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ORIGINAL ARTICLE

Acid hydrolysis optimization of pomegranate peels waste using response surface methodology for ethanol production

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Abstract

Agro-industrial wastes can be processed into valuable products. Successively, current investigation is an effort to optimize the acid hydrolysis of pomegranate peels waste (PPW) using central composite design (CCD) of response surface methodology (RSM) for ethanol production. Concentration of sulfuric acid, temperature, and time of hydrolysis were used as dependent variables, whereas reducing sugars, total carbohydrates, extractives, weight loss, hemicellulose, cellulose, and lignin contents were recorded as responses for PPW decomposition. The highest glucose level of 0.56 ± 0.04 mg mL^{-1} (with 5% acid concentration at 100 °C for 30 min) and carbohydrate contents of 1.53 ± 0.07 mg mL^{-1} (with 3% acid concentration at 75 °C for 45 min) were obtained. Subsequently, detoxification of hydrolysate was conducted employing 2.5% activated charcoal that reduced 62% of phenolic compounds. Detoxified hydrolysate was subjected to fermentation by ethanologenic yeasts: *Metschnikowia* sp. Y31, *Metschnikowia cibodasensis* Y34, and *Saccharomyces cerevisiae* K7 for 10 days experiment. Significant ethanol yield of 0.42 ± 0.08 g g^{-1} was noticed by *Metschnikowia* sp. Y31 on day 5 and 0.41 ± 0.07 g g^{-1} for *Metschnikowia cibodasensis* Y34 on day 2. The results demonstrated the hopeful prospect for bioethanologenesis using cellulosic wastes at marketable level.

Keywords Bioenergy · Biofuels · Biomass valorization · Fruit wastes · Statistical optimization · Waste management

1 Introduction

Waste management measures comprise of all the proceedings requisite to handle waste from its collection to disposal. The indecent disposal of wastes consequences the unhygienic environment that leads to pollution. Incineration and land filling are the general practices executed for waste management in Pakistan [1–3]. To reduce the piles of agro-industrial wastes, 3 R (reduce, reuse, recycle) strategy was also proposed to recuperate energy and management of municipal waste. Although these strategies can lessen the amount of waste to a huge amount, they also cause significant ecological contamination [4–6]. Alternately, an excellent way of waste management is its conversion into such valuable products that can be further used for mankind. In this regard, production of biofuels such as bioethanol from waste biomass, in the current scenario of depleting fossil fuels' reservoirs, can play a pivotal role in boosting the country's economy [7–9].

The main part of agricultural wastes comprises biodegradable substances that are composed of lignocellulosic biomass (LCB) [10]. Bioethanol produced through fermentation of the abovementioned wastes while utilizing microorganisms can be considered the chief liquid biofuel and an alternative additive to gasoline [11, 12]. Ethanol is a highly demanded fuel worldwide. Mixing of bioethanol and gasoline lessens the emission of greenhouse gasses to about 40–50% [13]. Being an agricultural country, abundant fruit wastes are produced in Pakistan throughout the year. The fruit wastes can be used as a potential feedstock for the production of bioethanol. Alternately, if the generated fruit wastes are not disposed of properly, they can lead to severe environmental issues [14].

Pomegranate is a tropical fruit and cultivated in Iran, Afghanistan, Northern India, Pakistan, Russia, Azerbaijan,

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California, and Mediterranean region. According to Food Agricultural Organization (FAO), approximately 1.5 million tons of waste is produced annually during industrial processing of pomegranates and has immense nutritional values. Pakistan is cultivating pomegranate in 13,000 ha, and the annual pomegranate fruit yield in Pakistan appeared about 0.5 million tons in 2010–2011 [15–17].

Basically, pomegranate fruit consists of peel, seeds, and aril. The exocarp/peel of a mature pomegranate fruit weighs up to 500 g kg^{-1}. The weight of edible part and arils reaches 100 and 400 g kg^{-1}, respectively [18–20]. The chief components of arils are water (80–85%), sugars (predominately C6 sugars: 10–14% glucose and fructose), few organic acids (ascorbic acid, malic acid, and citric acid), and other bioactive compounds (anthocyanins, antioxidants, pectin, phenolics, etc.) [21].

All parts of the pomegranate plant (roots, stem, leaves, and fruit) are utilized to treat various diseases. The plant consists of different dyes, alkaloids, antioxidants, flavonoids, and tannins [22, 23]. Pomegranate peels waste (PPW) is a good source of phenolic (tannins and flavonoids) compounds that contribute to antioxidant activity [24]. Due to having antioxidant potential, PP extract is used in food recipes, animal feedstocks, cosmetics, tinctures, and certain therapeutic formulae [25]. The compositional analysis of PP revealed the percent presence of proteins (5.1), fats (2.4), total sugars (30.5), crude fibers (12.61), insoluble fibers (30.003), lignin (29.4), phenolic compounds (40.53), tannins [26], and minerals [26–29]. PPW is used for biorefineries to get value-added products. Waste is modified physically, chemically, and microbially to get industrially important products such as dietary fibers, color pigments, dyes, medicinal components, and bioactive compounds. The modified PPW is also used for solvent extraction and as heavy metal absorbent. Dried PPW serves as substrate for the production of certain enzymes, biogas, and single-cell proteins [29].

Different statistical approaches have been employed in the last few years to optimize various steps involved in the pretreatment of LCB and production of different enzymes and biofuels [30–34]. Statistical approach of optimization proves to be a quicker and economical approach and provides real optima for the desired results [35].

