Paper Mulberry Fruit Juice: a Novel Biomass Resource For Bioethanol Production

Pleasure Chisom Ajayo
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Mei Huang
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Li Zhao
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Dong Tian
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Qin Jiang
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Shihuai Deng
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Yongmei Zeng
Institute of Ecological and Environmental Sciences, Sichuan Agricultural University, Chengdu, Sichuan 611130, P. R. China. Rural Environment Protection Engineering & Technology Center of Sichuan Province, Sichuan Agricultural University, Chengdu, Sichuan 6

Fei Shen (fishen@sicau.edu.cn)
Sichuan Agricultural University https://orcid.org/0000-0001-5693-170X

Research

Keywords: IG feedstock, Yeast fermentation, Response surface methodology, Optimization

Posted Date: October 5th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-934167/v1

Abstract

By way of broadening the use of diverse sustainable bioethanol feedstocks, the potentials of Paper mulberry fruit juice (PMFJ), as a non-food, sugar-based substrate, was for the first time evaluated for fuel ethanol production. Without any external nutrient supplementation, the suitability of PMFJ was proven, as maximum ethanol concentration (56.4 g/L), and yield (0.39 g/g), were achieved within half a day of the start of fermentation, corresponding to a very high ethanol productivity of 4.7 g/L/hr. Using Response Surface Methodology, established potentials were further maximized through statistical optimization of process conditions of temperature (20 – 40 ℃), yeast concentration (0.5 – 2 g/L), and pH (4 – 6). At the optimal temperature of 30 ℃, inoculum size of 0.55 g/L, and pH of 5, ethanol concentration, productivity, and yield obtained were 73.69 g/L, 4.61 g/L/hr, and 0.48 g/g, respectively. Under this ideal process conditions, bioethanol from PMFJ compares favorably with typical sugar-based energy crops, highlighting its resourcefulness as a high value biomass resource for fuel ethanol production.

Introduction

At the United Nations General Assembly of September 22, 2020, China's president Xi Jinping committed his country to achieving carbon neutrality by 2060, in line with the Paris Agreement target of limiting global warming to 1.5 ℃ over this period (UN News 2020). Seeing that the major source of carbon emissions is energy-related (from fossil fuel burning) (Heede 2014), utilization of energy from biomasses is a significant and sustainable strategy to achieving the goal of net-zero carbon emission (Zhang et al. 2021). Bioethanol produced from the fermentation of sugars from different biomasses into ethanol, is the most widely used and most demanded transport biofuel, accounting for approximately 71 % of global biofuel production in 2019 (IEA 2020). It has numerous advantages over fossil-derived fuel, including its renewability, sustainability, and carbon neutral nature (i.e., the overall carbon emitted through the biofuel combustion has been balanced or even outbalanced by the carbon absorbed through photosynthetic carbon sink, during the lifetime of the biomass feedstock) (Micic and Jotanovic 2015). In view of these facts, many countries have implemented policies mandating that a set percentage of this liquid biofuel be blended with gasoline. In China for instance, the Central Government in 2017 stipulated that the mandatory use of E10 gasohol (gasoline containing 10 % bioethanol) be expanded from 11 trial provinces to the entire nation by 2020 (Authur et al. 2017). Being the country with the highest carbon emissions since 2008 (bp-Statistical Review of World Energy 2021), this move is expected to contribute to a greener environment, a closer step to achieving the Paris Agreement Goal, and of course, a lesser dependence on crude oil. Meeting and keeping up with this national mandate thus require amongst other things, the intensification of research efforts on the use of diverse feedstocks, coupled with efficient technological conversion processes (Zhang et al. 2021). However, as a developing country with very large human population, grain-based production of fuel ethanol in China is currently prohibited due to food security concerns (Dyk et al. 2016). This makes the utilization of non-food biomasses a very attractive prospect, as it eliminates the food versus fuel debate, while further improving the economic competitiveness of bioethanol over fossil fuel.

Paper mulberry (*Broussonetia papyrifera* (L.) Vent.) is a non-food shrub or small tree that is indigenous to South west China, but now widely distributed in all of China, other Asian countries, the continent of Europe, as well as the Pacific Islands (Liao et al. 2014; Gonzalez-Lorca et al. 2015). Due to its aggressive invasiveness and wide adaptability to diverse ecologies, it is also gaining widespread dominance as an introduced specie in some African countries like Ghana and Uganda (Morgan and Overholt 2013; Pe et al. 2016; Adigbli et al. 2018; Abugre et al. 2019). Other attractive features of this tree include its strong germinating ability, high growth rate and biomass yield, prolific regeneration capability, strong adaptability to stress conditions, and low management requirements (Thaiutsa et al. 2001; Xianjun et al. 2014). Paper mulberry (PM) trees are grown both in an agroforestry system and monoculture, where they serve multiple functions as fallow crops/soil improvers (Saito et al. 2009; Anning et al. 2018), intercrop specie (Thaiutsa and Puangchit 2001), afforestation trees (Kyereh et al. 2014), avenue/urban plantations (Maan et al. 2021), and as excellent raw materials for production of high quality paper (Peng et al. 2019), textile (Peña-Ahumada et al. 2020), medicine (Park et al. 2017), and fabrication of modern bio-materials (Chen et al. 2017; Park et al. 2019; Kim et al. 2020). Relative to other components of PM tree such as the stem, stem bark, and roots, its fruits which reportedly contain considerable amounts of soluble sugars (Han et al. 2016), are being underutilized. Being a non-food fruit, they are mostly disregarded when ripe. Thus, they drop to the ground and rot, resulting to great loss of these sugar resources to the environment (personal communications by F. Shen). The rich sugar contents of PM fruit give clues to its potential as a possible feedstock for use in first generation (1G) bioethanol production. Apart from the research of Ding et al. (2016), that evaluated the use of its fruit juice as sugar baits for biological control of mosquitoes (*Culex pipiens pallens*), the utilization of the free sugars present in Paper mulberry fruit juice (PMFJ) remains largely unexplored.

Ethanol production using directly fermentable sugars (glucose, fructose and sucrose) in juices is technologically easier and more efficient, and produces higher ethanol titre compared to the use of starch or lignocellulosic biomass (Zabed et al. 2014; Cheng 2018). Yeast fermentation performance (as indicated by the concentration, amount, and rate of ethanol production) varies not only with the specie or strain involved, but also with prevailing fermentation conditions including carbon source (feedstock), temperature, pH, and other growth factors. At sub or supra-optimal levels of these conditions, ethanol production can be inhibited as a result of impaired viability and vitality of yeast cells. Thus in order to greatly enhance the production and profitability of fuel ethanol, optimal levels of these conditions must be established (Mohd Azhar et al. 2017). Among fermenting yeast organisms, *Saccharomyces cerevisiae* cells are mostly employed in industrial ethanol production due to reasons that include but are not limited to their greater fermenting efficiency and higher ethanol tolerance (Zabed et al. 2014). Using *S. cerevisiae*, varying process conditions of temperature, pH and yeast concentrations, have been reported for different sugar-based feedstocks (Dodić et al. 2009; Hadeel et al. 2011; Giri et al. 2013; Nasidi et al. 2013; Thangadurai et al. 2014; Matharasi et al. 2018; Dular 2019), a reason being the variations that exist in biomass composition.

