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Determination of Bioethanol Production from Apricot (*Prunus armeniaca)* Pomace

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HIGHLIGHTS

- Bioethanol production from apricot pomace was reported for the first time.

- Dilute acid and enzymatic hydrolyses can efficiently saccharify the apricot pomace.

- The washing step increased the fermentation efficiency significantly.

- The maximum ethanol was observed as 30.09 g/L from 20% washed apricot pomace.

Abstract: Fruit juice industry generates massive amount of lignocellulosic by-products annually which are excellent raw materials for bioethanol production. In the current study, bioethanol production from apricot (*Prunus armeniaca*) pomace by *Kluyveromyces marxianus* was investigated for the first time. Some key parameters for fermentation such as pretreatment methods, biomass and cellulase loading and time, were optimized. *Kluyveromyces marxianus* produced 30.09 g/L ethanol in the 20% washed apricot pomace and 120 FPU/g cellulose enzyme loading. The highest theoretical yield and $Y_{P/S}$ values were also observed as 94.7% and 0.50 g/g, respectively, when 15 FPU/g cellulose enzyme was used. These results depict that apricot pomace is a promising feedstock for bioethanol production.

INTRODUCTION

Renewable energy sources such as solar, wind or biomass are cheap and sustainable alternatives for fossil based fuels. To diminish the harmful effects of fossil fuels on the environment, comprehensive researches are carried out by scientists. These researches mainly focus on sustainable, renewable and cheap sources such as biofuels. Among these biofuels, bioethanol is considered one of the most promising ones [1,2]. Because bioethanol production from renewable sources can reduce CO_2 emissions up to 90% and reduce the dependence on fossil fuels, its usage in certain areas is a reasonable option for sustainable

energy production [3]. Bioethanol can be obtained from different feedstocks such as corn, wheat, lignocellulose, microalgae or genetically modified crops. Lignocellulosic materials are particularly advantageous for bioethanol production because these feedstocks do not require any additional land usage, and they do not have any impact on fibre or food production chain [4]. Furthermore, lignocellulose, which is abundant, cheap and one of the most underutilized raw material on Earth, does not compete with sugar containing crops such as sugarcane or sugar beet [5]. Cellulose, hemicellulose, and lignin are the three main components of lignocellulose. Lignin creates physical barrier for cellulose and hemicellulose degradation. For this reason, lignocellulose is resistant to disruption. However, to obtain fermentable sugars from cellulose and hemicellulose, this structure has to be pretreated [6,7].

Pretreatment is a significant parameter for bioethanol production. The efficient pretreatment step is necessary to obtain fermentable sugars, which will subsequently be used in fermentation. Dilute acid pretreatment followed by enzymatic hydrolysis is generally considered as one of the most effective methods for degradation of lignocellulose. In this method, sugars derived from hemicellulose release to the slurry while cellulose and lignin are partially disrupted. In this way, the structure of the lignocellulose changes and accessibility of the enzymes to the cellulose increases [8]. However, during dilute acid pretreatment, some inhibitory substances are formed [9]. Although washing of biomass with water is a suitable option for the elimination of these compounds, during the washing step, concentrations of the fermentable sugars decrease [10,11]. Therefore, using the whole slurry can be advantageous for bioethanol production. Whole slurry usage reduces the operational costs and allows it to utilize from a broader range of fermentable sugars [12].

In bioethanol production, determining the optimal enzyme loading is another critical parameter. In the presence of optimal enzyme concentration, fermentable sugars such as glucose have been turned into ethanol more effectively, and general bioethanol production costs are reduced [13].

Another important parameter for bioethanol production is initial biomass loading. Lower biomass loadings result in lower ethanol concentrations. Furthermore, efficient usage of high biomass loadings can reduce water usage, operational and total costs. However, there are some disadvantages of high biomass loadings, such as poor agitation quality, heat transfer limitations, the formation of inhibitors or high viscosity [14]. Therefore, investigations of the suitable materials and microorganisms for bioethanol production in the presence of high biomass loading are essential factors for the process.