A critical step for the production of bioethanol from LCB is the scarcity of efficient hydrolysis for releasing fermentable sugars [36]. The methodology of dilute acid hydrolysis for LCB proved to be an efficient and economical way to degrade biomass [37–39]. However, the drawback of this technique is the production of inhibitors such as hydroxyl methyl furfural (HMF), phenolic compounds, and acetic acid that reduces the growth of organisms used to ferment ethanol. Various methods have been used to enhance the yields of sugars and decrease the amount of toxins using diverse types of detoxification procedures [40]. Activated charcoal adsorption can prove to be very useful to palliate the inhibitors of hydrolysates, thus improving the microbial growth [41]. The focus of the current study was to develop low-cost ethanologenesis by using PPW. Initially, the biomass hydrolysis, being extremely a critical step, was optimized through central composite design (CCD) of response surface methodology (RSM) by Design Expert Software. The pretreated and detoxified biomass hydrolysate was then subjected to ethanol production via fermentative yeast keeping in view the incredible worth of some previously reported fermentative yeast species *Metschnikowia* sp. and *Metschnikowia cibodasensis* [42]. The low-cost ethanol production from wastes of pomegranate can be highly valuable not only for sustainable energy production but also for effective waste management.

2 Materials and methods

2.1 Proximate compositional analysis of PPW

PPW was collected from various locations in Lahore, Punjab, Pakistan, and rinsed with water. The waste was then placed in a hot-air oven at 60 °C for 2 days. The substrate after drying was ground and sieved to obtain fine powder (particle size ~ 1 mm). Different compositional contents such as carbohydrates, lipids, proteins, and reducing sugars were then estimated following phenol sulfuric acid method [43], method of Zollner and Kirsch [44], method of Folin Ciocalteu [45], and DNS method [46], respectively. The ash and moisture contents were estimated following protocols of AOAC [47]. Lignin, cellulose, and hemicellulose contents were measured by following the method proposed by Lin et al. [48].

2.2 Dilute sulfuric acid hydrolysis optimization by CCD

Sulfuric acid was used in dilute form to hydrolyze PPW. Peels and acid ratio for hydrolysis was 1:10. The reaction was carried out in conical flasks of 100-mL capacity covering with aluminum foil. Three parameters, i.e., acid concentration, hydrolysis temperature, and time, were executed by CCD into 20 runs. Experiments were performed in triplicates. The mixture in the flasks was agitated in shaking incubator at 100 rpm for specified temperature and time. After completion of reaction, the mixture was filtered and neutralized by NaOH pellets to keep the volume same. The neutralized mixture was filtered again and proceeded for detoxification step.

The PPW hydrolysis was executed using CCD by design expert software (version 6.0.8) for obtaining the highest reducing sugar contents that were vital for bioethanol production through fermentation [49].

Experimental plan for three dependent factors, viz., acid concentration (X_1, %) along with hydrolysis temperature

Table 1 Coded values of variables for central composite design of acid hydrolysis of pomegranate peels

Variable	Actual value of coded level			
	Coded symbol	Low level	Center point	High level
Acid concentration (%)	X_1	1	3	5
Hydrolysis temperature (°C)	X_2	50	75	100
Hydrolysis time (min)	X_3	30	45	60

(X_2, °C) and time (X_3, min), was described in Table 1, while 20 runs of the experimental design were shown in Table 2. The model was designed based on low and high levels for parameters with central points as 3% (X_1), 75 °C (X_2), and 45 min (X_3). The dependent parameters were selected on the basis of previous research emphasizing the influential impact of some key factors affecting biomass hydrolysis [50–53]. The subsequent optimization of the parameters for acid saccharification of PPW by RSM was performed in this investigation.

The following general quadratic equation "Y" [1] illustrated the relationship of input variables and different responses [reducing sugars (Y_1) and total carbohydrates (Y_2)] with the help of RSM as:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + e \quad (1)$$

where

Y = predicted response

β_0 = constant coefficient

$\beta 1$, $\beta 2$, and $\beta 3$ = linear coefficients

$\beta 11$, $\beta 22$, and $\beta 33$ = quadratic coefficients

$\beta 12$, $\beta 13$, and $\beta 23$ = cross products coefficients

X_1, X_2, and X_3 = input variables

e = residual error between the observed Y and the prediction ($\hat{Y}$)

Table 2 Central composite design matrix of three independent variables for responses of acid hydrolysis of pomegranate peels

Run no.	Acid conc. X_1 (%)	Hydrolysis temperature X_2 (°C)	Hydrolysis time X_3 (min)
1	3	75	45
2	5	50	30
3	5	100	60
4	5	100	30
5	1	100	30
6	3	75	45
7	5	50	60
8	3	75	45
9	1	100	60
10	1	50	30
11	1	50	60
12	3	75	45
13	6.36	75	45
14	0.36	75	45
15	3	75	70.23
16	3	75	45
17	3	75	19.77
18	3	75	45
19	3	117.04	45
20	3	32.96	45

2.3 Saccharification of PPW hydrolysate

After chemical hydrolysis, the PPW hydrolysate obtained was processed for the estimation of reducing and non-reducing sugars. The saccharification of PPW was estimated by the following formula [54]:

$$\text{Saccharification yield } \left(\text{mg mL}^{-1}\right) = \frac{\text{Reducing sugars in hydrolysate}}{\text{Reducing sugars in PPW}}$$

2.4 Detoxification of PPW hydrolysate

Some toxic phenolic compounds are considered an obstacle for microbial strains to ferment sugars. Thus, hydrolysate detoxification was well thought out a compulsory step prior to fermentation. For the purpose, PPW hydrolysate was detoxified with 2.5% activated charcoal [55]. The charcoal was removed with filter paper after agitation at 200 rpm for 1 h at 30 °C. Then it was centrifuged at 2000 rpm for 20 min. The supernatant was neutralized with pellets of NaOH. Total phenolic estimation of the hydrolysate was carried out by Folin-Ciocalteu method as described by Gonzalez et al. [56].

2.5 Production of ethanol from PPW hydrolysate

Bioethanol production was carried out by *S. cerevisiae* K7, *Metschnikowia* sp. Y31, and *M. cibodasensis* Y34 strains for optimum saccharification. *Saccharomyces cerevisiae* K7 granted by the Brewing Society in Japan (Tokyo, Japan) was considered standard yeast strain. *Metschnikowia* sp. Y31 and *M. cibodasensis* Y34 (isolated from flowers) have been evaluated previously for ethanol production [42].