In this study, the potentials of PM fruit juice (PMFJ) as a feedstock for bioethanol production was first evaluated. Then by the use of Response Surface Methodology, established potentials of this substrate was further maximized through statistical optimization of fermentation conditions of temperature, yeast concentration and pH. This research thus opened up a pathway for the optimal bioconversion process of a novel biomass resource into ethanol, which is a contributory step towards meeting the need for a cleaner, cheaper and sustainable energy.

Materials And Methods

2.1 Biomass preparation

The ripe fruits of PM were harvested from the trees at the farm of Sichuan Agricultural University, Chengdu, China. The whole fruits were weighed and the orange-coloured achenes (fruit part of interest) were separated from the seeds, and the core (green ball-like clusters of fleshy calyces). The separated achenes together with its juice were blended and sieved using a cheese cloth. The juice produced was recorded and immediately stored at -18 °C till subsequent analysis/use.

2.2 Development of yeast culture

Active dry yeast (*Saccharomyces cerevisiae*, Angel Yeast Co. Ltd., Yichang, China) was used for the bioconversion of juice sugars to ethanol. Using 50 mL of YPG media (2 g/L yeast extract powder, 20 g/L protein, and 20 g/L glucose), 5 g of dry yeast was activated in a 250 mL flask for 2 hours, at temperature of 35 °C and at 150 rpm. Thereafter, the activated yeast cells were separated from the nutrient media through centrifugation at 5000 rpm for 5 minutes. The cells were then repeatedly washed using autoclaved distilled water at the same conditions of centrifugation, until a clear supernatant was obtained. This was to prevent the transfer of any external nutrient from the activation period to the main fermentation experiment. The yeast slurry was dissolved in a certain volume of sterile water and the concentration determined, from which the different amounts of yeast cells required for fermentation were then calculated.

2.3 Batch fermentation experiments

To evaluate the potential of PMFJ as a feedstock for bioethanol production, preliminary batch fermentation was first performed. The pH of juice was adjusted to 6 using 2.5 M NaOH, and autoclaved at 115 °C for 15 minutes. Yeast concentration of 6 g/L was inoculated into the substrate aseptically and fermentation was carried out in an orbital shaker (150 rpm) at a temperature of 35 °C for 96 hours. This was repeated in triplicates. Samples were withdrawn at 12, 24, 48, 72 and 96 hours, and centrifuged at 10,000 rpm for 5 minutes. The supernatant was stored at -18 °C pending analysis of residual sugar and ethanol concentrations.

To maximize established potentials of PMFJ for bioethanol production, juice fermentation conditions at varying levels of temperature, yeast concentrations and pH were performed for optimization. pH was carefully adjusted either with 2.5 M NaOH or 2.5 M HCl. Fermentation process was carried out as outlined above, but samples were this time withdrawn at shorter intervals (every 8 hours for the whole incubation period of 80 hours).

2.4 Analytical methods

The pH of juice was directly measured using a pH meter (Shanghai Jingke Scientific Instrument Co., Ltd.), while titratable acidity determination was by the method of OECD (2005). Dinitrosalicylic acid (DNS) method was employed for the total reducing sugar analysis (Miller 1959; Salari et al. 2019). For the total soluble sugar determination, the fruit juice was first subjected to acid hydrolysis, to convert probably present sucrose to its monomeric sugars (Sewwandi et al. 2020). Thereafter, DNS method was used for the analysis of total sugars present. The concentration of metallic nutrients in juice was determined by the Inductively coupled plasma optical emission spectrometry (Bulska and Ruszczyńska 2017). For the fermentation products; residual sugar was analysed by DNS method, and the sugar consumed calculated (Eq. 1), while ethanol concentration was analysed using High Performance Liquid Chromatography (Flexar, PerkinElmer, Inc., Waltham, MA, USA), equipped with a column (SH1011, Shodex, Showa Denko America, Inc., New York, USA) and a refractive index detector. Operating conditions were; 0.05 mol/L H_2SO4 as mobile phase, flow rate of 0.8 mL/min, and temperature of column and detector set at 50°C and 60°C, respectively. From the detected ethanol concentration, ethanol yield (Eq. 1), productivity (Eq. 2), and fermentation efficiency (Eq. 3) were calculated.

$$Y_{ps} = P/S \quad \text{..... (1)}$$

Y_{ps}, P, and S represent the ethanol yield (g/g), ethanol produced (g), and sugar consumed (g), respectively. Sugar consumed = Initial sugar – residual sugar.

$$Q_p = P/T \quad \text{..... (2)}$$

Q_p is the ethanol productivity (g/L/h), and P and T respectively stand for the maximum ethanol concentration (g/L), and fermentation time (hrs.) at which it was obtained.

$$F_e = Y_{ps}/0.51 \times 100 \quad \text{..... (3)}$$

F_e and 0.511 are the fermentation efficiency (%), and maximum theoretical yield of ethanol from glucose, respectively.

2.5 Statistical optimization and analysis

Response surface methodology (RSM) is an effective statistical and predictive modelling approach that optimizes multiple variables using minimal number of experimental runs. To maximize the potential of bioethanol production from PMFJ using *S. cerevisiae*, Box Behnken design of RSM was used to optimize the three important fermentation conditions of temperature, yeast concentration, and pH. The coded and actual levels of each of these predictor variables were shown in Table 1, and were selected based on literature (Zabed et al. 2014). Design-Expert software (Stat-Ease Inc., V 8.0.6., Minneapolis, USA) was used to generate the orthogonal treatment combinations (comprising of 15 experimental runs including 3 central points), and was also utilized in the analysis of data obtained. Ethanol concentration, and ethanol productivity, as important indicators of fermentation performance were chosen as responses for optimization (response variables). A second order polynomial model was fitted to the obtained data of each response to evaluate the effect of the combined predictor variables on the response. Numerical optimization was next carried out, and the optimized fermentation conditions suggested by the model was verified by performing the corresponding experiment to establish their validity.

Table 1 Actual and coded levels of fermentation conditions of PMFJ

Variables	Variable names	Units	Coded and actual levels		
			-1	0	+1
X1	Temperature	°C	20	30	40
X2	Yeast conc.	g/L	0.5	1.25	2
X3	pH		4	5	6

