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Bioconversion of Napier grass to 2-phenylethanol: unveiling the synergy of pretreatment with deep eutectic solvents and yeasts

Elizabeth Jayex Panakkal[1], Yu-Shen Cheng[2], Kuan-Shiong Khoo[3], Pau-Loke Show[4], Prapakorn Tantayotai[5], Malinee Sriariyanun[1*]

[1] *Biorefinery and Process Automation Engineering Center, Department of Chemical and Process Engineering, The Sirindhorn International Thai-German Graduate School of Engineering, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand*

[2] *Department of Chemical and Materials Engineering, National Yunlin University of Science and Technology, Douliu, Yunlin, Taiwan*

[3] *Algal Bioseparation Research Laboratory, Department of Chemical Engineering and Materials Science, Yuan Ze University, Taoyuan, Taiwan*

[4] *Department of Chemical Engineering, Khalifa University, Abu Dhabi, United Arab Emirates*

[5] *Department of Microbiology, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand*

ABSTRACT

2-Phenylethanol (2-PE), a valuable flavor compound with high market demand, can be produced from readily available lignocellulosic biomass. This study explored the potential of Napier grass, a fast-growing energy crop, as a substrate for 2-PE production. Napier grass was pretreated using three different deep eutectic solvents (DES) formulated with choline chloride (ChCl) combined with lactic acid (LA), glycerol (Gly), or urea (Urea). DES pretreatment partially removed hemicellulose and increased cellulose content (up to 40%) in the biomass. Subsequent enzymatic hydrolysis revealed the highest glucose yield, from ChCl/LA pretreated biomass, almost 25% more than the native biomass. Fermentation optimization for temperature and nitrogen content identified strain-specific optimal conditions for 2-PE and ethanol production using two non-conventional yeasts, *Kluyveromyces marxianus* and *Pichia kudriavzevii*. Notably, ChCl/Gly pretreated biomass with *P. kudriavzevii* produced the highest 2-PE yield (510.51 ppm) alongside significant ethanol production (30.63 g/L). This study paves the way for a more sustainable approach to producing 2-PE, a valuable flavor compound, using readily available biomass and non-conventional yeasts.

Keywords: Biomass, Deep eutectic solvent, Pretreatment, 2-Phenylethanol, Volatile compounds, Value-added chemicals.

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Corresponding Author:
Malinee Sriariyanun
malinee.s@tggs.kmutnb.ac.th

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1. INTRODUCTION

Growing demand for processed and packaged food due to the change in lifestyle and modernization has brought a surge in the market size for a variety of food flavors. According to the Global Opportunity Analysis and Industry Forecast 2022–2030 report, packaged food's market size is expected to achieve a revenue of US$34,435 million during 2022–2030 with a Cumulative Annual Growth Rate (CAGR) of 6.03% (Research

Dive, 2022). Increased urbanization and time constraints for people have resulted in more dependence on packaged and processed food. This has ultimately increased the demand for food flavors, which are additives used in the food to improve its taste and smell perceived through mouth and nose (Fisher and Scott, 2007). The current methods of flavor production through chemical synthesis face limitations as the exact replication of a natural flavor is hard through chemical synthesis which results in difficulty keeping pace with the booming demand for diverse flavor profiles in the food industry.

Current flavor production techniques rely heavily on chemical synthesis, allowing for the creation of a wider range of flavors in a shorter timeframe (Panakkal et al., 2021b). While this method offers a wide variety of flavor options, unwanted by-products like hydroxycinnamic acid or some racemic mixtures can complicate separation, purification, and waste management (Liu et al., 2022). Driven by consumer interest in natural ingredients and health concerns, the food industry is seeking innovative ways to extract natural flavors. However, natural flavors from plants or animals often exist in low concentrations or complex mixtures, posing challenges such as the requirement of complicated extraction and purification techniques, which involve high cost (Mortzfeld et al., 2020). Therefore, the industry is seeking novel and greener route alternatives to extract substantial volumes of flavor compounds that retain their natural character. Promising solutions involve microbial biosynthesis and bioconversion, where microbes produce desired flavor compounds during fermentation (Panakkal et al., 2021b).