The synthetic medium was prepared by following the protocol used by Bonciu et al. [57]. For the culturing of ethanologenic yeasts, Malt Yeast Glucose (MYG) medium was prepared for different inocula. All the yeast strains were revived separately in MYG medium (10 mL) by incubating overnight at 30 ± 0.2 °C.

The fermentation medium was composed of 5 mL of yeast as inoculum in 45 mL of the synthetic medium and 50 mL of the detoxified hydrolysate with the condition of having maximum reducing sugar contents as evaluated previously. The inoculated fermentation medium was placed in incubator without agitation for 10 days at 30 ± 0.2 °C and taken out after every 24-h interval on regular basis and evaluated for variations in reducing sugar contents and ethanol production. All the experiments were performed in triplicates.

Reducing sugars were measured by DNS method, and acid dichromate test was performed for ethanol estimation [58]. The growth of fermentative microorganisms was measured spectrophotometrically (CE-2041UK) by examining optical densities of fermentation media at 600 nm [59].

2.6 Statistical analysis

All runs in CCD for optimization experiment were performed in triplicates. For CCD data analysis, Design Expert Software (ver. 6.0.8 Software, Stat-Ease, Minneapolis, MN 55413) was used by following ANOVA and regression for the response surface quadratic model. The experimental data obtained from fermentation experiments were analyzed by one-way ANOVA following Duncan multiple range test (SPSS Version. 16.0. Software, Chicago, IL, USA).

3 Results

3.1 Proximate compositional analysis of PPW

The compositional analysis of PPW showed that it has good potential for the growth of microorganisms (Table 3). PPW has been appeared as good a substrate for ethanologens due to the presence of fermentable sugars in considerable amount [60, 61]. Cellulose and hemicellulose contents present in PPW were also previously subjected to chemical and enzymatic hydrolysis for better ethanol production by different microbes [62]. This study depicted 0.767 ± 0.08 mg mL^{-1} of moisture contents, 0.235 ± 0.01 mg mL^{-1} reducing sugars, 0.786 ± 0.016 mg mL^{-1} total sugars, 0.033 ± 0.002 mg mL^{-1} total lipids, and 0.166 ± 0.005 mg mL^{-1} total proteins in PPW in addition to hemicellulose (2.93 ± 0.126 mg mL^{-1}), lignin (1.43 ± 0.125 mg mL^{-1}), and cellulose (3.53 ± 0.030 mg mL^{-1}) contents.

Table 3 Compositional analysis of pomegranate peels

Parameter	Quantity
Moisture content (%)	7.67 ± 0.08
Reducing sugar content (g L^{-1})	23.5 ± 0.01
Total carbohydrate (g L^{-1})	78.6 ± 0.01
Total lipid (g L^{-1})	3.3 ± 0.001
Hemicellulose content (%)	29.30 ± 1.26
Lignin content (%)	14.3 ± 0.12
Cellulose content (%)	35.3 ± 0.03
Ash content (%)	12.4 ± 0.002

3.2 Dilute sulfuric acid hydrolysis optimization by CCD

For optimization of acid hydrolysis of biomass, different parameters were investigated and tabulated with statistical interpretation of the model (Table 4). The quadratic regression (Eq. (2)) for the release of reducing sugars (Y_1) was:

$$Y_1 = 0.38 - 0.040\,X_1 + 0.053\,X_2 - 0.055\,X_3 + 0.003\,X_1^2 - 0.020\,X_2^2 + 0.0069\,X_3^2 - 0.0016\,X_1X_2 - 0.0064\,X_1X_3 - 0.053\,X_2X_3 + 0.00045 \quad (2)$$

The plus signs represented the synergistic association, whereas minus signs indicated antagonistic relations among variables. In determining the relationship between the response Y and the factors Y = f (X1, X2) in the statistical model, X1, X2 showed linear interaction, while $X1^2$, $X2^2$ exhibited quadratic relationship. In the current study, the equation is useful for identifying the relative impact of hydrolysis parameters, i.e., acid concentration (X1), hydrolysis temperature (X2), and hydrolysis time (X3), on reducing sugars (Y1) by comparing the factor coefficient. Individual factor, for instance, acid concentration ($-0.040\,X_1 + 0.053\,X_2$), increased the level of reducing sugars by hydrolyzing PPW. It did not have any interaction effects with other factors studied in the experiment, thus showing synergistic relationship. Similarly, same rule applies for negative signs.

Table 4 Central composite design matrix of three independent variables for reducing sugars, total carbohydrates, weight loss, extractive, hemicellulose, lignin, and cellulose contents by sulfuric acid hydrolysis of pomegranate peels