Results And Discussion

3.1 Composition of Paper mulberry fruit juice (PMFJ) and preliminary evaluation of its fermentability

The ripe fruits of PM were highly juicy, constituting almost half of the fresh fruit weight (Table 2). This confers on it a succulent and delicate structure, and a consequent increased susceptibility to microbial degradation of its sugars (Choosung et al. 2019). As typical of sugar-based biomasses; prompt harvest, swift juice extraction and immediate storage of juice under appropriate conditions prior to fermentation, are very important steps to ensure sugar preservation (Klasson and Boone 2021). The total reducing sugar content of PM juice (glucose and fructose; 160.7 g/L) was basically the same with the total fermentable sugar (glucose, fructose and sucrose; 161.7 g/L) (Table 2), an indication that the juice contained trace or no amount of sucrose sugar. Similarly, the total soluble sugar composition in ripe fruits of Mulberry (*Morus alba* L.) which belonged to the same Moraceae family as Paper mulberry, had been reported to be made up of 80 % of reducing sugars (Lee and Hwang 2017). A sugar concentration of 150 – 200 g/L is considered desirable in industrial bioethanol production (Zabed et al. 2014). The rich fermentable sugar present in PMFJ is thus one of the indicators of its suitability as a high value feedstock for commercial bioethanol production. Furthermore, with almost all of the fermentable sugars being present in the forms of glucose and fructose monosaccharides, ethanol production might be initiated earlier due to rapid passage of directly fermentable sugar monomers into the yeast cells without prior hydrolysis in the yeast plasma membrane (D'Amore et al. 1989). It is interesting to note that the concentration of fermentable sugars in PMFJ compares favourably with that of some notable sugar-based bioenergy crops, except for sugar beets (table 3). However, remarkable variations exist in their sugar composition, whereby unlike PMFJ, sucrose is the dominant saccharide present in the juices of those sugar crops. The concentrations of minerals essential to yeast activities in PMFJ are shown in table 2. The proportions of each of these ions observed, are in agreement with an earlier study on the mineral composition of Paper mulberry fruits (Sun et al. 2012). The nutrient ions present in PMFJ are adequately sufficient to support a robust fermentation process, as all the essential metal ions, both macro and trace, were above the critical level required for yeast growth and metabolism (Walker 2014). This eliminates the need for external nutrient supplementation along with its associated costs, which is a big advantage in industrial bioethanol production.

Table 2 Paper mulberry fruit juice composition

Constituents	Concentration
Juice content (g/kg fruit)	442.86 ± 0.73
pH	5.12 ± 0.01
Total titratable acidity (g/L)	1.60 ± 0.00
sugar composition (g/L)	
Total fermentable sugar	161.70 ± 1.04
Total reducing sugar	160.70 ± 0.21
mineral composition (mg/L)	
K	2460.34 ± 5.2
Ca	303.65 ± 1.7
Mg	241.33 ± 3.3
Fe	25.40 ± 0.01
Zn	2.96 ± 0.00
Cu	0.82 ± 0.00
Mn	0.61 ± 0.00
Co	0.31 ± 0.00

Each parameter value is the mean of triplicate values ± standard deviation.

Table 3 Fermentable sugars in Paper mulberry fruit juice in comparison to juices of typical energy crops

Crop	Total fermentable sugar (g/L)	Principal fermentable sugar	References
Paper mulberry	**161.7**	**Reducing sugars; 99 %**	**Current study**
Sweet sorghum	94.5 – 170.0	Sucrose; 45 – 80 %	(Luo et al. 2014; Barcelos et al. 2016)
Sugar cane	151.0 – 187.0	Sucrose; 83 – 91 %	(Silva et al. 2017; Thammasittirong et al. 2017)
Sugar beet	240.6 – 679 8*	Sucrose; 92 – 99.5 %	(Grahovac et al. 2012; Gumienna et al. 2014)

**Expressed as g/kg of juice dry matter*

To actually evaluate the potential of PMFJ as a viable feedstock for bioethanol production, preliminary batch fermentation study was carried out using 6 g/L yeast loading, for an incubation period of 96 hours, and at temperature and pH conditions of 35 C and 6, respectively. At the first 12 hours of fermentation, the sugar concentration in the fermentation broth had dropped drastically from 161.7 to 17.6 g/L, which corresponded to sugar consumption of 89.12 % by the yeast organisms (Fig. 1). Within the subsequent 12 hours, a relatively lower amount of sugar was taken up. Afterwards, no further uptake was observed due to depleting substrate concentration. With the high rate of sugar consumption, bioethanol was rapidly metabolized in the yeast cells, and moved from the intracellular membranes into the fermentation broth; leading to an ethanol concentration of 56.4 g/L, produced at a very high rate (productivity) of 4.7 g/L/hr within the first 12 hours (Fig. 1). This concentration was above the minimum level (40 g/L) required for a cost effective down-stream ethanol distillation process (Chen et al. 2016). At subsequent periods, concentration remained relatively constant, indicating that stationary phase of ethanol production was already achieved within half a day of the start of fermentation. The presence of metal ions (such as potassium, magnesium, zinc, calcium, manganese, iron, cobalt, and copper) in fermentation media play very crucial role in yeast cell metabolism as they primarily act as co-factors for a large number of enzymes involved in the production of bioethanol (Walker and Walker 2018). The inherent yeast-essential mineral nutrients in PMFJ were all above the threshold level required, which undoubtedly resulted to its excellent fermentability in terms of ethanol concentration, and productivity. Additionally, the quick rate of sugar uptake suggested the absence of components in the sugar substrate that could prove inhibitory to yeast cells, such as some toxic ions (Walker 2014). The rapid rate of sugar uptake by the yeast cells also seemingly confirmed our earlier speculation that with the directly fermentable glucose and fructose sugars being mainly present, movement of sugars into the yeast cells would be faster, as there would be no prior sucrose hydrolysis into its monomers in the yeast plasma membrane.

Bioethanol yield represents the amount of ethanol produced relative to the amount of sugar consumed. The higher the yield, the higher the portion of the total consumed sugar that was actually incorporated into the metabolic pathway of producing the desired product (bioethanol). Based on stoichiometric mass balance, the maximum theoretical yield of bioethanol from 1 g of consumed fermentable sugar monomer is 0.51 g. On a practical basis though, some sugars will expectedly be used up in some side reactions necessary for ethanol synthesis. Therefore, bioethanol yield corresponding to at least 90 % of the maximum theoretical yield (fermentation efficiency) is seen as being good in practice (Zabed et al. 2014). The obtained bioethanol yield of 0.39 g/g from fermentation of PMFJ was equivalent to 76.5 % of the maximum theoretical yield, which fell short of the minimal level. Nevertheless, from the overall performance of PMFJ during this preliminary trial, it can be concluded that this sugar substrate has great potentials for utilization in bioethanol production. In a subsequent evaluation, the fermentation performance of this novel biomass resource was further improved through optimization of process conditions.

3.2 Optimization of bioethanol production from Paper mulberry fruit juice

Response Surface Methodology (RSM) is one of the experimental models for obtaining optimum settings for a range of factors affecting a response variable(s) of interest. Three fermentation factors each at three coded levels were evaluated using Box-Behnken design of RSM to optimize ethanol concentration and productivity. Table 1 under Sect. 2.5 displays the fermentation conditions evaluated for the optimization. Unlike the preliminary study, minimal amounts of yeasts were this time employed (0.5–2 g/L), bearing in mind the nature of sugar substrate and its rapid uptake, as well as, economic considerations. The maximum temperature was extended to 40 °C, with minimum of 20 °C, while the pH values ranged from 4–6. Samples were withdrawn every eight hours for a whole duration of 80 hours. At the 16th hour, most of the treatment combinations had achieved stationary phases of sugar uptake and ethanol production. Therefore, data collected at this time-point were used for evaluation.

