Kluyveromyces marxianus. Energy Sources, Part A Recover Util Environ Eff 0:1–10. https://doi.org/10.1080/15567036.2019.1600621

Djelal H, Chniti S, Jemni M, Weill A, Sayed W, Amrane A (2017) Identification of strain isolated from dates (Phœnix dactylifera L.) for enhancing very high gravity ethanol production. Environ Sci Pollut Res 24:9886–9894. https://doi.org/10.1007/s11356-016-8018-x

Evcan E, Tari C (2015) Production of bioethanol from apple pomace by using cocultures: conversion of agro-industrial waste to value added product. Energy 88:775–782. https://doi.org/10.1016/j.energy.2015.05.090

Farah Amani AH, Toh SM, Tan JS, Lee CK (2018) The efficiency of using oil palm frond hydrolysate from enzymatic hydrolysis in bioethanol production. Waste and Biomass Valorization 9:539–548. https://doi.org/10.1007/s12649-017-0005-z

Fernandes MC, Ferro MD, Paulino AFC, Mendes JAS, Gravitis J, Evtuguin DV, Xavier AMRB (2015) Bioresource technology Enzymatic saccharification and bioethanol production from Cynara cardunculus pretreated by steam explosion. Bioresour Technol 186:309–315. https://doi.org/10.1016/j.biortech.2015.03.037

Frederick N, Zhang N, Ge X, Jianfeng X, Matt P, Elizabeth M, Carrier DJ (2014) Poplar (Populus deltoides L.): the effect of washing pretreated biomass on enzymatic hydrolysis and fermentation to ethanol. ACS Sustain Chem Eng 2:1835–1842. https://doi.org/10.1021/sc500188s

García-Aparicio MP, Oliva JM, Manzanares P, Ballesteros M, Ballesteros I, Negro AGMJ (2011) Second-generation ethanol production from steam exploded barley straw by Kluyveromyces marxianus CECT 10875. Fuel 90:1624–1630. https://doi.org/10.1016/j.fuel.2010.10.052

Günan Yücel H, Aksu Z (2015) Ethanol fermentation characteristics of Pichia stipitis yeast from sugar beet pulp hydrolysate: use of new detoxification methods. Fuel 158:793–799. https://doi.org/10.1016/j.fuel.2015.06.016

Gupta R, Sharma KK, Kuhad RC (2009) Separate hydrolysis and fermentation (SHF) of Prosopis juliflora, a woody substrate, for the production of cellulosic ethanol by Saccharomyces cerevisiae and Pichia stipitis-NCIM 3498. Bioresour Technol 100:1214–1220. https://doi.org/10.1016/j.biortech.2008.08.033

Hasnaoui N, Wathelet B, Jiménez-Araujo A (2014) Valorization of pomegranate peel from 12 cultivars: dietary fibre composition, antioxidant capacity and functional properties. Food Chem 160:196–203. https://doi.org/10.1016/j.foodchem.2014.03.089

Horn SJ, Nguyen QD, Westereng B, Nilsen PJ, Eijsink VGH (2011) Screening of steam explosion conditions for glucose production from non-impregnated wheat straw. Biomass and Bioenergy 35:4879–4886. https://doi.org/10.1016/j.biombioe.2011.10.013

Johanningsmeier SD, Harris GK (2011) Pomegranate as a functional food and nutraceutical source. Annu Rev Food Sci Technol 2:181–201. https://doi.org/10.1146/annurev-food-030810-153709

Josefsson A (2013) Effect of process conditions on high solid enzymatic hydrolysis of pre-treated pine

Jung Y, Kim IJ, Kim HK, Kim KH (2013) Dilute acid pretreatment of lignocellulose for whole slurry ethanol fermentation. Bioresour Technol 132:109–114. https://doi.org/10.1016/j.biortech.2012.12.151

Jung HY, In J, Kim J, Kyung H, Kim KH (2014) Whole slurry fermentation of maleic acid-pretreated oil palm empty fruit bunches for ethanol production not necessitating a detoxification process. 659–665. https://doi.org/10.1007/s00449-013-1035-y

Kharytonov M, Pidlisnyuk V, Stefanovska T, Babenko M, Martynova N, Rula I (2019) The estimation of Miscanthus×giganteus' adaptive potential for cultivation on the mining and post-mining lands in Ukraine. Environ Sci Pollut Res 26:2974–2986. https://doi.org/10.1007/s11356-018-3741-0