Run no.	Acid conc. (%)	Temp (°C)	Time (min)	Reducing sugars (g L^{-1})	Total carbohydrates (g L^{-1})	Weight loss (%)	Extractives (%)	Hemicellulose (%)	Soluble lignin (%)	Crude cellulose + insoluble lignin (%)
1	3	75	45	33.1 ± 0.05	121.5 ± 0.01	68.11 ± 0.22	19.87 ± 0.72	24.76 ± 1.25	26.61 ± 1.30	28.76 ± 1.86
2	5	50	30	27.0 ± 0.01	123.3 ± 0.01	66.00 ± 0.39	20.67 ± 4.17	20.35 ± 2.10	23.80 ± 1.52	35.18 ± 2.44
3	5	100	60	48.1 ± 0.03	118.2 ± 0.04	68.22 ± 0.73	15 ± 0.97	25.51 ± 0.21	28.72 ± 0.85	30.77 ± 1.97
4	5	100	30	56.3 ± 0.04	122 ± 0.03	71.89 ± 0.68	20.53 ± 1.18	15.91 ± 1.26	32.11 ± 0.84	31.44 ± 0.99
5	1	100	30	29.2 ± 0.04	98.6 ± 0.09	62.89 ± 0.40	17.95 ± 0.72	26.27 ± 1.57	23.79 ± 1.83	31.99 ± 2.70
6	3	75	45	31.1 ± 0.07	100.9 ± 0.03	73.89 ± 6.23	16.9 ± 0.40	19.09 ± 0.36	26.67 ± 1.06	37.34 ± 1.16
7	5	50	60	44.6 ± 0.02	117.3 ± 0.14	68.44 ± 0.62	17.59 ± 0.65	18.41 ± 1.09	29.34 ± 2.15	34.66 ± 1.84
8	3	75	45	29.2 ± 0.03	119.1 ± 0.08	68.56 ± 0.80	15.53 ± 0.33	19.72 ± 1.44	28.21 ± 1.07	36.54 ± 2.17
9	1	100	60	40.4 ± 0.04	138.2 ± 0.02	62.22 ± 0.95	16.83 ± 1.36	21.02 ± 2.24	24.19 ± 1.27	37.97 ± 2.04
10	1	50	30	42.1 ± 0.11	105.8 ± 0.08	64.78 ± 0.44	19.57 ± 1.41	20.74 ± 0.89	34.62 ± 1.54	25.07 ± 1.09
11	1	50	60	40.3 ± 0.08	150.3 ± 0.07	61.00 ± 0.19	15.39 ± 0.93	18.47 ± 1.79	35.47 ± 3.23	30.67 ± 2.34
12	3	75	45	31.2 ± 0.06	137.3 ± 0.09	74.11 ± 1.93	20.06 ± 0.81	23.13 ± 2.17	24.26 ± 1.91	32.55 ± 4.25
13	6.36	75	45	38.2 ± 0.07	104.8 ± 0.26	77.33 ± 0.19	20.09 ± 0.83	20.84 ± 1.50	24.85 ± 1.87	34.22 ± 3.09
14	0.36	75	45	28.0 ± 0.03	119.1 ± 0.06	56.11 ± 0.44	22.52 ± 0.90	17.97 ± 0.34	20.69 ± 3.12	38.81 ± 2.42
15	3	75	70.23	30.1 ± 0.02	136.9 ± 0.19	72.56 ± 0.44	19.85 ± 0.63	19.68 ± 0.47	23.87 ± 4.51	36.60 ± 4.48
16	3	75	45	33.3 ± 0.03	1.44 ± 0.02	69.00 ± 0.88	17.52 ± 0.95	19.56 ± 0.61	26.97 ± 3.74	35.95 ± 4.25
17	3	75	19.77	39.4 ± 0.08	150.0 ± 0.16	68.78 ± 0.11	21.36 ± 0.68	14.04 ± 0.62	25.28 ± 0.49	39.32 ± 1.77
18	3	75	45	29.5 ± 0.09	150.3 ± 0.03	68.00 ± 0.19	20.49 ± 0.70	17.46 ± 0.25	27.5 ± 0.86	34.55 ± 0.22
19	3	117.04	45	36.3 ± 0.02	121.2 ± 0.21	68.44 ± 0.73	18.31 ± 0.09	24.11 ± 1.47	21.45 ± 3.78	36.12 ± 2.52
20	3	32.96	45	35.5 ± 0.00	120.7 ± 0.10	67.56 ± 0.22	19.86 ± 0.30	26.07 ± 0.88	20.7 ± 3.70	33.37 ± 3.04

All values represent the mean of triplicates ± SEM

The linear interactive effect between X1 and X2 on Y1 and Y2 corresponds to the B3 slope. If B3 is positive (the interactive effect is positive), then it means that X2 is more positive, and the effect of X1 on response becomes more positive. This is interpreted as synergistic association. Alternatively, the more negative X2 is, the more negative effect of X1 on Y becomes, i.e., antagonistic effect. These effects are also dependent on high/low levels of X1 or high/low levels of X2. In this study, linear relationship of two factors (+X1X2, X2X3, X1X3) mutually had positive effect on reducing sugars in saccharification process as explained above.

The optimum experimental and predicted value (g L^{-1}) was 52.3 ± 0.01 and 47, respectively, at 3% of H_2SO_4 concentration at 100 °C temperature for 30 min of hydrolysis. The highest reducing sugar contents observed were 56.3 ± 0.04 g L^{-1} at 5% H_2SO_4 concentration at 100 °C for 30 min. The reliability of model was explored by ANOVA (Table 5) by RSM. The F value of model was 4.42, and the p value was 0.0187 showing significance of the model. The values of coefficient of R^2 and Adj R^2 appeared as 0.8154 and 0.6308, respectively (Table 6). For "Adeq Precision," a value of 8.782 was calculated. Basically, R-squared (R^2) is a statistical measure that represents the proportion of the variance for a dependent variable that is explained by an independent variable or variables in a regression model. R-squared values range from 0 to 1 and are commonly stated as percentages from 0 to 100%. The R-squared values such as $0.3 < r < 0.5$, $0.5 < r < 0.7$, and $r > 0.7$ are generally considered weak/low, moderate, and strong effect size. The values for R^2 were calculated by regression using Design Expert Software (ver. 6.0.8 Software, Stat-Ease, Minneapolis, MN 55413). Usually, the larger the R^2, the better the regression model fits the observations. In the current study, the R^2 value was 0.8154 for response Y1.

Adequate precision measures the signal-to-noise ratio. It compares the range of the predicted values at the design points to the average prediction error. A ratio greater than 4 is desirable. The present investigation showed ratios of 8.782 for Y1, which indicated an adequate signal to confirm that the model can be used to navigate the design space. A ratio greater than four is desirable. Adequate precision was calculated by regression analysis using design expert software. In the current model, value 8.782 for Y1 was calculated.