3.2.1 Bioethanol concentration and productivity responses to fermentation conditions of Paper Mulberry Fruit Juice

With the use of the quadratic polynomial function, the relationships of ethanol concentration and productivity with the three fermentation conditions of temperature, yeast concentration, and pH were described (Eqs. 1 and 2).

$$Y_{\text{Ethanol concentration}} = 71.12 + 29.19X_1 - 0.20X_2 + 1.31X_3 - 1.68\,X_1X_2 - 0.59\,X_1X_3 - 1.21\,X_2X_3 - 28.43X_1^2 - 0.63X_2^2 + 1.18X_3^2 \quad \text{..... (1)}$$

$$Y_{\text{Ethanol productivity}} = 4.44 + 1.19X_1 + 0.02X_2 + 0.11X_3 - 0.18X_1X_2 - 0.08\,X_1X_3 - 0.08\,X_2X_3 - 1.14X_1^{2\,-}\,0.04X_2^2 + 0.08X_3^2 \quad \text{..... (2)}$$

The analysis of variance (ANOVA) for the quadratic models of ethanol concentration, and productivity were highly significant, as $p < 0.0001$, and $p = 0.0001$, respectively (Table 4). This indicated that the models for the regression terms were adequate, and that a higher order model would not be needed. As seen in the R-square values of the models, more than 99 % of variations in the both responses could be explained by the factors of fermentation conditions, reflecting the model reliability. The models for the two responses passed the lack of fit test, as p values were higher than 0.05, showing that the experimental data fitted well to the model design, and could suitably be used for prediction purpose. The less than 5 % coefficient of variation (CV) was a proof of the reproducibility and reliability of experimental data.

Table 4 ANOVA for the quadratic models of ethanol concentration (g/L), and productivity (g/L/hr.)

Sources of variance	Ethanol conc.			Ethanol prod.		
	Sum of sq.	F value	P value	Sum of sq.	F-value	P value
Model	9885.36	226.42	<0.0001	16.47	69.87	0.0001
Temperature - X1	6818.78	1405.66	<0.0001	11.28	430.61	<0.0001
Yeast conc. - X2	0.30	0.06	0.8122	0.00	0.17	0.6953
pH - X3	13.73	2.83	0.1533	0.09	3.45	0.1225
X1X2	11.39	2.33	0.1876	0.13	4.81	0.0798
X1X3	1.39	0.28	0.6151	0.03	1.04	0.3548
X3X4	5.86	1.21	0.3219	0.02	0.86	0.3966
$X1^2$	2985.41	615.43	<0.0001	4.81	183.70	<0.0001
$X2^2$	1.47	0.30	0.6062	0.01	0.27	0.6225
$X3^2$	5.14	1.06	0.3505	0.02	0.92	0.3813
Lack of fit	20.87	4.12	0.2016	0.12	5.37	0.1610
R^2	0.9976			0.9921		
CV	3.92			3.85		

Based on the p values of the three fermentation conditions considered, only temperature had a highly significant main linear effects on the two dependent variables (Table 4). There were positive responses of ethanol concentration and productivity to increases in temperature, with linear coefficients of 29.19, and 1.19, respectively, (Eqs. 1 and 2). None of the interaction effects of the fermentation factors on the both responses were significant, which made generation of the 3D surface plot of the experimental factors unnecessary. This non-significant interaction effects also signalled that the remarkable impact exhibited by temperature basically remained the same, irrespective of the prevailing yeast concentration and pH within the range considered. Bioethanol concentration and productivity exhibited no significant quadratic responses to yeast concentration and pH, but had a highly significant curve relationship with fermentation temperature. However, unlike the temperature main effect, quadratic impact of temperature caused a significant reduction in the responses, as indicated by the negative values of the coefficients in the polynomial functions. Therefore, the optimal region for each dependent variable in response to temperature, was a maximum rather than minimum (i.e., the curvature is convex). This meant that while bioethanol concentration and productivity initially responded positively to increases in temperature, a further unit increase in temperature above the optimal level, would result to significant reductions in these responses at magnitudes of -28.43, and – 1.14, respectively, (Eqs. 1 and 2).

The observed and predicted values of ethanol concentration and productivity as a function of fermentation conditions were shown in Table 5. The observed values varied from 9.61–76.51 g/L, and 1.72–4.78 g/L/hr, respectively. Based on the amount of substrate consumed, this corresponded to yields of 0.18–0.51 g/g (35–98 % of the maximum theoretical yields/ fermentation efficiencies). The predicted values of the responses by the model matched closely with the actual experimental data obtained, as revealed by the very small residual values. Yeast concentration and pH within the ranges evaluated were not critical process conditions influencing ethanol titre and rate of formation, though generally there were slight negative responses at lower values of these predictor factors. At same conditions of yeast loading and pH, an increase in temperature above 20 □C resulted to significant improvements in ethanol concentration. There were increases from 16.21–72.31 g/L (runs #4 vs #10), 14.12–72.70 g/L (runs #11 vs #14),

9.61–72.47 g/L (runs #2 vs #8), and 15.00–71.14 g/L (runs #7 vs #13). The same trend was also observed in the rate of ethanol production, from 2.36–4.52 g/L/hr (runs #4 vs #10), 2.08–4.54 g/L/hr (runs #11 vs #14), 1.72–4.53 g/L/hr (runs #2 vs #8), and finally from 2.34–4.44 g/L/hr (runs #7 vs #13). These tremendous increases matched well with the rate of sugar consumption. At just 16 hours of fermentation, stationary phase of sugar uptake had been achieved by most runs at which fermentation temperature was above 20 ℃. On the other hand, sugar metabolism was really slow at 20 ℃, resulting to a much later attainment of stationary phase at 32–40 hours (table S1).

Table 5 The actual and predicted values for ethanol concentration (g/L) and productivity (g/L/hr.)

Runs	Codes			Ethanol concentration			Ethanol productivity		
	X1	X2	X3	Observed	Predicted	Residual	Observed	Predicted	Residual
1	30	2	4	69.25	71.38	-2.13	4.33	4.47	-0.14
2	20	0.5	5	9.61	11.38	-1.77	1.72	1.87	-0.15
3	30	0.5	6	76.51	74.39	2.12	4.78	4.64	-0.14
4	20	1.25	6	16.21	16.57	-0.36	2.36	2.38	0.00
5	30	1.25	5	69.82	71.12	-1.30	4.36	4.44	-0.08
6	30	1.25	5	71.12	71.12	0.00	4.44	4.44	-0.00
7	20	2	5	15.00	14.35	0.65	2.34	2.27	0.07
8	40	0.5	5	72.47	73.13	-0.65	4.53	4.60	0.09
9	30	1.25	5	72.42	71.12	1.30	4.53	4.44	-0.07
10	40	1.25	6	72.31	73.78	-1.47	4.52	4.59	0.08
11	20	1.25	4	14.12	12.77	1.47	2.08	2.01	0.15
12	30	0.5	4	69.64	69.35	0.29	4.35	4.28	-0.00
13	40	2	5	71.14	69.38	1.76	4.44	4.29	-0.08
14	40	1.25	4	72.70	72.34	0.36	4.54	4.55	-0.01
15	30	2	6	71.28	71.58	-0.29	4.46	4.54	-0.08