K. marxianus, which was used in this current study is a very suitable yeast for bioethanol production from lignocellulosic biomass, because of its fast growth rate and capacity to assimilate a broad range of fermentable sugars in the lignocellulosic materials. In addition to these unique properties, *K. marxianus* can produce adequate amounts of bioethanol in the presence of high biomass loadings [15].

The type of raw material is another crucial parameter for bioethanol production. Therefore, in the current study, apricot pomace was used as feedstock for bioethanol production. Apricot (*Prunus armeniaca*) is an important fruit product of the juice industry. Apricot pomace (AP) has a rich fermentable sugar profile and contains fermentable sugars such as glucose, xylose, galactose and fructose [16]. It is estimated that 20.000.000 tons of apricot have been produced worldwide in 2018 [17]. Furthermore, in 2018, 750.000 tons of apricot were produced in Turkey. It can be calculated that 187.500-412.500 tons of apricot pomace (AP) were generated in the same year [18]. After the apricot processing, a considerable amount of fermentable sugars remain in the apricot pomace (AP). In a study, it was shown that 47 g/L glucose was recovered from apricot pomaces [19]. In another study, sucrose, glucose and fructose contents of the 13 different apricot cultivars were determined between 31.53-85.63 g/kg, 14.93-54.65 g/kg and 10.61-44.29 g/kg, respectively [20]. Because of its rich fermentable sugar content, AP provides a suitable growth environment for biotechnologically important microorganisms. For instance, it has been shown that *Aspergillus sojae* was able to produce a high amount of polygalacturonases, such as 380 U/mL in the presence of apricot pomace and orange peel [21]. In another study, Dulf and coauthors [22] showed the phenolic, flavonoids, antioxidant compounds production potentials of *Aspergillus niger* and *Rhizopus oligosporus* in the presence of AP as a carbon source. However, there is no report about the usage of fermentable sugars of AP for bioethanol production in the literature. Therefore, considering its potential, AP was used as a raw material for bioethanol production for the first time in the current study. To the best of our knowledge, this is the first report which shows that AP is a promising growth medium for bioethanol producer microorganisms.

For these reasons which are mentioned above, dilute acid pretreatment, and enzymatic hydrolysis were performed to obtain fermentable sugars from AP. *K. marxianus*, which is a good bioethanol producer, was used for fermentation experiments. Critical parameters such as pretreatment methods (washed fraction and the whole slurry of AP), fermentation time, sugar consumption profile of raw material, initial biomass loading (5%, 10% and 20%) and enzyme loading were optimized.

Furthermore, it is reported that the ethanol and sugar concentrations should be above 4% and 8%, respectively, for commercial bioethanol production [23]. It is seen in the literature that these values are generally can be obtained when the raw material concentration is above 20%. In the current study, 3.81% (30.09 g/L) bioethanol was obtained. The goal of this work is to suggest a commercially available raw material and fermentation agent to the literature. By this context, this study indicates that AP is promising raw material for commercial ethanol production.

MATERIAL AND METHODS

Microorganisms and fermentation media

AP was obtained from Erkon Nar Meyve Suyu Ltd./Niğde/Turkey. AP was dried in an oven overnight at 70 ºC. After that step, dried AP was kept in screw cap bottles and directly used in the experiments. *K. marxianus* was provided from Ankara University Culture Collection. Until experiments yeast were kept at slant PDA (potato dextrose agar) medium at 4 ºC. Before fermentation experiments, yeast was inoculated to GPY medium (glucose 20 g/L, peptone 10 g/L, yeast extract 3.0 g/L) for 24 hours.