Response surface graph exhibited the outcome of optimum value and variables by response using sulfuric acid hydrolysates. Figure 1a showed an increase in reducing sugar contents because of a decrease in the acid

Table 5 Single factor ANOVA ($p < 0.05$) of fitted quadratic regression model for reducing sugars and total carbohydrates examined by sulfuric acid treated hydrolysates

Content	Source	Sum of Suqares	*df	Mean square	*F* value	*p* value
Reducing sugar	Model	0.13	9	0.015	4.42	0.0187
	Residual	0.030	9	0.00332		Significant
	Lack of fit	0.029	5	0.00589	51.84	0.0010
	Pure error	0.00045	4	0.00011		Significant
	Cor total	0.17	19			
Total carbohydrate	Model	0.48	9	0.054	3.27	0.0463
	Residual	0.15	9	0.016		Significant
	Lack of fit	0.10	5	0.021	1.87	0.2814
	Pure error	0.044	4	0.011		Not significant
	Cor total	0.70	19			

*df is the degree of freedom that attributes to the blocks and generally equate one less the blocks number

concentration as well as an increase in temperature of hydrolysis. Figure 1b depicted a decrease in reducing sugars with increase in both acid concentration plus time. The results elucidate that increase in temperature exhibited sharp elevation in contents of reducing sugars, whereas increased time showed slight elevation (Fig. 1c).

Total carbohydrates (Eq. (3)) were also observed in PPW hydrolysate and demonstrated by an equation that explained the effect of different variables.

$$\begin{aligned} Y_2 = {} & 1.40 + 0.036\ X_1 \\ & + 0.012\ X_2 - 0.093\ X_3 - 0.051\ {X_1}^2 - 0.039\ {X_2}^2 \\ & + 0.046\ {X_3}^2 + 0.039\ X_1\ X_2 + 0.035\ X_1\ X_3 \\ & + 0.17\ X_2\ X_3 + 0.044 \end{aligned} \quad (3)$$

The more positive symbols are the indications for a consistent equation. The experimental value recorded for total carbohydrates was 1.12 ± 0.01 mg mL^{-1}, while the predicted value was 1.21 mg mL^{-1} at optimum conditions. The maximum total carbohydrates were 1.53 ± 0.07 mg mL^{-1} with 3% sulfuric acid concentration for 45 min at 75 °C. The model was significant with *F* value (3.27) and *p* value (0.0463), while R^2 (0.7656) and Adj R^2 (0.5312) were also calculated. In the current study, the R^2 value was 0.7656 for response Y2, i.e., total carbohydrates after saccharification. The model accounts for 76.56% of the variance/variations that indicated reliability in predicting increase in carbohydrates after saccharification. The more variance of model indicated that the data points will fall closer to the fitted regression line. The model explained a lot of variation within the data and is significant. The three parameters in the model supported for the response.

Table 6 Exploitation of different fruit wastes for the production of bioethanol

Lignocellulosic material	Pretreatment method	Microorganism	Ethanol yield	Reference(−s)
Pomegranate peels	Dilute sulfuric acid	*Saccharomyces cerevisiae* K7	0.42 ± 0.08 g g^{-1} (12.76%)	This study
Pomegranate peels	Dilute sulfuric acid	*Metschnikowia* sp. Y31	0.41 ± 0.07 g g^{-1} (12.18%)	This study
Pomegranate peels	Dilute sulfuric acid	*Metschnikowia cibodasensis* Y34	0.44 ± 0.09 g g^{-1} (11.89%)	This study
Pomegranate peel	Dilute acid	*Saccharomyces cerevisiae*	5.5 g L^{-1} (0.5%)	Demiray et al. [62]
Pomegranate peel	Dilute acid + enzymatic hydrolysis	*Kluyveromyces marxianus*	14.3 g L^{-1} (1.43%)	Demiray et al. [63]
Orange peels	Dilute sulfuric acid + enzymatic hydrolysis (potato dextrose agar for enzyme production)	*Saccharomyces cerevisiae* for 1 week	19.17%	Maina et al. [64]
Apple pomace	Dilute acid + enzymatic hydrolysis	*Saccharomyces cerevisiae* + *Zymomonas mobilis*	134 g/kg dry apple pomace (i.e., 13.4%)	Magyar et al. [65]
Date palm	Sulfuric acid	*Saccharomyces cerevisiae*	19.7%	Boulal et al. [66]
Mango peels	Dilute sulfuric acid + enzymatic hydrolysis	*Saccharomyces cerevisiae*	9.68%	Arumugam and Manikandan [67]
Banana peels	Dilute sulfuric acid + enzymatic hydrolysis	*Saccharomyces cerevisiae*	13.84%	Arumugam and Manikandan [67]