Among the most popular flavors in the market such as chocolate, fruity, and floral, 2-phenylethanol (2-PE) with a rose-like smell is widely used in cosmetic and pharmaceutical industries. It is also known as 2- phenylethyl alcohol and it has a very high demand due to its applicability (Mitri et al., 2022). It is also used in the food and beverage industry as an organoleptic enhancer (Cordero-Soto et al., 2020). Various world organizations including the Food and Drug Administration (FDA), the Flavor and Extract Manufacturers Association, the Joint Expert Committee on Food Additives, and the Council of Europe recognized the usage of 2-PE as safe, which has added value to the product (Scognamiglio et al., 2012). Most of the 2-PE production globally is done by chemical synthesis which costs around 3.5–5 US$/kg. However, the synthesis of 2-PE through natural routes by extracting bioactive compounds derived from the plant sources is expensive and may cost up to the 1000 US$/kg (Mitri et al., 2022). The production of 2-PE through the microbial route is estimated to reduce the synthetic cost to 220 US$/kg (Martínez-Avila et al., 2020).

Generally, industries synthesize 2-PE chemically by three different methods such as Friedel-Crafts reaction, hydrogenation of styrene oxide and oxidation of propylene (Conde-Báez et al., 2019; Mitri et al., 2022). However, chemical synthesis of 2-PE involves toxic carcinogens (e.g., benzene, styrene oxide) and requires extreme conditions, leading to undesirable by-products and complex purification steps (Mitri et al., 2022). Though plant-based extraction is an alternative, it is costly due to low compound concentration in flowers like jasmine and daffodil. Even though chemical synthesis is more cost-effective, fermentation-derived products tend to be more expensive. Despite its higher cost, fermentation-based 2-PE appeals to consumers seeking natural products, as it can command a premium price. Hence, biotechnological production of 2-PE through microbial fermentation is gaining interest as the US FDA and European Legislation would classify the product as natural if the substrate used in the production is of natural origin. Microbial fermentation using yeast offers a sustainable approach to producing 2-PE naturally, using L-Phenylalanine or glucose as substrates (Garavaglia et al., 2007).

Several yeast strains have been identified with the potential to produce 2-PE by utilizing carbon and nitrogen sources through enzyme-catalyzed reactions. Apart from the *S. cerevisiae*, non-conventional yeasts such as *Kluyveromyces marxianus*, *Kluyveromyces lactis*, *Pichia anomala*, *Candida utilis*, *Candida tropicalis*, *Yarrowia lipolytica*, *Pichia kudriavzevii* were also reported to produce 2-PE (Mitri et al., 2022). Yeast cells can produce 2-PE via two pathways: the de novo synthesis (Shikimate pathway) and bioconversion of L-Phenylalanine (L-Phe) (Ehrlich pathway) (Wang et al., 2017). The Ehrlich pathway converts L-Phe to 2-PE through transamination, decarboxylation and reduction reactions, with high L-Phe concentrations promoting this pathway (Zhou et al., 2023). Even though yeasts can produce 2-PE, the amount of production will depend on the yeast species, media composition, and fermentation conditions (Garavaglia et al., 2007). Hence optimization of media composition and fermentation condition is essential to enhance the production of 2-PE. However, to make the process more economical, highly productive yeasts and cheap feedstock must be used as raw materials for fermentation. Hence, agro-waste can be utilized to support microbial growth and fermented to flavor compounds.

Agro-waste, rich in lignocellulosic materials, offers significant potential for converting waste into valuable products like biofuels, biochemicals, and bioplastics due to its availability and low cost (Akbarian et al., 2022; Ashokkumar et al., 2022). However, its complex structure of cellulose, hemicellulose, and lignin resists chemical and enzymatic processing (Yan et al., 2020). Effective utilization relies on pretreatment methods—such as organosolv, ionic liquid, and deep eutectic solvents (DES)—which break down these components (Wei et al., 2021; Lee et al., 2022; Pakchamni et al., 2022). Recent studies have focused on pretreatment with a green solvent, DES, which is less toxic, biodegradable, and recyclable (Fakayode et al., 2021; Kumar et al., 2021; Lee et al., 2022). However, the choice and optimization of pretreatment or hydrolysis methods are essential and must be tailored to the specific biomass. Key factors include the duration and cost-

effectiveness of pretreatment and avoiding byproducts that could inhibit microbial growth in later stages (Kılmanoğlu et al., 2021).