Pretreatment of AP

Sulfuric acid (1% H_2SO_4) was used for all pretreatment experiments. For pretreatment three different AP loading (5%, 10%, 20%) were tested. 1% H_2SO_4 (v/v) was added to AP and autoclaved immediately at 121 ºC for 15 min. The effect of different AP media compositions (the whole slurry of AP and washed fraction of AP) on bioethanol production was also examined. The whole slurry contained liquid and solid AP residues together. For the washing step, AP was filtered through Whatman No:1 paper, and solid residues were washed with tap water until AP reached neutral pH value. These two AP media and three different biomass loadings were used in fermentation experiments. All experiments were performed at 250 mL ErlenMeyer flasks working volume with 100 mL.

Enzymatic hydrolysis of AP

CellicCTec 2 (Sigma/USA), a commercial cellulase, was used for enzymatic hydrolysis. To determine the effect of different enzyme loading on bioethanol production, four different enzyme concentrations (15, 30, 60 and 120 FPU/g cellulose) were added to AP media. After pretreatment, the pH of the AP media were adjusted to 4.8 in the presence of 50 mM citrate buffer. Enzymatic hydrolysis was carried out in 50 ºC for 72 hours [24]. The agitation speed was 100 rpm. In order to stop enzymatic hydrolysis and obtain stable sugar yield, AP media were boiled at the water bath for 10 min.

Fermentation assays

For fermentation, 5 g/L peptone, 3 g/L yeast extract, 0.5 g/L $MgSO_4.7H_2O$, 1 g/L KH_2PO_4, 0.1 g/L $CaCl_2$ and 0.05 g/L $ZnSO_4$ were supplemented to AP media. Seperate hydrolysis and fermentation (SHF) experiments were carried out. By this reason, fermentation was started after enzymatic hydrolysis. All fermentation experiments were carried out at 30 ºC for 72 hours. The agitation speed was 100 rpm Fermentation experiments were performed at 100 mL Erlenmeyer flasks working volume with 50 mL. All experiments were performed as triplicate.

Analytical methods

Ethanol concentrations were detected with gas chromatography (GC2010-Shimadzu/Japan). Before measurements, 2 mL samples were centrifuged at 11180 g for 10 min. The supernatant was filtered through 0.22 membrane filter and 1 µL sample was injected through the injection port. Restek Rtx-Wax column (60 m length, 0.25 mm id.) and flame ionization detector (FID) was used for ethanol determination. The temperature of the injection port and detector were set as 140 ºC and 160 ºC respectively. The initial column temperature was 50 ºC, and the column temperature was increased to 150 ºC within 19 min. Column flow was 1.86 mL/min, and nitrogen was used as carrier gas [25].

HPLC (Shimadzu) system equipped with BioRad Aminex 87-P column using an isocratic mobile phase (5 mM H_2SO_4) and refractive index detector (RID) were used for the determination of the sugar profile of AP. Before analysis, 2 mL samples were centrifuged at 11180 g for 10 min. The supernatant was filtered through

0.22 membrane filter. The column oven temperature was held as 80 ºC, and the total flow was set at 0.6 mL/min. Samples were analyzed for 30 min [26].

Total reducing sugar was determined with the DNS method (Miller 1959). Filter paperase unit (FPU) of the enzyme was determined according to method described by Adney and Baker [27].

Theoretical ethanol yields were calculated according to the Equation (1) which presented below [28].

$$\text{Eq. (1): } Theoretical\ ethanol\ yield\ (\%) = \frac{ethanol\ (g/L)}{initial\ sugar\ (g/L) \times 0.511} \times 100$$

Volumetric ethanol productivity (Q_p) was calculated according to the Equation (2), as described elsewhere [29].

$$\text{Eq. (2): } Q_p(g/Lh) = \frac{ethanol\ (g/L)}{h_{\text{maximum ethanol}}}$$

Ethanol yield ($Y_{P/S}$) was determined according to the Equation (3) [30]:

$$\text{Eq. (3): } Y_{P/S}\ (g/g) = \frac{maximum\ ethanol\ (g/L)}{consumed\ sugar\ (g/L)}$$

Cellulose amount of AP was determined by an external laboratory, namely Düzen Norwest/Ankara.