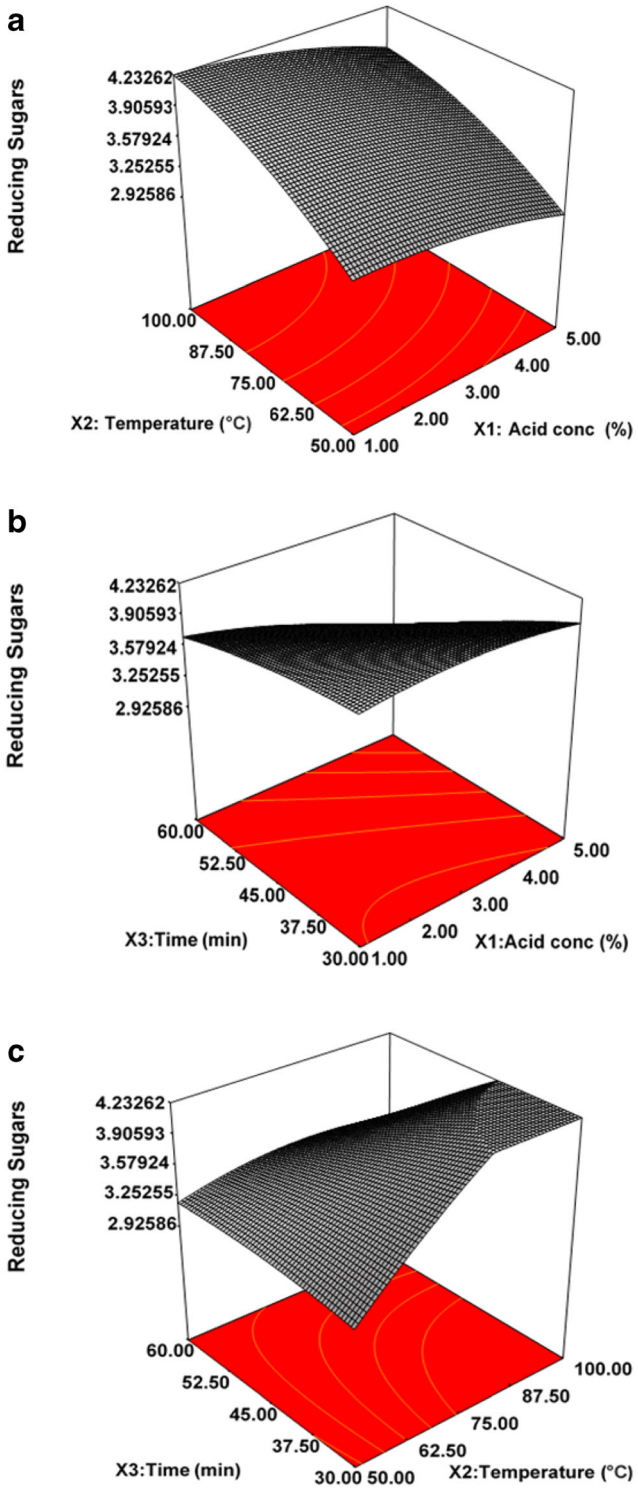

Fig 1 .Response surface plot for reducing sugars (mg mL^{-1}) in PPW from various treatments of sulfuric acid concentration with different hydrolysis temperature (**a**), time (**b**), and temperature with varying time (**c**)

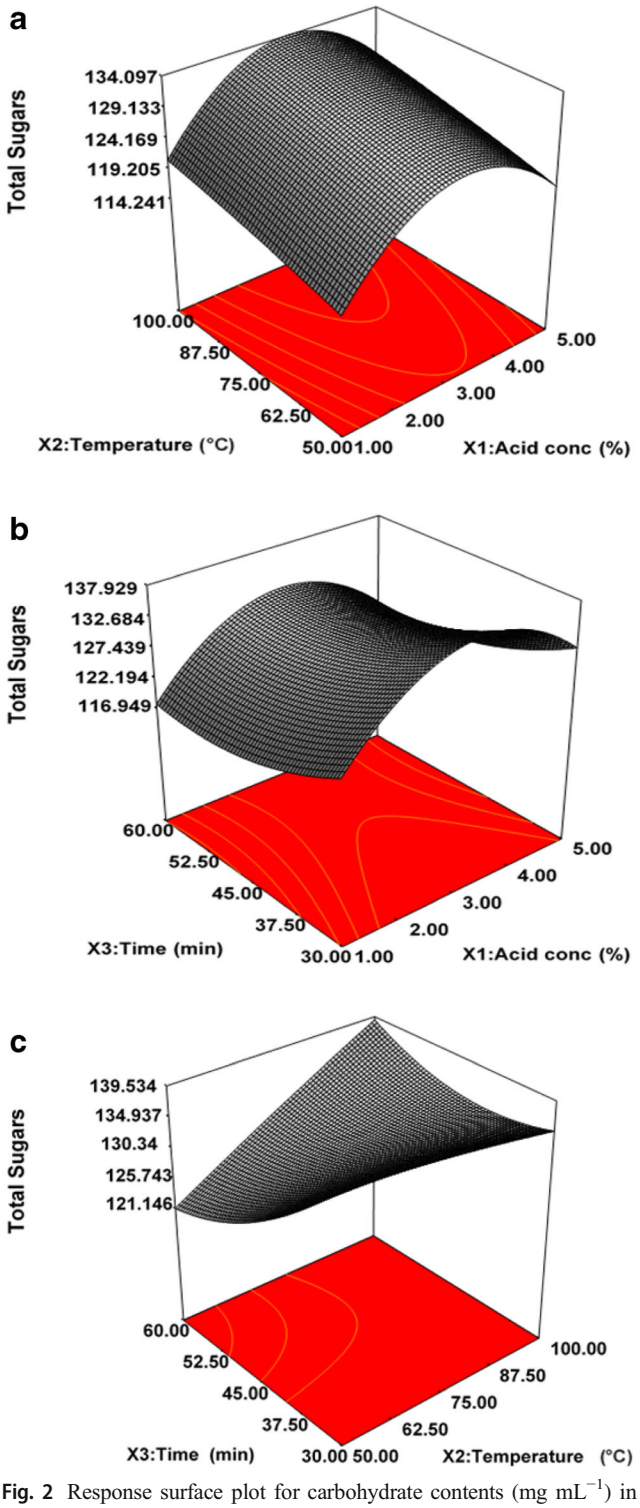

Fig. 2 Response surface plot for carbohydrate contents (mg mL^{-1}) in PPW from various treatments of sulfuric acid concentration with different hydrolysis temperature (**a**), time (**b**), and temperature with time (**c**)

Statistical methods are required to ensure that data are interpreted correctly and that apparent relationships are meaningful (significant) and not simply chance occurrences. The significance of model helps to interpret hypothesis that cellulosic and hemicellulosic biomass of PPW was hydrolyzed into monomers (reducing sugars). The reducing sugars were subjected to fermentation for ethanologenesis. The values of F, p,