This research work aims to evaluate the potential of using DES as a pretreatment solvent to pretreat Napier grass in producing 2-PE under optimized fermentation conditions using two non-conventional yeasts, *Kluyveromyces marxianus* and *Pichia kudriavzevii.* Napier grass was chosen as the biomass in the study as it is a perennial grass with a high growth rate and less fertilizer requirement (Panakkal et al., 2022). Moreover, it is also regarded as an energy crop due to its biomass composition. Napier grass was pretreated with three different DES (i.e., ChCl/LA, ChCl/Gly, and ChCl/Urea) to enhance the sugar production from biomass without the generation of inhibitors. These released sugars after hydrolysis were fermented with *K. marxianus* and *P. kudriavzevii* and evaluated for their ability to produce 2-PE. To enhance the production of 2-PE, the fermentation conditions for the yeasts were optimized for temperature and media composition using the one-factor-at-a-time (OFAT) approach. Although, there are studies on producing 2-PE from agro-waste using solid-state fermentation and synthetic media (Martínez et al., 2018; Martínez-Avila et al., 2020; Mitri et al., 2022), however, to date there are no reports on the production of 2-PE using DES-pretreated biomass. In addition to this, ethanol production as an additional value-added product from DES pretreated Napier grass fermented with *K. marxianus* and *P. kudriavzevii* was also analyzed. Overall, this research provides insight into the utilization of DES-pretreated Napier grass to produce 2-PE on a lab scale. More studies must be done to identify and understand the limitations and methods to improve 2-PE yield on the industrial scale using DES-pretreated biomass.

2. MATERIALS AND METHODS

2.1 Biomass Collection

Napier grass utilized in this research was sourced locally from Bangkok, Thailand. For the preparation of the biomass for subsequent analysis, it was dried using a hot air oven (WOF-50, Diahan Scientific, Gangwon-do, Korea) set to the 80ºC overnight until a stable dried weight was obtained. Following this, the dried biomass was ground in a household blender and then passed through an aluminum sieve (10-mesh size), ensuring that the biomass particles were of uniform and consistent size. The powdered biomass was stored in sealed packages until further use.

2.2 Synthesis of Deep Eutectic Solvents and Pretreatment of Biomass

In this study, three different deep eutectic solvents (DES) were prepared by mixing choline chloride (ChCl) with lactic acid (LA), glycerol (Gly), and urea (Urea) at a 1:2 molar ratio. The mixtures were processed in a rotary evaporator at 60ºC for ChCl/LA and at 80ºC for ChCl/Gly and ChCl/Urea until a clear, colorless liquid was achieved. The resulting liquids were transferred to closed bottles and stored in a desiccator until needed (Sai and Lee 2019; Li et al., 2021).

The DES pretreatment followed the procedure outlined in our previous work, adhering to the optimal conditions specified in Table 1 (Panakkal et al., 2022; Panakkal et al., 2024). Napier grass was pretreated under the conditions detailed in Table 1 using the specified DES in a stirring mantle (Mtop, Model: MS-ES 302, Korea), equipped with a condenser to prevent solvent evaporation. For the ChCl/LA pretreated biomass, washing was done with water using a centrifuge at 8000 xg for 10 min until the wash liquid reached a neutral pH. In contrast, biomass pretreated with ChCl/Gly and ChCl/Urea, due to their high viscosity, was washed with both water and ethanol to remove any residual DES (Panakkal et al., 2022; Panakkal et al., 2024). The pretreated solids were then separated from the slurry via vacuum filtration and dried in a hot air oven (WOF-50, Daihan Scientific, Gangwon-do, Korea) at 60ºC overnight until a constant dry weight was obtained. The dried solids were stored in sealed bags until further use.

Table 1. Pretreatment conditions for three different DES

DES	Biomass loading (w/w)	Temperature (ºC)	Time (hrs)
ChCl/LA	1:6.5	80	5
ChCl/Gly	1:8	130	0.5
ChCl/Urea	1:20	130	5

The native biomass washed with water and ethanol was used as a control in the study to account for solids removed during the washing step. These control biomass samples were also dried in a hot air oven and stored until needed. The outline of the study is represented in Fig. 1.

2.3 Enzymatic Hydrolysis

Enzymatic hydrolysis was conducted to assess sugar production from both untreated and pretreated Napier grass. The biomass was introduced into a 50 mM citrate buffer (pH 4.8) with a solid loading of 2.5% (w/v), supplemented with sodium azide (Ajax Finechem, Brooklyn, MA, USA) to prevent microbial contamination and Ctec2 enzyme at a concentration of 30 FPU/g biomass. The hydrolysis mixture was incubated for 72 hrs at 50ºC and 150 rpm in an orbital shaker. Following the incubation, the mixture was heated in a water bath at 100ºC for 5 min to halt the enzymatic activity. Subsequently, the mixture was centrifuged at 8000 xg for 10 min and the solids were separated from the hydrolysate. The sugar content in the hydrolysate was subsequently measured using the 3,5-dinitrosalicylic acid (DNS) method and high-performance liquid chromatography (HPLC) (Miller, 1959). Briefly, DNS assay was performed by mixing an aliquot of the sample with DNS reagent in an Eppendorf tube. Further, the mixture was incubated in a boiling water bath for 5 min. Further, the mixture was cooled using ice for 5 min. The absorbance of the mixture was read using a