Temperature has been implicated as the top factor having strong impact on fermentation performance by yeast cells (Lin et al. 2012; Zabed et al. 2014; Bhadana and Chauhan 2016; Mohd Azhar et al. 2017). For one, it affects fluidity of yeast membranes, subsequently impacting on the passage of solutes into and out of cells (Zabed et al. 2014). Over a 168-hour incubation, Lin et al. (2012) observed that increasing the temperature from 10 to 20, and then 30 ℃ shortened the exponential growth period of yeast cells to 120 and 48 hours, respectively. He concluded that the quicker onset of

stationary phase was initiated as a result of increased cell division and metabolic activities. Similarly in our study, at each evaluated temperature, a comparison of the residual sugar in fermentation broth with the corresponding bioethanol concentration and rate of production, revealed a strong inverse relationship (fig. S1). The poor fermentation performance at the low temperature of 20 ℃ was therefore a consequence of reduced uptake of fermentable sugar molecules for conversion into bioethanol, owing to a decreased yeast metabolic rate. With increase in temperature beyond 20 ℃ and up to a point, sugar uptake was improved tremendously (varying from 89.5–95.2 % consumption). Bioethanol was rapidly metabolized in the yeast cells, and moved from within the cells into the fermentation broth leading to a high ethanol concentration, and attainment of stationary phase at just the 16th hour of incubation. However, much higher increase in temperature up to 40 ℃ presented a stress factor to yeast cells, which led to significant reductions in ethanol production. There was also a corresponding increase in the amount of residual sugar, indicating inhibited substrate uptake (table S1). The metabolic and physical mechanisms behind this inhibition have been reported to include inactivation of regulatory enzymes, denaturation of yeast ribosomes, and change in fluidity of yeast membranes which hindered inter and intracellular solute movement, resulting to accumulation of toxins in yeast cells and reduced uptake of the much needed carbon substrate (Walker 1998). It is worth stating that even at extreme temperature condition of 40 ℃, the concentrations of bioethanol from PMFJ (71.14–72.70 g/L) was still above the minimum requirement (40 g/L) for industrial fermentation, and the maximum productivity realized (4.52–4.54 g/L/hr) exceeded many reported values in literature from the fermentation of other sugar substrates (Table 7). This could be related to the abundant availability of minerals in the juice especially Mg^{2+} ions, as this mineral has been reported to exert a membrane protective effects on yeast cells, enabling an enhanced ethanol production even under temperature stress (Eardley and Timson 2020; Walker and Basso 2020).

Table 7 Bioethanol production from PMFJ compared to some notable sugar-based substrates using *S. cerevisiae*

Feedstocks	Initial total sugar conc. (g/L)	Dominant sugar	Temp. (℃)	Yeast conc. (g/L)	pH	Nutrient addition	Time (hrs.)	Ethanol conc. (g/L)	Fermentation efficiency (%)	Ethanol productivity (g/L/hr)	References
Paper mulberry fruit juice	**162**	**Reducing sugars; 99 %**	**35**	**0.55**	**5**	**Nil**	**16**	**73.7**	**94**	**4.6**	**Current work**
Sweet sorghum juice	95	Sucrose; 45 %	35	1	5	Nil	72	49.5	101	2.4	(Luo et al. 2014)
	162	Sucrose; 78 %	37	12	4.5	Nil	11	72	87	6.5	(Barcelos et al. 2016)
Sugar cane juice	230	Sucrose	30	20	5	Nil	24	79.2	-	3.3	(Giri et al. 2013)
	153 – 187	Sucrose; 87 – 93 %	37	5 x 10^5 cells/ml	5	Nil	36	9.1 – 10.7	87 – 90	0.25 – 0.30	(Thammasittirong et al. 2017)
Sugar beets thin juice concentrate	200	Sucrose	30	1	5	Yes	72	91.2	86	1.3	(Kawa-Rygielska et al. 2013)
Sugar beets thick juice	210	Sucrose; 99%	30	3	5	Nil	46	86.3	94	1.9	(Grahovac et al. 2012)
Sugar beets raw juice	136	Sucrose	28	10	5	Nil	20	66.3	94	4.2	(Dodić et al. 2012)
Banana fruit waste	485	-	35	50	6	Yes	168	24.1	-	-	(Matharasi et al. 2018)
Grape fruit waste	-	-	30	10	5.6	Nil	36	58.2	-	1.6	(Dular 2019)
Jamaica cherry fruit juice	-	-	34	80	6	Yes	630	74.0	-	-	(Thangadurai et al. 2014)

Varying literature reports exist with respect to the influence of yeast concentration on bioethanol production. According to the findings of Matharasi et al. (2018) on batch fermentation of Banana fruit waste, increasing the yeast loadings from 1–5 %, progressively improved bioethanol concentration significantly. Conversely, in a review of several studies on yeast bioethanol production, Mohd Azhar et al. (2017) reported that while higher inoculum sizes had no effect on the final ethanol concentration, it markedly influenced the rate of ethanol formation (productivity), through reduction of incubation period, due to more rapid sugar uptake by the large yeast cells population. In an optimization modelling of bioethanol production from Sweet sorghum juice, Luo et al. (2014) noted no significant effect on both the final ethanol concentration and ethanol productivity, under the evaluated yeast loadings of 0.5–2 g/L. Similarly in the present research, increase in the yeast cell concentrations within the range used (0.5–2 g/L), had no significant effect on ethanol concentration, and productivity. Even if higher levels of yeast loadings were to be used in our study, the possibility of

observing a significant effect is not justified. This is in consideration of the fact that during the preliminary investigations to evaluate fermentability of PMFJ, the ethanol productivity and concentration realized using 6 g/L of yeast cells (Fig. 1), were respectively at par with and even lower than that obtained under the minimal yeast levels used in the optimization study, at similar temperature and pH conditions (Table 5). Therefore, the excellent performance of PMFJ even at very low yeast inputs could be due to substrate-related factors, which include its rich essential mineral nutrients' status, the fermentable sugars being mostly composed of glucose and fructose monosaccharides which facilitated quicker conversion to bioethanol, as well as the absence of any yeast-inhibitory factor in the must that could impair cells activities.

The H^+ concentration (pH) of the fermentation broth affects nutrients permeability into the yeast cells, which in extension influences yeast metabolism, ethanol production, and by-product formation (Lin et al. 2012; Zabed et al. 2014) In our study, while there were negative responses of ethanol concentration, and productivity to low pH value of 4, the impact of pH was not significant.

3.2.2 Numerical optimization and validation of model prediction

Optimization was achieved based on the criteria of maximizing bioethanol concentration, and productivity, while keeping the temperature, yeast concentration and pH in range settings. The optimized fermentation conditions predicted by the model were temperature of 35 °C, yeast concentration of 0.55g/L, and pH of 5.0, which would result to ethanol concentration, and productivity of 79.14g/L, and 4.78 g/L/hr, respectively. These optimal fermentation conditions suggested by the model were verified by performing the corresponding experiment to establish its validity. The actual responses of ethanol concentration, and productivity subsequently obtained were all within the 95 % confidence interval (Table 6), confirming the model prediction.