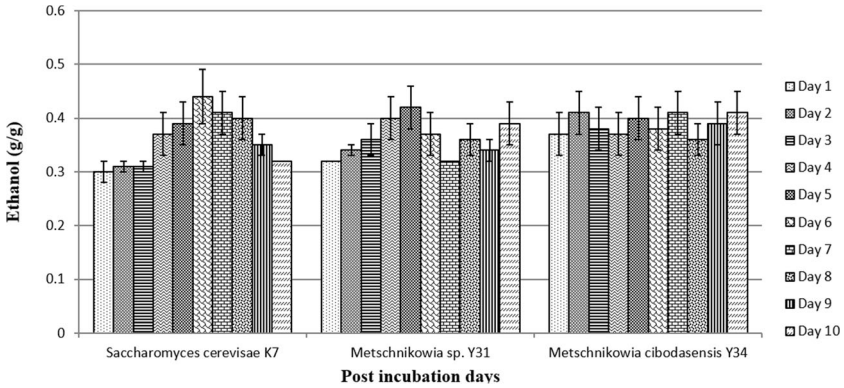

Fig. 3 Ethanol production (mg mL^{-1}) by *S. cerevisiae* K7, *Metschnikowia* sp. Y31, and *M. cibodasensis* Y34 isolates using PPW hydrolysate

R-squared, and adequate precision will help to interpret the significance of model. The desired values will be > 4 for *F* and adequate precision, near to 1 for R^2, and < 0.05 for probability. The model's values for *F* (4.42), *p* (0.0187), coefficient of R^2 (0.8154), and adequate precision (8.782) indicated the significance of model and predicted the optimum values for hydrolysis.

It can been seen from the Fig. 2a that increase in sugar contents was achieved by increasing the temperature. In case of studying the effect of acid concentration, maximum total sugars were recorded at 3% H_2SO_4. The sugar yield decreased above/below this value. Figure 2b showed that slight increase in carbohydrates contents was obtained by increasing hydrolysis time, whereas sharp increase was obtained at 4% acid concentrations. Figure 2c exhibited that maximum carbohydrates contents were recruited at initial values of hydrolysis temperature and time (i.e., 50 °C, 30 min) and increase in both parameters resulted in decreased carbohydrate contents.

3.3 Percent saccharification and detoxification of PPW hydrolysate

Percent saccharification yield of reducing sugars estimated after hydrolysis was 2.26%. The 62% reduction in phenol contents was estimated after detoxification by using 2.5% activated charcoal. The amount of phenolic compounds (mg mL^{-1}) in PPW hydrolysate before detoxification was 1.50 ± 0.01, and the amount lessened to 0.93 ± 0.06 after detoxification.

3.4 Production of ethanol from PPW hydrolysate

Maximum ethanol yield (0.42 ± 0.08 g g^{-1}) was noticed with *Metschnikowia* sp. Y31 at day 5 and 0.41 ± 0.07 g g^{-1} with *M. cibodasensis* Y34 at days 2, 7, and 10. The *S. cerevisiae* K7 manifested 0.44 ± 0.09 g g^{-1} ethanol yield at day 6 (Fig. 3). In terms of percentage, 12.18, 11.89, and 12.76% contents of ethanol were obtained from three yeast isolates, i.e., *Metschnikowia* sp. Y31, *M. cibodasensis* Y34, and *S. cerevisiae* K7, respectively. The reduction in reducing sugar contents was noticed day wise because of ethanol bioproduction (Fig. 4). Stability in growth of yeast (*Metschnikowia* sp. Y31 and *M. cibodasensis* Y34) subsequent to day 5 and 7 envisaged that these organisms could be promising candidates as they have the capacity to tolerate ethanol (Fig. 5).

4 Discussion

The waste management performance through recycling has been increased and being used extremely in various countries

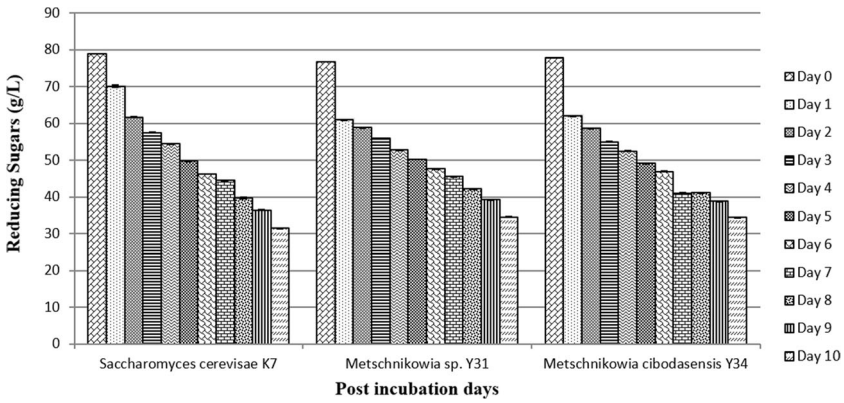

Fig. 4 Periodic reduction in reducing sugars (g L^{-1}) by *S. cerevisiae* K7, *Metschnikowia* sp. Y31, and *M. cibodasensis* Y34

nowadays [68]. The chief renewable fuel to meet the measurement of waste management is ethanol [69]. In the current study, management of LCB after pretreatment with dilute acid then fermentation with yeast isolates for the production of ethanol was carried out.

Dilute acid pretreatment was performed with sulfuric acid. The same pretreatment was also reported by Jennings and Schell [70] to break down the cellulose and hemicellulose into simple sugars. The CCD was applied by Design Expert Software for the sake of optimization of conditions for hydrolysis. The same design was reported for the optimization to produce bioethanol from glycerol [71] and wastes of citrus fruits [72].