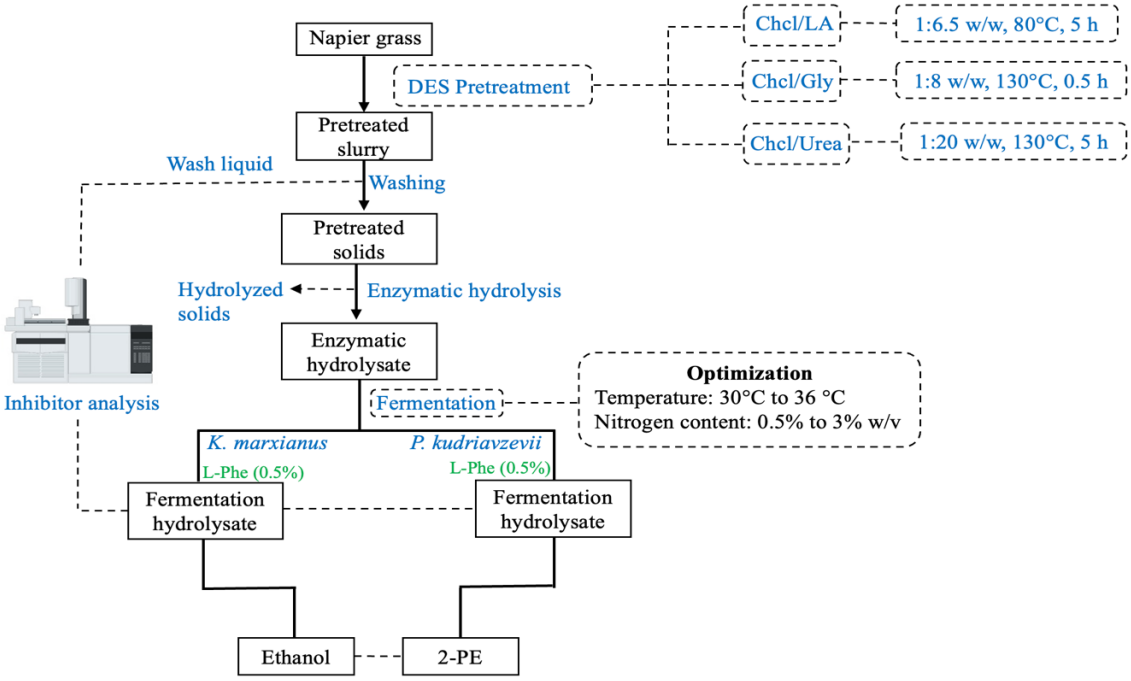

Fig. 1. Outline of the study

spectrophotometer at 540 nm.

2.4 Optimization of Fermentation Condition for 2-PE Production

The production of 2-phenylethanol (2-PE) alongside ethanol through fermentation using two non-conventional yeast strains, *K. marxianus,* and *P. kudriavzevii* were selected based on their temperature tolerance, rapid growth rates, and ability to utilize diverse sugars (Martínez-Avila et al., 2020; Baptista and Domingues, 2022). Previous research has employed these yeasts to produce bioflavor from waste biomass (Garavaglia et al., 2007; Conde-Báez et al., 2017; Chreptowicz et al., 2018; Martínez et al., 2018). However, the production of 2-PE will depend on the yeast species, media composition, and fermentation conditions (Garavaglia et al., 2007). Hence, optimization of media composition and fermentation condition is essential to enhance the production of 2-PE. To optimize fermentation conditions and media composition for improved 2-PE production, the OFAT method was utilized, focusing on temperature (30ºC, 33ºC, 36ºC) and nitrogen content (peptone at 0.5%, 1.75%, 3% w/v). This method evaluates the impact of one variable while keeping others constant. The optimal conditions were determined based on the highest mean relative abundance of 2-PE in the hydrolysate fermentation.