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. The analysis of variance (ANOVA) was performed in order to determine the effect of different AP pretreatments, type of sugar and enzyme loading. All experiments were performed as triplicate.

RESULTS AND DISCUSSION

Fermentable sugar composition of AP before enzymatic hydrolysis

AP is an important by product of the food industry. In the literature, there are some reports about the structural composition of AP. In one of them, cellulose content of the apricot cell wall was found between 16.9-22.2 g/100 g in the unpeeled apricots. Moreover, in the peeled apricots, these values varied from 18.5 to 21.6 g/100 g [31]. In our current study, the cellulose amount of the raw AP was found as 21.5%. The relative sugar percentages of the AP was shown in Table 1. It is clearly seen that the most abundant sugar of the AP was found glucose with 52% relative percentage. Ucuncu and coauthors [32] determined the highest total reducing sugar concentration of dilute acid pretreated crude apricot pomace as 49.16%. They also found that glucose and fructose are the dominant sugars of AP. Other fermentable sugars of the AP were determined as galactose (1.7%), fructose (11.5%), arabinose (26.5%) and xylose (8.3%), respectively.

Table 1. Relative sugar percentages of apricot pomace after dilute acid pretreatment (Pre-treatment conditions: 121 ºC with 1% H_2SO_4 for 15 min. Biomass loading: 20%).

Sugar Type	Relative Percentage (%)
Glucose	52.0
Galactose	1.7
Fructose	11.5
Arabinose	26.5
Xylose	8.3

Effect of pretreatment and initial AP loading on bioethanol production

In order to investigate the effect of initial AP loading and different AP media compositions on bioethanol production, whole slurry and washed fractions of AP were tested with three different initial AP loadings (5%, 10%, 20%). To determine the appropriate biomass amount and pretreatment type, 15 FPU/g cellulose enzyme loading was used in all fermentation media preparing with increasing AP loading. During enzymatic hydrolysis, the highest concentration of sugars in both media were obtained at the end of the 72 hours in the presence of 20% initial biomass loading. It was also observed that the whole slurry of AP had higher sugar concentrations when compared with the washed fraction of AP in all initial AP loadings. Sugar concentrations of 5%, 10% and 20% of whole slurry were 32.0 g/L, 66.78 g/L and 110.15 g/L, respectively. On the other hand, lower sugar concentrations such as 22.61 g/L, 30.70 g/L and 45.09 g/L were obtained when the 5%,

10% and 20% solid fractions of AP were washed respectively (Table 2). The data depicts that, before enzymatic hydrolysis, no sugar was detected in the washed fraction of AP. However, 16.05 g/L, 30.17 g/L and 57.39 g/L sugar were obtained when 5%, 10% and 20% of the whole slurry of AP were used.

Table 2. Sugar concentrations of increasing washed and whole slurry apricot pomace (Pretreatment conditions: 121 ºC with 1% H_2SO_4 for 15 min. Enzyme loading: 15 FPU/g cellulose, pH: 4.8, T: 50 ºC).

	Biomass Loading (%)	Sugar Concentration (g/L)			
		Before enzymatic hydrolysis	After enzymatic hydrolysis		
			24 h	48 h	72 h
Washed fraction of AP	5	ND	8.12±0.2[a]	15.72±0.2[d]	22.61±1.2[g]
	10	ND	16.90 ±0.1[b]	23.17±0.5[e]	30.70±0.3[h]
	20	ND	29.76±0.3[c]	35.98±1.1[f]	45.09±0.8[i]
Whole slurry of AP	5	16.05±1.5[a]	22.09±0.6[d]	28.11±0.8[g]	32.00±2.4[j]
	10	30.17±2.2[b]	38.83±3.2[e]	43.76±0.4[h]	66.78±2.1[k]
	20	57.39±0.9[c]	71.08±2.4[f]	83.78±1.7[i]	110.15±4.3[l]