Table 6 Confirmation of the optimized fermentation conditions predicted by the model

Responses	Predicted value	95 % CI*[a]	Observed value
Concentration (g/L)	79.14	72.47 – 85.83	73.69*[b]
Productivity (g/L/hr)	4.78	4.29 – 5.27	4.61

*[a] *Confidence interval*

*[b] *Based on the amount of sugar consumed, this represented a yield of 0.48g/g (94 % of the maximum theoretical yield)*

With the use of *S. cerevisiae* in batch fermentation, different optimal process conditions have been reported for several sugar-based feedstocks (table 7). While the ideal temperature and pH established for the fermentation of PMFJ were well within the ranges generally reported in literatures, the yeast concentration optima differed greatly. Interestingly, even at relatively very low yeast concentration and non-supplementation of external nutrients, bioethanol production from PMFJ compared favourably with some notable sugar-based energy crops, and even exceeded most other sugar-based feedstocks, which boosts its economic suitability by way of reductions of process time and cost. This novel biomass can actually be utilized as a great resource for bioethanol production, having met and surpassed the industrial conditions for acceptability including, sugar concentration (150 – 200 g/L), ethanol titre (> 40 g/L), ethanol productivity (> 1 g/L/hr), and fermentation efficiency (> 90 %).

Conclusions

For the first time, the biotechnological viability and optimal fermentation conditions of the fruit juice of Paper mulberry tree for bioethanol production was successfully evaluated. This sugar and nutrient rich juice offer great promises as a viable feedstock for conversion to ethanol, comparing favourably with the juices of typical sugar energy crops. As an important indigenous tree species in southwest China, its non-food fruit juice can be usefully exploited in the area of 1G ethanol production, which will add to feedstock diversity, and thus contribute towards meeting the need for a cleaner, cheaper and sustainable energy.

Abbreviations

PM	Paper mulberry
PMFJ	Paper mulberry fruit juice
DNS	Dinitrosalicylic acid
RSM	Response surface methodology

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material statement

We declare that all data generated or analysed during this study are included in this published article and its supplementary information file.

Competing interests

The authors declare that they have no competing interests.

Fundings

This work was supported by the National Key Research and Development Program of China [grant number: 2020YFD1100704]; and the National Natural Science Foundation of China [grant number: 21978183].

Authors' contributions

PCA was responsible for data acquisition, analysis and interpretation; drafting and revisions of manuscript. MH, LZ, DT, QJ, SD and YZ conceived the project. FS conceived and designed the project, and performed manuscript revisions. All authors read and approved the final manuscript.

Acknowledgements

We are grateful to Babatope Samuel Ajayo (College of Agronomy, Sichuan Agricultural University, Chengdu, China) for the insightful discussions with him on approaches to Response Surface Methodology.

References

1. Abugre S, Nyamaah E, Adusu D (2019) Natural Regeneration of Indigenous Tree Seedlings Under Broussonetia Papyrifera Stand. Journal of Energy Natural Resource Management 5:1–6
2. Adigbli DM, Anning AK, Adomako JK, Fosu-mensah BY (2018) Effects of Broussonetia papyrifera invasion and land use on vegetation characteristics in a tropical forest of Ghana. Journal of Forestry Research. https://doi.org/10.1007/s11676-018-0691-9
3. Anning AK, Gyamfi B, Effah AT (2018) Broussonetia papyrifera controls nutrient return to soil to facilitate its invasion in a tropical forest of Ghana. Journal of Plant Ecology 11:909–918. https://doi.org/10.1093/jpe/rtx058
4. Authur R, Hayes D, Li M, Wang X, Yang Y, Zhang W (2017) China's New Nationwide E10 Ethanol Mandate and Its Global Implications. https://www.extension.iastate.edu/agdm/articles/others/Li Nov 17.html. Accessed 9 March 2021
5. Barcelos CA, Maeda RN, Santa Anna LMM, Pereira N (2016) Sweet sorghum as a whole-crop feedstock for ethanol production. Biomass Bioenerg 94:46–56. https://doi.org/10.1016/j.biombioe.2016.08.012
6. Bhadana B, Chauhan M (2016) Bioethanol production using Saccharomyces cerevisiae with different perspectives: substrates, growth variables, inhibitor reduction and immobilization. Fermentation Technology 5:2–5. https://doi.org/10.4172/2167-7972.1000131
7. Birch RM, Walker GM (2000) Influence of magnesium ions on heat shock and ethanol stress responses of Saccharomyces cerevisiae. Enzyme Microbial Technology 26:678–687. https://doi.org/10.1016/S0141-0229(00)00159-9
8. bp-Statistical Review of World Energy (2021) https://www.bp.com/content/dam/bp/business -sites/en/global/corporate/pdfs/energy-economics/statistical-review/bp-stats-review 2021-co2-emissions.pdf. Accessed 9 Sept 2021
9. Bulska E, Ruszczyńska A (2017) Analytical techniques for trace element determination. Physical Sciences Reviews 2:1–14. https://doi.org/10.1515/psr-2017-8002
10. Chen J, Tang C, Yue Y, Qiao W, Hong J, Kitaoka T, Yang Z (2017) Highly translucent all wood plastics via heterogeneous esterification in ionic liquid/dimethyl sulfoxide. Ind Crops Prod 108:286–294. https://doi.org/10.1016/j.indcrop.2017.06.054
11. Chen X, Kuhn E, Jennings EW, Nelson R, Tao L, Zhang M, Tucker MP (2016) DMR (deacetylation and mechanical refining) processing of corn stover achieves high monomeric sugar concentrations (230 g L-1) during enzymatic hydrolysis and high ethanol concentrations (> 10% v/v) during fermentation without hydrolysate purification or concentration. Energy Environmental Science 9:1237–1245. https://doi.org/10.1039/c5ee03718b
12. Cheng J (2018) Biological process for ethanol production. In: Cheng J (ed) Biomass to renewable energy processes, second. Taylor & Francis, Boca Raton, pp 144–190
13. Choosung P, Utto W, Boonyaritthongchai P, Wasusri T, Wongs-Aree C (2019) Ethanol vapor releasing sachet reduces decay and improves aroma attributes in mulberry fruit. Food Packaging Shelf Life 22:100398. https://doi.org/10.1016/j.fpsl.2019.100398
14. D'Amore T, Russell I, Stewart GG (1989) Sugar utilization by yeast during fermentation. J Ind Microbiol 4:315–323. https://doi.org/10.1007/BF01577355