The enzymatic hydrolysis process as described in Section 2.3, was performed without sodium azide (Panakkal et al., 2021a), and the resulting hydrolysate was served as the fermentation medium. Nineteen milliliters of this medium were inoculated with 1 mL of yeast inoculum at an optical density (A600) of approximately 1.0. To support yeast growth and acclimatization, 0.5% glucose, and 1% yeast extract were added, along with peptone according to the optimization requirements, varying from 0.5% to 3% (w/v) (Table 2–4). The mixture was incubated in an orbital shaker (Model: JSSI-100C, JS Research Korea) at the specified temperature varying from 30–36ºC for 120 hrs with continuous shaking at 150 rpm (Table 2–4). Post-fermentation, the hydrolysate was centrifuged at 8000 xg for 10 min to collect the supernatant, which was then analyzed for 2-PE content using GC-MS to determine the mean relative abundance.

The optimal fermentation conditions were further tested in triplicate with adding L-Phenylalanine at 0.5% (w/v) to enhance 2-PE production. L-Phenylalanine has been reported to boost 2-PE production as it is converted by yeast via the Ehrlich pathway (Martínez-Avila et al., 2020).

2.5 Inhibitor Analysis

Potential inhibitors such as 5-hydroxymethyl furfural (5-HMF), furfural, and acetic acid in the pretreatment liquid and fermentation hydrolysate were identified using gas chromatography-mass spectrometry (GC-MS). The analysis was conducted on a GC-MS instrument (Shimadzu, Tokyo, Japan) equipped with a DB-wax column (Agilent J & W GC column, USA) tailored for inhibitor detection. Helium served as the carrier gas, flowing through the column at 1.25 mL/min. The GC inlet operated with a split ratio of 30, and the column oven temperature was maintained at 50ºC, while the injector temperature was set to 250ºC. A 1 µL sample was injected into the system.

The GC program started at 50°C with a 1-min hold, then increased to 120°C at a rate of 4°C /min with a 2-min hold. The temperature was then ramped up to 170°C, held for 1 min, and finally raised to 240°C at a rate of 10°C /min with a 10 min hold. For the MS program, the ion source temperature was set at 200°C, and mass detection was scanned in the range of 40 to 600 m/z.

2.6 Ethanol Analysis from Fermentation Hydrolysate

Fermentation hydrolysate collected at 72 hrs and processed as described in Section 2.4 was used to determine ethanol production by non-conventional yeasts. The hydrolysate was analyzed spectrophotometrically to calculate the ethanol concentration as reported previously (Sriariyanun et al., 2019). Briefly, the extraction of ethanol from hydrolysate used Tri-n-butyl phosphate (TBP). A 1 mL liquid sample was thoroughly mixed with 1 mL of TBP (Sigma Aldrich, USA) using a vortex for 1 min. The mixture was then subjected to centrifugation at 3420 xg for 5 min, resulting in two separate phases. The upper, clear layer was identified as the TBP layer, and the lower layer was the aqueous phase. Subsequently, 500 µL of the TBP layer was transferred into a new microtube and combined with 500 µL of a dichromate reagent containing 10% (w/v) $K_2Cr_2O_7$ in 5 M H_2SO_4. This mixture was vortexed for 1 min and then left to stand at room temperature for 10 min, allowing the oxidation products in the lower phase to develop a blue-green color. To measure the optical density, 100 µL of the oxidation product was diluted with 900 µL of deionized water. The absorbance of this diluted sample was measured at 595 nm using a UV/Vis spectrophotometer (T80+ UV/Vis Spectrometer, PG Instrument Ltd., USA). The ethanol concentration was determined by utilizing a standard curve for ethanol.

2.7 2-PE Analysis

The presence of 2-PE in the fermentation hydrolysate was assessed using GC-MS analysis. The hydrolysate collected at 120 hrs was centrifuged at 8000 xg for 10 min, and the supernatant was analyzed using a GC-MS system (Shimadzu, Tokyo, Japan) equipped with a DB-wax column (Agilent J & W GC column, USA). Helium served as the carrier gas with a flow rate of 1.22 mL/min. The GC was operated in split mode with a split ratio of 30. The injector temperature was maintained at 250°C, and the injection volume was 1 µL. The GC program started at an initial temperature of 50°C (held for 1 min), then ramped up to 200°C (held for 5 min) at a rate of 20°C /min. The MS program used an ion source temperature of 200°C and scanned a mass range from 30 to 600 m/z. The quantification of 2-PE in the hydrolysate for optimization studies used Ethyl acetate as an internal standard (IS) to determine the mean relative abundance (Güneşer et al., 2015). However, 2-PE was quantified in the optimum fermented samples using a standard curve for 2-PE.