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

In contrast to sugar concentrations, in this part of the study, higher ethanol amounts were obtained when washed fraction of AP was used. Furthermore, in all biomass loadings, the highest ethanol concentrations were obtained at the end of the 24 hours fermentation time. After 24 hours, it was observed that almost all fermentable sugars of the washed fraction of AP were depleted, and ethanol contents were decreased in all biomass loadings. In washed fraction, ethanol concentrations increased with the increased initial AP loading and the maximum ethanol concentration was detected as 20.9 g/L in the presence of 20% washed fraction AP. The highest ethanol concentrations were 7.95, and 12.19 g/L in 5% and 10% washed fraction of AP at the end of the 24 hours, respectively (Figure 1A). On the other hand, in the whole slurry, the maximum ethanol was detected as 13.28 g/L in the presence of 10% initial AP loading, and ethanol content significantly decreased in the presence of 20% whole slurry. In the presence of 5% and 20% initial AP loadings, maximum ethanol concentrations of the whole slurry of AP were observed as 8.24 and 4.45 g/L, respectively. Similar to the washed fraction of AP, ethanol concentrations were decreased after 24 hours in the whole slurry of AP. However, fermentable sugars were not consumed completely (Figure 1B). Because the highest ethanol concentrations were obtained from 20% washed slurry of AP, further studies were carried out with 20% washed slurry.

In this part of the study, significant inhibition was observed in increased whole slurry biomass loadings. Similar results were observed in a previous study that compared bioethanol production from the whole slurry of spruce. It was shown that higher biomass loadings resulted in lower ethanol concentrations. In the presence of 5% biomass and 10 FPU/g substrate, loadings ethanol concentration was 18.1 g/100g. On the other hand, ethanol concentration decreased to 2.4 g/100g when the initial biomass loading increased to 7.5% in the presence of the same enzyme loading [33]. In another study, *K. marxianus* activity was totally inhibited in the presence of 5% whole slurry of barley straw, however, when the 14% (w/v) washed barley straw was used for fermentation, bioethanol production of *K. marxianus* reached to 30.2 g/L [34]. Furthermore, in some previous reports, it was shown that the pretreatment of 20% washed biomass resulted in good ethanol performance inconsistency with our findings. Dong and coauthors (2018) [35], for instance, showed that the ethanol concentration of the 20% washed momentary pine slurry was 15.5% higher than non washed samples.

Higher theoretical ethanol yields and Q_p values in all biomass loadings were determined when the AP was washed. The highest theoretical ethanol yield was 90.7% when the 20% washed AP was used. On the other hand, the theoretical ethanol yield was only 7.9% in the 20% whole slurry of AP. The maximum Q_p value was found as 0.87 g/L.h in the presence of the washed fraction of AP. In the whole slurry of AP, Q_p value decreased to 0.18 g/L.h. Similarly, the highest $Y_{P/S}$ was obtained from the washed fraction of AP as 0.47 g/g, while the $Y_{P/S}$ of the whole slurry of AP was 0.29 g/g (Table 3).

Furthermore, *K. marxianus* reached 68.8%, 79.3% of the theoretical ethanol yields in the presence of 5% and 10% washed fraction of AP, respectively. Same values at the whole slurry were 50.3%, 77.0% for 5% and 10% AP loading respectively. Q_p values of the 5% and 10% washed fraction of AP were 0.33, 0.50

g/L.h. On the other hand, in the whole slurry of AP, these values were 0.17, 0.50 g/L.h, for the same initial AP loadings. *K. marxianus* yielded 0.36, and 0.43 g/g ethanol for the 5% and 10% washed fraction of AP. $Y_{P/S}$ values for the whole slurry were 0.43 and 0.43 g/g in the presence of 5% and 10% AP, respectively (Table 3).