15. Ding YM, Hu Y, Yu BT, Mo XC, Mo JC (2016) Laboratory evaluation of differential attraction of Culex pipiens pallens to fruit-based sugar baits. Acta Trop 163:20–25. https://doi.org/10.1016/j.actatropica.2016.07.016
16. Dodić JM, Vučurović DG, Dodić SN, Grahovac JA, Popov SD, Nedeljković NM (2012) Kinetic modelling of batch ethanol production from sugar beet raw juice. Appl Energy 99:192–197. https://doi.org/10.1016/j.apenergy.2012.05.016
17. Dodić S, Popov S, Dodić J, Ranković J, Zavargo Z, Jevtić Mučibabić R (2009) Bioethanol production from thick juice as intermediate of sugar beet processing. Biomass Bioenerg 33:822–827. https://doi.org/10.1016/j.biombioe.2009.01.002
18. Dular P (2019) Bioethanol production from rotten fruit. IJRASET 7:1555–1560. https://doi.org/10.22214/ijraset.2019.4282
19. Dyk JS Van, Li L, Barros D, Hu J, Zhang X, Tan T, Saddler J (2016) The Potential of Biofuels in China. IEA Bioenergy 39:32
20. Eardley J, Timson DJ (2020) Yeast Cellular Stress: Impacts on Bioethanol Production. Fermentation 6:109. https://doi.org/10.3390/fermentation6040109
21. Giri R, Kundu BS, Diwan P, Raj K, Wati L (2013) Ethanol production from direct sugarcane and juice by yeast. Agricultural Science Digest - A Research Journal 33:188. https://doi.org/10.5958/j.0976-0547.33.3.005
22. Gonzalez-Lorca J, Rivera-Hutinel A, Moncada X, Lobos S, Seelenfreund D (2015) Ancient and modern introduction of Broussonetia papyrifera ([L.] Vent.; Moraceae) into the Pacific: genetic, geographical and historical evidence. NZ J Bot 53:75–89. https://doi.org/10.1080/0028825X.2015.1010546
23. Grahovac J, Dodić J, Jokić A, Dodić S, Popov S (2012) Optimization of ethanol production from thick juice: A response surface methodology approach. Fuel 93:221–228. https://doi.org/10.1016/j.fuel.2011.10.019
24. Gumienna M, Szambelan K, Jeleń H, Czarnecki Z (2014) Evaluation of ethanol fermentation parameters for bioethanol production from sugar beet pulp and juice. J Inst Brew 120:543–549. https://doi.org/10.1002/jib.181
25. Hadeel A, Hossain ABMS, Latifa K, ALNaqeb H, Abear J, Norah AH (2011) Bioethanol fuel production from rambutan fruit biomass as reducing agent of global warming and greenhouse gases. Afr J Biotech 10:10157–10165. https://doi.org/10.5897/ajb11.790
26. Han Q, Wu Z, Huang B, Sun L, Ding C, Yuan S, Zhang Z, Chen Y, Hu C, Zhou L, Liu J, Huang Y, Liao J, Yuan M (2016) Extraction, antioxidant and antibacterial activities of Broussonetia papyrifera fruits polysaccharides. Int J Biol Macromol 92:116–124. https://doi.org/10.1016/j.ijbiomac.2016.06.087
27. Heede R (2014) Tracing anthropogenic carbon dioxide and methane emissions to fossil fuel and cement producers, 1854–2010. Clim Change 122:229–241. https://doi.org/10.1007/s10584-013-0986-y
28. IEA (2020) Renewables 2020 Analysis and forecast to 2025. In: International Energy Agency. https://www.iea.org/reports/renewables-2020/transport-biofuels. Accessed 29 Mar 2021
29. Kawa-Rygielska J, Pietrzak W, Regiec P, Stencel P (2013) Utilization of concentrate after membrane filtration of sugar beet thin juice for ethanol production. Biores Technol 133:134–141. https://doi.org/10.1016/j.biortech.2013.01.070
30. Kim S, Seo AY, Lee TG (2020) Functionalized cellulose to remove surfactants from cosmetic products in wastewater. Carbohyd Polym 236:116010. https://doi.org/10.1016/j.carbpol.2020.116010
31. Klasson KT, Boone SA (2021) Bioethanol fermentation of clarified sweet sorghum (Sorghum bicolor (L.) Moench) syrups sealed and stored under vegetable oil. Ind Crops Prod 163:113330. https://doi.org/10.1016/j.indcrop.2021.113330
32. Kyereh B, Agyeman VK, Abebrese IK (2014) Ecological characteristics that enhance Broussonetia papyrifera's invasion in a semideciduous forest in Ghana. Journal of Ecosystems 2014:1–6. https://doi.org/10.1155/2014/270196
33. Lee Y, Hwang KT (2017) Changes in physicochemical properties of mulberry fruits (Morus alba L.) during ripening. Sci Hortic 217:189–196. https://doi.org/10.1016/j.scienta.2017.01.042
34. Liao SX, Deng ZH, Cui K, Cui YZ, Zhang CH (2014) Genetic diversity of Broussonetia papyrifera populations in southwest China. Genet Mol Res 13:7553–7563
35. Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S, Kong H (2012) Factors affecting ethanol fermentation using Saccharomyces cerevisiae BY4742. Biomass Bioenerg 47:395–401. https://doi.org/10.1016/j.biombioe.2012.09.019
36. Luo Z, Wang L, Shahbazi A (2014) Optimization of ethanol production from sweet sorghum (Sorghum bicolor) juice using response surface methodology. Biomass Bioenerg 67:53–59. https://doi.org/10.1016/j.biombioe.2014.04.003
37. Maan I, Kaur A, Singh HP, Batish DR, Kohli RK (2021) Exotic Avenue plantations turning foe: Invasive potential, distribution and impact of Broussonetia papyrifera in Chandigarh, India. Urban Forestry Urban Greening 59:127010. https://doi.org/10.1016/j.ufug.2021.127010
38. Matharasi A, Uma C, Sivagurunathan P, Sampathkumar P (2018) Determination of bioethanol potential from banana waste using indigenous yeast (Saccharomyces cerevisiae. KX033583). Journal of Pharmacognosy Phytochemistry 7:2041–2047
39. Micic V, Jotanovic M (2015) Bioethanol as fuel for internal combustion engines. Zas Mat 56:403–408. https://doi.org/10.5937/ZasMat1504403M
40. Miller GL (1959) Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030
41. Mohd Azhar SH, Abdulla R, Jambo SA, Marbawi H, Gansau JA, Mohd Faik AA, Rodrigues KF (2017) Yeasts in sustainable bioethanol production: A review. Biochemistry Biophysics Reports 10:52–61. https://doi.org/10.1016/j.bbrep.2017.03.003