2.8 Statistical Analysis

All experiments were performed in triplicates and the statistical analysis was carried out using IBM SPSS software. The analysis of variance (ANOVA) was tested for comparison with the untreated sample following LSD and Duncan test with a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Biomass Compositional Analysis

A compositional analysis of Napier grass was carried out to determine the effectiveness of DES pretreatment on biomass. Each of the DES chosen in this study differ in their chemical nature. ChCl/LA provides an acidic medium for pretreatment while ChCl/Gly acts as a polyol based near neutral DES. On the other hand, ChCl/Urea is alkaline in nature and provides alkaline medium for pretreatment of Napier grass. Pretreatment with these DES hence will help to evaluate the effect of different types of DES on Napier grass pretreatment. Napier grass has previously been reported to have 28%–46% cellulose, 10%–34% hemicellulose, and 17–25% lignin in it (Kongkeitkajorn et al., 2020; Manokhoon and Rangseesuriyachai, 2020; Chinwatpaiboon et al., 2021). Napier grass in this study had 26.50% cellulose, 18% hemicellulose, and 21% lignin content in it. DES pretreatment has helped improve the biomass's cellulose content, which can be observed in Fig. 2. Pretreatment with ChCl/LA has enhanced the cellulose content from 26.50% to 35.10% and has resulted in partial removal of hemicellulose. This trend was noted previously with acidic DES pretreatment on corn stover where ChCl/LA pretreatment enhanced the glucan content and caused the xylan removal (Ao et al., 2022).

Another study on phloem biomass pretreatment with acidic DES also reported removal of hemicellulose and lignin while retaining cellulose in the biomass (Nie et al., 2024). Along with lignin removal, the acidic condition in acidic DES causes hemicellulose degradation with the help of protons (Tian et al., 2020). The enhanced cellulose content is also obvious in the Napier grass pretreated with ChCl/Gly (Fig. 2). Generally, polyol-based DES has been reported as inefficient in biomass fractionation (Oh et al., 2020). On the other hand, pretreatment with ChCl/Gly at higher temperatures has also proved to help in biomass digestibility (Procentese et al., 2017). In this study, the ChCl/Gly has improved cellulose content which could be due to the higher pretreatment temperature that could have improved biomass digestibility. Neutral DES has been stated to have a weaker ability to break β-O-4 bond (Li et al., 2023). This could be the reason that ChCl/Gly has resulted in less lignin and hemicellulose removal. The pretreatment with ChCl/Urea has also resulted in enhanced cellulose content Amide DES containing ChCl and Urea has previously been reported in removing lignin and hemicellulose from kenaf bast by deprotonation of phenolic hydroxyl groups in different lignin units (Nie et al., 2023).

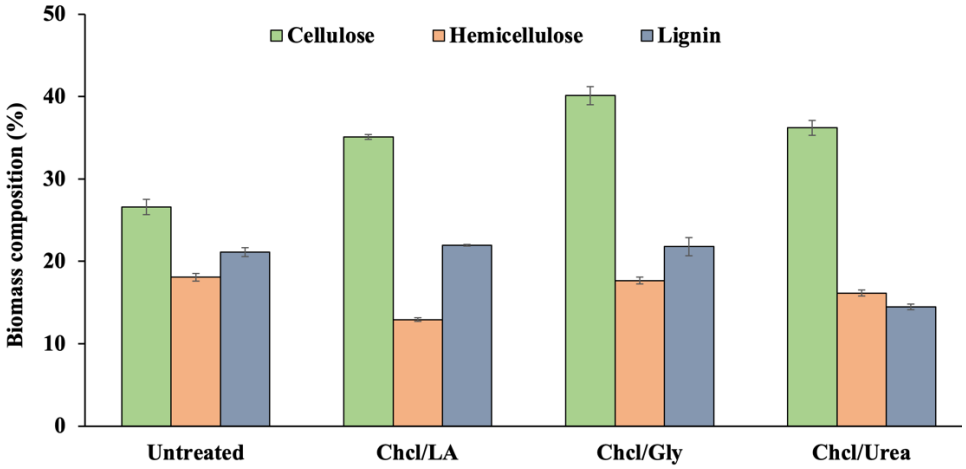

Fig. 2. Biomass composition of untreated and pretreated Napier grass

In this study also ChCl/Urea pretreatment has resulted in partial removal of hemicellulose and lignin, thereby increasing the cellulose content. This enhancement in cellulose content from DES pretreatment of Napier grass can be beneficial in enzymatic hydrolysis as this can be converted to monomeric sugars.