Kim S, Ho C (2014) Evaluation of whole Jerusalem artichoke (Helianthus tuberosus L.) for consolidated bioprocessing ethanol production. Renew Energy 65:83–91. https://doi.org/10.1016/j.renene.2013.07.025

Kim TH, Lee YY (2005) Pretreatment of corn stover by soaking by soaking in aqueous ammonia. Appl Biochem Biotechnol 121:1119–1131

Kumar P, Barrett DM, Delwiche MJ, Stroeve P (2009a) Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res 48(8):3713–3729

Kumar S, Singh SP, Mishra IM, Adhikari DK (2009b) Ethanol and xylitol production from glucose and xylose at high temperature by Kluyveromyces sp. IIPE 453:1483–1489. https://doi.org/10.1007/s10295-009-0636-6

Lansky EP, Newman RA (2007) Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 109:177–206. https://doi.org/10.1016/j.jep.2006.09.006

Lu Y, Wang Y, Xu G, Chu J (2010) Influence of high solid concentration on enzymatic hydrolysis and fermentation of steam-exploded corn stover biomass. 360–369. https://doi.org/10.1007/s12010-008-8306-0

McIntosh S, Vancov T, Palmer J, Morris S (2014) Ethanol production from cotton gin trash using optimised dilute acid pretreatment and whole slurry fermentation processes. Bioresour Technol 173:42–51. https://doi.org/10.1016/j.biortech.2014.09.063

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030

Naik SN, Goud VV, Rout PK, Dalai AK (2010) Production of first and second generation biofuels: a comprehensive review. Renew Sustain Energy Rev 14:578–597. https://doi.org/10.1016/j.rser.2009.10.003

Narra M, James JP, Balasubramanian V (2015) Bioresource technology Simultaneous saccharification and fermentation of delignified lignocellulosic biomass at high solid loadings by a newly isolated thermotolerant Kluyveromyces sp. for ethanol production. Bioresour Technol 179:331–338. https://doi.org/10.1016/j.biortech.2014.11.116

Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates I & II. Bioresour Technol 74:25–33

Pessani NK, Atiyeh HK, Wilkins MR, Bellmer DD, Banat IM (2011) Simultaneous saccharification and fermentation of Kanlow switchgrass by thermotolerant Kluyveromyces marxianus IMB3: the effect of enzyme loading, temperature and higher solid loadings. Bioresour Technol 102:10618–10624. https://doi.org/10.1016/j.biortech.2011.09.011

Petersen MØ, Larsen J, Thomsen MH (2009) Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass and Bioenergy 33:834–840. https://doi.org/10.1016/j.biombioe.2009.01.004

Pocan P, Bahcegul E, Oztop MH, Hamamci H (2018) Enzymatic hydrolysis of fruit peels and other lignocellulosic biomass as a source of sugar. Waste and Biomass Valorization 9:929–937. https://doi.org/10.1007/s12649-017-9875-3

Roca C, Olsson L (2003) Increasing ethanol productivity during xylose fermentation by cell recycling of recombinant Saccharomyces cerevisiae. Appl Microbiol Biotechnol 60:560–563. https://doi.org/10.1007/s00253-002-1147-9

Romaní A, Ruiz HA, Pereira FB, Teixeira JA, Domingues L (2014) Integrated approach for effective bioethanol production using whole slurry from autohydrolyzed Eucalyptus globulus wood at high-solid loadings. Fuel 135:482–491

Rosas-Burgos EC, Burgos-Hernández A, Noguera-Artiaga L, Kacaniova M, Hernandes-Garcia F, Cardenas-Lopez JL, Carbonell-Barrachina AA (2017) Antimicrobial activity of pomegranate peel extracts as affected by cultivar. J Sci Food Agric 97:802–810. https://doi.org/10.1002/jsfa.7799

Saha BC, Kennedy GJ, Bowman MJ, Qureshi N, Dunn RO (2019) Factors affecting production of itaconic acid from mixed sugars by Aspergillus terreus. Appl Biochem Biotechnol 187:449–460. https://doi.org/10.1007/s12010-018-2831-2

Santos JRA, Lucena MS, Gusmão NB, Gouveia ER (2012) Optimization of ethanol production by Saccharomyces cerevisiae UFPEDA 1238 in simultaneous saccharification and fermentation of delignified sugarcane bagasse. Ind Crops Prod 36:584–588. https://doi.org/10.1016/j.indcrop.2011.10.002

Shi J, Sharma-shivappa RR, Chinn M, Howell N (2009) Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production. Biomass Bioenergy 33:88–96. https://doi.org/10.1016/j.biombioe.2008.04.016