Table 3. Kinetic parameters of *K. marxianus* in the presence of increasing washed and whole slurry apricot pomace (Pretreatment conditions: 121 ºC with 1% H_2SO_4 for 15 min. Enzyme Loading: FPU/g cellulose. pH 4.8, T: 30 ºC).

	Biomass loading (%)	Theoretical ethanol yield (%)	Q_P (g/L.h)	$Y_{P/S}$ (g/g)
Washed fraction of AP	**5**	68.8	0.33	0.36
	10	79.3	0.50	0.43
	20	90.7	0.87	0.47
Whole slurry of AP	**5**	50.3	0.17	0.43
	10	77.0	0.50	0.43
	20	7.90	0.18	0.29

In a previous report, the theoretical yield of washed samples of dilute acid pretreated wheat straw was found as 45% in *Pichia stipitis*, whereas unwashed sample's theoretical yield was only 39.5% [36]. Liu and Chen [37] reported that theoretical ethanol yields of *Saccharomyces cerevisiae* were 87% and 76% when the steam-exploded (1.5 MPa for 6 min) 20% washed and unwashed corn stover were used respecively in the presence of 15 FPU/g substrate. In another study, total inhibition was observed at the *K. marxianus* when the non washed wheat straw used. On the other hand, in the presence of 12% washed wheat straw, theoretical ethanol yield, Q_p and $Y_{P/S}$ values of the *K. marxianus* were calculated as 59.7%, 0.73 g/Lh and 0.30 g/g respectively [38]. Similarly, in this part of the study, the highest theoretical ethanol yield, Q_p and $Y_{P/S}$ values, were found as 90.7%, 0.87 g/Lh and 0.47 g/g for *K. marxianus* respectively in the presence of 20% washed fraction of AP.

Effect of enzyme loading on bioethanol production

It is important to reduce overall bioethanol production costs. By this context, determining the optimal enzyme loading is a critical step for the overall cost of the process [39]. In the current study, to determine the effect of increasing enzyme concentrations on bioethanol production, four different enzyme loadings (15, 30, 60, 120 FPU/g cellulose) were tested on 20% washed fraction of AP. Enzymatic hydrolysis was performed for 72 hours, and sugar concentrations of increased enzyme loadings were monitored periodically. Final sugar concentrations obtained after enzymatic hydrolysis with increasing enzyme concentrations were shown in Table 4. The initial sugar concentrations were found as 20.91 g/L, 24.17 g/L, 29.25 g/L and 32.64 g/L, at 15, 30, 60 and 120 FPU/g cellulose enzyme loadings, respectively at the end of the 24 hours of enzymatic hydrolysis and it was observed that sugar concentrations increased during the enzymatic hydrolysis day by day. Additionally, increased enzyme concentrations resulted in increased sugar concentrations. The highest sugar concentration was obtained in the presence of 120 FPU/g cellulose as 70.78 g/L. Sugar concentrations of the 15, 30 and 60 FPU/g cellulose were 41.17, 52.80 and 64.35 g/L, respectively. There was no significant difference between sugar concentrations of 60 FPU and 120 FPU enzyme contained groups.

Table 4. The effect of increasing enzyme loadings on sugar concentrations of washed apricot pomace (Pretreatment conditions: 121 ºC with 1% H_2SO_4 for 15 min. Biomass loading: 20% AP, pH: 4.8, T: 50 ºC).

Enzyme Loading (FPU/g cellulose)	Sugar concentration (g/L)		
	24 h	48 h	72 h
15	20.91±2.1[a]	31.88±0.4[d]	41.17±4.5[f]
30	24.17±1.8[a]	35.99±1.9[d]	52.80±3.8[f]
60	29.25±0.4[b]	42.02±2.8[e]	64.35±2.4[g]
120	32.64±0.9[c]	48.22±3.1[e]	70.78±3.7[g]

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

Sugar profiles of AP with the increased enzyme loadings were given in Table 5 and glucose percentages of 20% AP, which hydrolyzed with 15 FPU, 30 FPU, 60 FPU and 120 FPU were found as 48.6%, 51.6%, 55.6% and 60.2%, respectively. According to the Table, high enzyme loadings such as 60 and 120 FPU/g

cellulose resulted in significantly higher glucose amounts in comparison to 15 and 30 FPU/g cellulose. This situation can be attributed to excessive enzyme requirement of high biomass loadings [40].