3.2 Enzymatic Hydrolysis

Hydrolysis study of the untreated and pretreated Napier grass was conducted enzymatically using cellulase enzyme at 50ºC for 72 hrs. This could reveal the release of glucose and xylose from the pretreated biomass compared to the untreated biomass. Fig. 3 represents the sugar yield from the untreated and pretreated Napier grass. The untreated Napier grass produced 152.42 mg/g of glucose and 23.15 mg/g of xylose through the enzymatic hydrolysis. This sugar yield was noted to increase when the biomass was pretreated with ChCl/LA and ChCl/Gly. The pretreatment with ChCl/LA could produce nearly 25% more glucose and 51% more xylose than the native biomass. This could be due to the improved enzyme accessibility owing to the increase in porosity and surface area of pretreated biomass that has resulted in removal of amorphous materials (Raj and Singh, 2024). Similarly, pretreatment with ChCl/Gly has also increased the sugar yield by up to 10% when compared with the untreated biomass. Pretreatment with ChCl/Gly has previously been reported to release amorphous cellulose from miscanthus and thereby improve the sugar yield upon hydrolysis (Hassan and Mutelet, 2022). Moreover, severe DES pretreatment with ChCl/Gly also can release more sugar monomers during the enzymatic hydrolysis (Procentese et al., 2017). In this study, the pretreatment of Napier grass at 130ºC with ChCl/Gly may have promoted the glucose and xylose release from the amorphous cellulose generated during pretreatment. While pretreatment with ChCl/LA and ChCl/Gly has improved the sugar yield, ChCl/Urea was unable to produce more sugar yield than the native Napier grass. A similar trend was observed in the total reducing sugar (TRS) content from pretreated biomass where the sugar yield improved by pretreatment with ChCl/La and ChCl/Gly, while TRS yield reduced in ChCl/Urea pretreatment (Fig. S1). Even though compositional analysis showed improved cellulose content in ChCl/Urea pretreatment, it could not generate more sugar monomers in hydrolysis. A decreased sugar yield on pretreatment with ChCl/Urea was also noted in the pretreatment of rice straw (Pan et al., 2017). This was attributed to the increased crystalline cellulose with more extensive inter and intra-molecular hydrogen bonds in addition to the Van der Waals interaction that was difficult to hydrolyze (Pan et al., 2017). Nevertheless, DES pretreatment followed by enzymatic hydrolysis in this study could release sugar monomers which can be utilized by the microbes for fermentation.

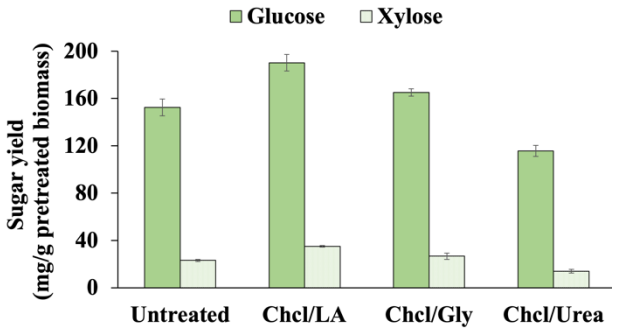

Fig. 3. Sugar yield from untreated and pretreated Napier grass

3.3 Optimization of Fermentation Conditions

Fermentation for flavor production was conducted using two yeast strains, *K. marxianus,* and *P. kudriavzevii*, utilizing enzymatic hydrolysates derived from pretreated samples. To enhance the production of 2-PE, the fermentation conditions were optimized only for pretreated biomass with each yeast strain. The study varied temperature (30–36ºC) and nitrogen content (peptone: 0.50% to 3% w/v) as two factors for optimization, using the method to determine the optimal conditions based on the

Table 2. Optimization of fermentation conditions for production of 2-PE by *K. marxianus*

Biomass	Temperature (°C)	Nitrogen content (%)	Mean relative abundance (mg/g biomass) *
Untreated	30	1.75	0.76 ± 0.91[a]
	30		1.01 ± 0.25[a]
ChCl/LA	33	1.75	3.23 ± 0.04[b]
	36		8.93 ± 0.44[c]
	30		1.10 ± 0.41[a]
ChCl/Gly	33	1.75	3.56 ± 0.27[a]
	36		15.23 ± 0.10[b]
	30		1.52 ± 1.63[a]
ChCl/Urea	33	1.75	3.17 ± 0.46[a]
	36		16.29 ± 0.20[b]
		0.5	0.79 ± 0.34[a, b]
ChCl/LA	36	1.75	0.65 ± 0.01[a]
		3	1.31 ± 0.35[b]
ChCl/Gly		0.5	0.62 ± 0.20[a]
	36	1.75	0.63 ± 0.10[a]
		3	1.32 ± 0.14[b]
		0.5	0.90 ± 0.24[b]
ChCl/Urea	36	1.75	0.49 ± 0.18[a]
		3	1.26 ± 0.14[b]