Singh A, Bajar S, Bishnoi NR (2014) Enzymatic hydrolysis of microwave alkali pretreated rice husk for ethanol production by Saccharomyces cerevisiae, Scheffersomyces stipitis and their coculture. Fuel 116:699–702. https://doi.org/10.1016/j.fuel.2013.08.072

Söderström J, Galbe M, Zacchi G (2004) Effect of washing on yield in one- and two-step steam pretreatment of softwood for production of ethanol. Biotechnol Prog 20:744–749

Song Y, Cho EJ, Park CS, Oh CH, Park BJ, Bae HJ (2019) A strategy for sequential fermentation by Saccharomyces cerevisiae and Pichia stipitis in bioethanol production from hardwoods. Renew Energy 139:1281–1289. https://doi.org/10.1016/j.renene.2019.03.032

Talekar S, Patti AF, Vijayraghavan R, Arora A (2018) An integrated green biorefinery approach towards simultaneous recovery of pectin and polyphenols coupled with bioethanol production from waste pomegranate peels. Bioresour Technol 266:322–334. https://doi.org/10.1016/j.biortech.2018.06.072

Tomás-Pejó E, Oliva JM, Ballesteros M, Olsson L (2008) Comparison of SHF and SSF processes from steam-exploded wheat straw for ethanol production by xylose-fermenting and robust glucose-fermenting Saccharomyces cerevisiae strains. Biotechnol Bioeng 100:1122–1131. https://doi.org/10.1002/bit.21849

Tomás-Pejó E, Oliva JM, González A, Ballesteros I, Ballesteros M (2009) Bioethanol production from wheat straw by the thermotolerant yeast Kluyveromyces marxianus CECT 10875 in a simultaneous saccharification and fermentation fed-batch process. Fuel 88:2142–2147. https://doi.org/10.1016/j.fuel.2009.01.014

Toquero C, Bolado S (2014) Bioresource Technology Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. Bioresour Technol 157:68–76. https://doi.org/10.1016/j.biortech.2014.01.090

Tsegaye B, Balomajumder C, Roy P (2018) Biodegradation of wheat straw by Ochrobactrum oryzae BMP03 and Bacillus sp. BMP01 bacteria to enhance biofuel production by increasing total reducing sugars yield. Environ Sci Pollut Res 25:30585–30596. https://doi.org/10.1007/s11356-018-3056-1

Tutt M, Kikas T, Olt J (2012) Influence of different pretreatment methods on degradation of wheat straw. Agron Res 10:269–276

Van Dyk JS, Pletschke BI (2012) A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes — factors affecting enzymes, conversion and synergy. Biotechnol Adv 30:1458–1480. https://doi.org/10.1016/j.biotechadv.2012.03.002

Vohra M, Manwar J, Manmode R, et al (2014) Journal of Environmental Chemical Engineering Bioethanol production: Feedstock and current technologies. Biochem Pharmacol 2:573–584. https://doi.org/10.1016/j.jece.2013.10.013

Wistara NJ, Pelawi R, Fatriasari W (2016) The effect of lignin content and freeness of pulp on the bioethanol productivity of jabon wood. Waste and Biomass Valorization 7:1141–1146. https://doi.org/10.1007/s12649-016-9510-8

Xiros C, Olsson L (2014) Comparison of strategies to overcome the inhibitory effects in high-gravity fermentation of lignocellulosic hydrolysates. Biomass and Bioenergy 65:79–90. https://doi.org/10.1016/j.biombioe.2014.03.060

Yasoubi P, Barzegar M, Sahari MA, Azizi MH (2007) Total phenolic contents and antioxidant activity of pomegranate (Punica granatum L.) peel extracts. J Agric Sci Technol 9:35–42

Zabed H, Sahu JN, Suely A, Boyce AN, Faruq G (2017) Bioethanol production from renewable sources: current perspectives and technological progress. Renew Sustain Energy Rev 71:475–501. https://doi.org/10.1016/j.rser.2016.12.076

Zhang K, Pei Z, Wang D (2016) Organic solvent pretreatment of lignocellulosic biomass for biofuels and biochemicals: a review. Bioresour Technol 199:21–33. https://doi.org/10.1016/j.biortech.2015.08.102

Zhu CP, Zhai XC, Li LQ, Wu XX, Li B (2015) Response surface optimization of ultrasound-assisted polysaccharides extraction from pomegranate peel. Food Chem 177:139–146. https://doi.org/10.1016/j.foodchem.2015.01.022