Table 5. The effect of increasing enzyme concentrations on relative sugar percentages of washed apricot pomace (Pretreatment conditions: 121 ºC with 1% H_2SO_4 for 15 min. Enzymatic hydrolysis conditions: 20% washed fraction of apricot pomace, pH: 4.8, T: 50 ºC).

Sugar Type	Relative sugar percentage (%)			
	15 FPU	30 FPU	60 FPU	120 FPU
Glucose	48.6±0.4[a]	51.6±1.2[a]	55.6±0.3[b]	60.2±0.6[c]
Galactose	3.3±0.2[a]	3.0±0.1[a]	2.9±0.4[a]	2.4±0.1[a]
Fructose	19.6±0.7[a]	16.7±1.1[a]	16.3±0.5[a]	11.0±1.6[b]
Arabinose	8.4±0.2[a]	9.4±0.6[a]	7.0±0.7[a]	8.3±1.1[a]
Xylose	20.1±0.6[a]	19.3±1.4[a]	18.2±0.6[a]	18.1±1.3[a]

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

In a study about pretreatment and enzymatic hydrolysis of rice straw, it was found that increased enzyme and biomass concentrations increased the sugar yields. In the presence of 15% biomass and 20 FPU/g, reducing sugar was found as 0.45 g/g biomass. On the other hand, when the enzyme concentrations were increased to 80 FPU/g in the same biomass loading, reducing sugar yield were found as 0.55 g/g. By this way, four times higher enzyme loading caused 22.0% higher sugar yield [41]. Similarly, in our study, 120 FPU/g cellulose gave 34.0% higher sugar concentrations than 30 FPU/g cellulose.

Ethanol and reducing sugar concentrations of 20% washed AP media hydrolyzed with different enzyme loadings were shown in Figure 2. It was detected that ethanol concentrations increased with the increased enzyme loadings, and maximum ethanol concentrations were obtained at the end of the 24 hours. After 24 hours, ethanol concentrations decreased. It was also observed that 60 FPU/g cellulose and 120 FPU/g cellulose enzyme loading gave similar ethanol concentrations. The highest ethanol was detected as 30.09 g/L in the presence of 120 FPU/g cellulose. On the other hand, the ethanol concentration of the 60 FPU/g cellulose enzyme loading was 29.49 g/L. In 15 and 30 FPU/g cellulose enzyme loading, 19.93 and 25.07 g/L ethanol was found respectively. Narra and coauthors [42] also reported that the highest ethanol concentrations were obtained in the presence of higher enzyme loadings. Researchers tested three different enzyme concentrations (6, 9, 12 FPU/g substrate) in rice straw, wheat straw and sugarcane bagasse for bioethanol production from *K. marxianus* higher ethanol concentrations were observed at 9 FPU/g substrate and *12* FPU/g substrate than 6 FPU/g substrate.

In 120 FPU, ethanol and sugar concentrations were higher than 15, 30 and 60 FPU enzyme loadings (Figure 2). In a previous report, It was shown that increased enzyme loadings resulted in increased ethanol concentrations. The highest ethanol concentration was observed as 21.0 g/L from potato peel wastes at 6% (w/v) enzyme loading, which was the highest amount used [43].

The maximum theoretical ethanol yield was observed at 15 FPU/g cellulose as 94.7%. On the other hand, in the presence of 30, 60 and 120 FPU/g cellulose enzyme loadings, *K. marxianus* reached 92.9%, 89.6% and 83.2% of the theoretical yield, respectively. A slight decrease was observed at the theoretical ethanol yields with the increased enzyme loadings (Table 6).