The highest level of reducing sugars was 52.3 ± 0.10 g L^{-1} measured at 3% sulfuric acid concentration at 100 °C for 30 min, while the value predicted was 47.95 g L^{-1}. The *F* value (4.42) and *p* value (0.0187) showed that the model was significant. The reducing sugars were increased because acidic pretreatment of PPW resulted in conversion of cellulose and hemicellulose into soluble sugars [73]. Previously, similar results with maximum release of reducing sugar contents (56.07 g L^{-1}) with 2.0% HCl concentration for 45 min were reported for the hydrolysis of durian peels [74]. In durian peels, glucose was the main sugar released after pretreatment. According to Aguilar et al. [75], acid hydrolysis helped to release glucose along with other sugars in the liquor. Dilute acid pretreatment not only converted hemicellulose into monomers but also caused structural changes to form hollow porous zones in lignocellulosic biomass to be made accessible for better enzymatic activity for cellulose [76–78].

The optimum carbohydrate contents (1.12 ± 0.01 mg mL^{-1}) were observed at the same optimum condition. The predicted value (1.21) in case of carbohydrate contents was close to the experimental value. The results were significant having the *F* value and *p* value of 3.27 and 0.0463, respectively. Unhasirikul et al. [74] reported the increase in reducing sugars, total sugars, and acid hydrolysis efficiency by performing HCl hydrolysis with durian peels. In the present investigation, the increase in acid concentration contributed to release more sugars (g L^{-1}), i.e., 52.3 ± 0.01 (3%) and 56 ± 0.04 (5%) at 100 °C for 30 min of sulfuric acid hydrolysis. The increase in acid leads to release more sugar and produce more inhibitors by degradation of sugars into hydroxymethylfurfural (HMF) and furfural and levulinic acid [79–82]. The production of toxic compounds such as furans and phenols that inhibit microbial fermentation of sugars could be removed by using suitable detoxification methods like activated charcoal treatment [55]. By performing acid hydrolysis, lignin is partially hydrolyzed into phenolics and became the part of fermentation medium by retaining in hydrolysate. Consequently, acid hydrolysis of lignocellulose resulted in formation of inhibitors that have negative impact on microbial metabolism in fermentation. The concentration of inhibitors not only affects the end product of fermentative metabolism but sometimes blocks the process completely [83, 84]. The production of inhibitors at low temperature is lower than the higher temperature for same time of hydrolysis. At higher temperature, the sugar degradation rate into inhibitors is high in hydrolysis. But at temperature higher than 100 °C, HMF, furfural, and phenolics are destructed along with sugar degradation. This effect is likely to increase by increasing hydrolysis time [85].

The growth rate of yeasts in hydrolysate showed the same log phase up to day 8 then stationary phase on days 9 and 10. Significant ethanol yield was recorded in log phase from day 5 to day 7. A study reported that the high substrate inhibited the growth rate of yeast because of high medium osmolality [86]. The significant amount of ethanol (0.42 ± 0.08 g g^{-1}) was observed on day 5 by *Metschnikowia* sp. Y31, while it was 0.44 ± 0.09 g g^{-1} on day 6 by *S. cerevisiae* K7. In terms of percentage, 12.18, 11.89, and 12.76% contents of ethanol were obtained from three yeast isolates, i.e., *Metschnikowia* sp. Y31, *M. cibodasensis* Y34, and *S. cerevisiae* K7, respectively. These results are comparable with the previous findings (Table 6). In the present study, *Metschnikowia* sp. Y31 showed 12.18% of ethanol employing PPW that is significantly improved as compared to some previously performed fermentative studies [62, 68]. A study using pomegranate peel under statistically optimal condition was also recorded by using a different strain *Candida tropicalis* [48].

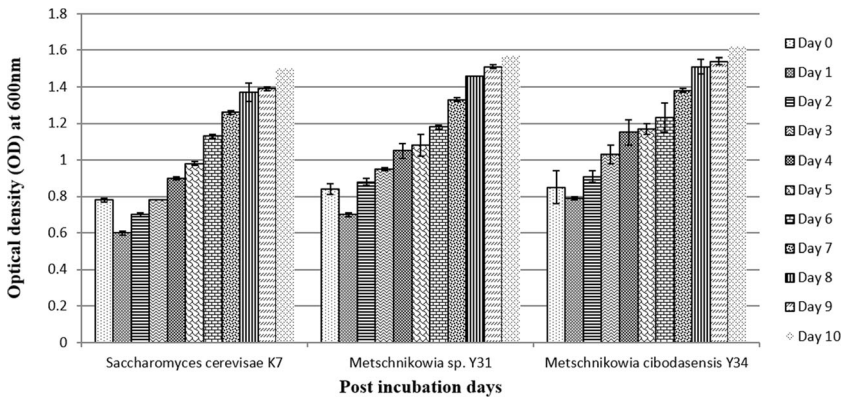

Fig. 5 Growth of *S. cerevisiae* K7, *Metschnikowia* sp. Y31, and *M. cibodasensis* Y34 in PPW hydrolysate

5 Conclusions

The present study arrived at the conclusion that maximum reducing sugars' release was 0.56 ± 0.04 mg mL^{-1} recorded by dilute sulfuric acid hydrolysis at 5% acid concentration with 100 °C of hydrolysis temperature for 30 min with ethanol production of 0.42 ± 0.08 g g^{-1} using *Metschnikowia* sp. Y31 as fermentative yeast after an incubation period of 5 days. Significant yield of ethanol was achieved while using treated pomegranate peels waste as substrate. In prospects to the control of agro-industrial waste and its bioconversion into value-added products such as ethanol, the optimal way of hydrolyzing the PPW as necessitated in this study will be highly valuable scientifically as well as economically. The main obstacle in utilizing cellulosic biomass as fuels' substrate is the selection of optimally efficient pretreatment and hydrolysis methodology. Our findings of the present investigation deals with the efficient employment of locally abundant LCB; i.e., PPW, involving low-cost acid hydrolysis, will be extremely helpful in developing efficient and economical way to valorize cellulosic wastes into bioethanol.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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