42. Morgan EC, Overholt WA (2013) Wildland Weeds: Paper Mulberry, Broussonetia. 1–2
43. Nasidi M, Agu R, Deeni Y, Walker G (2013) Fermentation of stalk juices from different Nigerian sorghum cultivars to ethanol. Bioethanol 1. https://doi.org/10.2478/bioeth-2013-0003
44. OECD-Organisation for economic cooperation and development (2005) Tests to Determine Quality of Fruits and Vegetables and Dry and Dried Produce. https://www.oecd.org/agriculture/fruitvegetables/publications/Guidelines_on_Objective… Accessed 9 Jan 2021
45. Park J, Park CW, Han SY, Kwon GJ, Kim NH, Lee SH (2019) Effects of pH on Nanofibrillation of TEMPO-Oxidized Paper Mulberry Bast Fibers. Polymers 11:414. https://doi.org/10.3390/polym11030414
46. Park JY, Yuk HJ, Ryu HW, Lim SH, Kim KS, Park KH, Ryu YB, Lee WS (2017) Evaluation of polyphenols from Broussonetia papyrifera as coronavirus protease inhibitors. J Enzyme Inhib Med Chem 32:504–512. https://doi.org/10.1080/14756366.2016.1265519
47. Pe J, Olivares G, Moncada X, Payacán C (2016) Sex Distribution of Paper Mulberry (Broussonetia papyrifera) in the Pacific. PLoS ONE 1–19. https://doi.org/10.1371/journal.pone.0161148
48. Peña-Ahumada B, Saldarriaga-Córdoba M, Kardailsky O, Moncada X, Moraga M, Matisoo-Smith E, Seelenfreund D, Seelenfreund A (2020) A tale of textiles: Genetic characterization of historical paper mulberry barkcloth from Oceania. PLoS One 15:e0233113. https://doi.org/10.1371/journal.pone.0233113
49. Peng X, Liu H, Chen P, Tang F, Hu Y, Wang F, Pi Z, Zhao M, Chen N, Chen H, Zhang X, Yan X, Liu M, Fu X, Zhao G, Yao P, Wang L, Dai H, Li X, Xiong W, Xu W, Zheng H, Yu H, Shen S (2019) A Chromosome-Scale Genome Assembly of Paper Mulberry (Broussonetia papyrifera) Provides New Insights into Its Forage and Papermaking Usage. Mol Plant 12:661–677. https://doi.org/10.1016/j.molp.2019.01.021
50. Saito K, Linquist B, Keobualapha B, Shiraiwa T, Horie T (2009) Broussonetia papyrifera (paper mulberry): its growth, yield and potential as a fallow crop in slash-and-burn upland rice system of northern Laos. Agroforestry System 76:525–532. https://doi.org/10.1007/s10457-009-9206-1
51. Salari M, Sowti Khiabani M, Rezaei Mokarram R, Ghanbarzadeh B, Samadi Kafil H (2019) Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media. Int J Biol Macromol 122:280–288. https://doi.org/10.1016/j.ijbiomac.2018.10.136
52. Sewwandi SDC, Arampath PC, Silva ABG, Jayatissa R (2020) Determination and Comparative Study of Sugars and Synthetic Colorants in Commercial Branded Fruit Juice Products. J Food Qual 2020:1–11. https://doi.org/10.1155/2020/7406506
53. Silva LA, Gasparini K, Assis C, Ramos R, Kist V, Barbosa MHP, Teófilo RF, Bhering LL (2017) Selection strategy for indication of crosses between potential sugarcane genotypes aiming at the production of bioenergy. Ind Crops Prod 104:62–67. https://doi.org/10.1016/j.indcrop.2017.04.025
54. Sun J, Liu S-F, Zhang C-S, Yu L-N, Bi J, Zhu F, Yang Q-L (2012) Chemical composition and antioxidant activities of broussonetia papyrifera fruits. PLoS ONE 7:1–8. https://doi.org/10.1371/journal.pone.0032021
55. Thaiutsa B, Puangchit L (2001) Paper Mulberry as an Alternative Crop of Agroforestry Systems in Thailand. Faculty of Forestry, Kasetsart University, Bangkok 10900, Thailand
56. Thaiutsa B, Puangchit L, Sommeechai M (2001) Effects of Spacings on Growth and Yields of Paper Mulberry at Different Harvesting Age. Faculty of Forestry, Kasetsart University, Bangkok 10900, Thailand
57. Thammasittirong SNR, Chatwachirawong P, Chamduang T, Thammasittirong A (2017) Evaluation of ethanol production from sugar and lignocellulosic part of energy cane. Ind Crops Prod 108:598–603. https://doi.org/10.1016/j.indcrop.2017.07.023
58. Thangadurai D, Bhat SV, Sangeetha J, Mundaragi AC (2014) Production of Bioethanol from Muntingia calabura Fruits using Saccharomyces cerevisiae and Schizosaccharomyces pombe. In: International Symposium on Agriculture and Environment, Faculty of Agriclture, University of Rahuna Kamburupitaya, Sri Lanka, 27 Nov 2014
59. UN News (2020) Global perspectives Human stories. https://news.un.org/en/story/2020/09/1073052. Accessed 22 Sep 2021
60. Walker GM (1998) Yeast physiology and biotechnology. John Willey & Sons
61. Walker GM (2014) Fermentation (Industrial): Media for Industrial Fermentations. In: Batt CA, Tortorello ML (eds) Encyclopedia of Food Microbiology, Second. Elsevier Ltd, Academic Press, pp 769–777
62. Walker GM, Basso TO (2020) Mitigating stress in industrial yeasts. Fungal Biology 124:387–397. https://doi.org/10.1016/j.funbio.2019.10.010
63. Walker GM, Walker RSK (2018) Enhancing Yeast Alcoholic Fermentations. In: Advances in Applied Microbiology. Elsevier, pp 87–129
64. Xianjun P, Linhong T, Xiaoman W, Yucheng W, Shihua S (2014) De Novo Assembly of Expressed Transcripts and Global Transcriptomic Analysis from Seedlings of the Paper Mulberry (Broussonetia kazinoki x Broussonetia papyifera). PLoS ONE 9:e97487. https://doi.org/10.1371/journal.pone.0097487
65. Zabed H, Faruq G, Sahu JN, Azirun MS, Hashim R, Nasrulhaq Boyce A (2014) Bioethanol production from fermentable sugar juice. The Scientific World Journal 2014. https://doi.org/10.1155/2014/957102
66. Zhang A, Gao J, Quan J, Zhou B, Lam SK, Zhou Y, Lin E, Jiang K, Clarke LE, Zhang X, Yu S, Kyle GP, Li H, Zhou S, Gao S, Wang W, Guan Y (2021) The implications for energy crops under China's climate change challenges. Energy Econ 96:105103. https://doi.org/10.1016/j.eneco.2021.105103

Figures

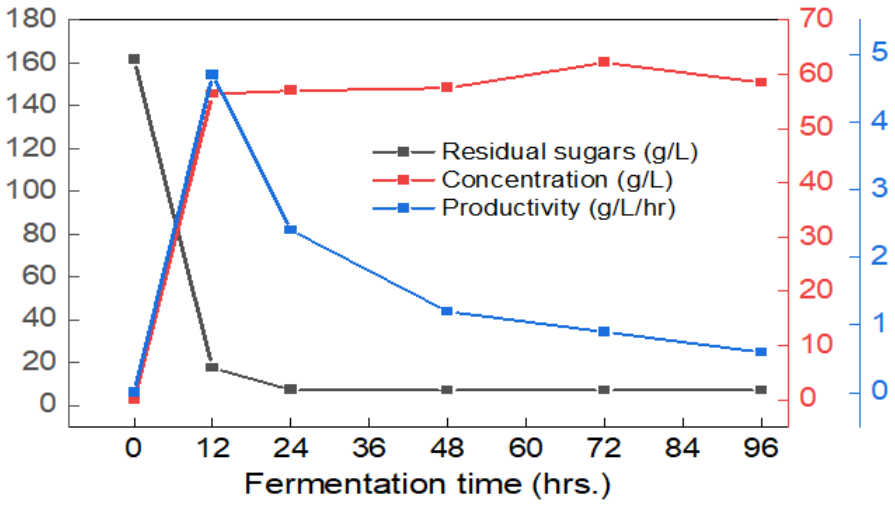

Figure 1

Fermentation profile of Paper mulberry fruit juice

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Ajayoetal21BOIBSupplementaryfile.docx
- Graphicalabstract.jpg