A similar observation was reported previously by Han and coauthors [44]. The authors found that in the presence of 20 FPU/g cellulose and 30 CBU/g *β*-glucosidase enzyme loading, the initial glucose concentration of cassava stem was 12.62 g/L and the theoretical ethanol yield was 93.11%. On the other hand, theoretical yield decreased to 89.6% when initial glucose concentration increased to 15.51 g/L.

Contrary to theoretical yield, the highest volumetric productivity rate (Q_p) was detected in the presence of 120 FPU as 1.25 g/L.h because of its higher sugar concentration. Q_p values of 15, 30 and 60 FPU/g cellulose were 0.83, 1.04 and 1.22 g/L.h, respectively (Table 6). Similarly, in 20% barley straw loading, which is enzymatically hydrolyzed, it was observed that the Q_p value was 0.547 g/L.h. When enzyme loading was increased, the same value increased to 0.968 g/L.h [45]. In another study, Rivera and coauthors [39] showed that the Q_p value of the sugarcane bagasse hydrolyzed with 17.4 FPU/100 mL cellulase was 0.230 g/L.h. When the enzyme loading increased to the 90 FPU/100 mL, Q_p was found as 0.273 g/L.h.

There was no significant difference between $Y_{P/S}$ values of the *K. marxianus* in the presence of increased enzyme loadings. In a study about bioethanol production from cassava peel, $Y_{P/S}$ was found as 0.37 g/g in the presence of 10% biomass loading which hydrolyzed with 100 FPU/g substrate enzyme, when the biomass

loading increased to 20% and hydrolyzed with same enzyme concentration, $Y_{P/S}$ was observed as 0.35 g/g [46].

In a study in the literature, researchers found $Y_{P/S}$ values as 0.461, 0.46 and 0.46 g/g respectively in the presence of 50, 100 and 150 g/L glucose supplemented sugarcane bagasse chips media [47]. Theoretically, 0.51 g ethanol can be obtained from 1 g glucose. In the current study, the highest $Y_{P/S}$ value was observed as 0.50 g/g in the growth media, which contains only AP as a sole carbon source. Therefore, our results depict that the fermentable sugars of AP were turned into ethanol effectively by *K. marxianus* (Table 6).

Table 6. Effect of increasing enzyme concentrations on kinetic parameters of *K. marxianus* (Pretreatment conditions: 121 ºC with 1% H_2SO_4 for 15 min. Biomass loading: 20% washed fraction of apricot pomace, pH: 4.8, T: 30 ºC).

Enzyme Loading (FPU/ g cellulose)	Theoretical Ethanol Yield (%)	Q_P (g/L.h)	$Y_{P/S}$ (g/g)
15	94.7	0.83	0.50
30	92.9	1.04	0.49
60	89.6	1.22	0.49
120	83.2	1.25	0.47

CONCLUSIONS

In this study, AP was effectively hydrolyzed with enzymatically and evaluated for the bioethanol production. It is crucial to determine the suitable raw materials for the renewable energy industry. By this context, we examined the effect of pretreatment methods (whole slurry and washed fractions of AP) and enzyme concentrations on fermentation in order to increase bioethanol concentrations using by *K. marxianus.* The highest ethanol was obtained from the washed fraction of apricot pomace in the presence of 120 FPU as 30.09 g/L with the 1.25 g/ L.h volumetric productivity. On the other hand, in the presence of 60 FPU, 29.49 g/L ethanol was found with 1.22 g/L.h productivity, which is very close to those obtained from 120 FPU. Furthermore, the highest theoretical ethanol yield and $Y_{P/S}$ were found as 94.7% and 0.50 g/g in the presence of 15 FPU, respectively. Our results showed that AP could be a suitable feedstock for commercially bioethanol production because of its high bioethanol production capacity.

Conflict of Interest: The authors declare no conflict of interest.

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