*: Different letters above the numerals represents different subsets of alpha

highest mean relative abundance of 2-PE (Table 2 and 3). From Table 2 and 3, it was observed that the optimal temperature for both *K. marxianus* and *P. kudriavzevii* was 36°C, where the mean relative abundance of 2-PE was the highest. The 2-PE abundance increased with rising temperatures, consistent with a previous study on 2-PE production from grape must culture using *K. marxianus* CBS 6556, which reported 37°C as the optimal temperature for maximum 2-PE production (Garavaglia et al., 2007). The study used a central composite design to optimize the temperature, pH, and L-Phenylalanine concentration for enhancing the 2-PE production (Garavaglia et al., 2007). Similarly, a study on 2-PE production from beet molasses medium with *K. marxianus CBS 600* indicated 35°C as the optimal temperature, with a decline in production observed at temperatures above 41°C (Etschmann et al., 2003). Another study on 2-PE production by *P. fermentans L-5* also identified the 30–35°C range as ideal, with a decrease in production at 40°C (Chung et al., 2000). Thus, the optimal temperature of 36 °C obtained in this study aligns with the previously reported range of 30–37°C.

Similarly, for the nitrogen content optimization, *K. marxianus* produced the maximum 2-PE at a peptone concentration of 3% (w/v) for pretreated biomass (Table 2). In contrast, *P. kudriavzevii* required different peptone concentrations, of 0.50% and 3% w/v, depending on the pretreated samples (Table 3). This variation is attributed to changes in the biomass structure due to different DES pretreatments, which may necessitate adjustments in the nutritional needs of yeasts for their optimal growth and metabolism (Baksi et al., 2023). A previous study evaluating the effects of various nitrogen sources (yeast extract, peptone, and ammonium sulfate) and carbon sources (glucose, fructose, and lactose) on flavor metabolite production demonstrated that *K. marxianus* produced more flavor compounds when yeast extract was used as the nitrogen source, followed by peptone and ammonium sulfate (Gethins et al., 2015). Another study on the impact of carbon and nitrogen sources on volatile compound production using *K. marxianus* showed an increase in fusel alcohol (2-PE and isoamyl alcohol) production with an increased nitrogen source (yeast extract) (İşleten Hoşoğlu, 2018). The optimal fermentation conditions obtained in this study based on the mean relative abundance of 2-PE for pretreated biomass are depicted in Table 4.

3.4 Inhibitor Analysis

To identify any potential inhibitors that could negatively impact fermentation and product yield, an inhibitor analysis was conducted. Inhibitors are typically generated under severe pretreatment conditions such as high temperatures and extended pretreatment durations. These harsh conditions can cause the degradation of sugar monomers, leading to the release of inhibitors that reduce the yield from the biomass (Agrawal et al., 2021). Common inhibitors include furfurals, 5-hydroxymethyl furfural (5-HMF), acetic acid, formic acid, and levulinic acid. Hexose sugars degrade into 5-HMF, while pentose sugars form furfural. Hemicellulose degradation results in the formation of formic acid, acetic acid, and levulinic acid (Yan et al., 2021). In this study, the pretreatment hydrolysate obtained after treating Napier grass, as well as the fermentation hydrolysate, was analyzed for the presence of 5-HMF, furfural, and acetic acid using GCMS. Only negligible

Table 3. Optimization of fermentation conditions for production of 2-PE by *P. kudriavzevii*

Biomass	Temperature (°C)	Nitrogen content (%)	Mean relative abundance (mg/g biomass) *
Untreated	30	1.75	0.84 ± 0.03[a]
ChCl/LA	30	1.75	1.17 ± 0.70[a]
	33		2.07 ± 0.29[b]
	36		3.98 ± 0.05[c]
ChCl/Gly	30	1.75	1.41 ± 0.29[a]
	33		2.44 ± 0.17[b]
	36		6.05 ± 0.24[c]
ChCl/Urea	30	1.75	1.42 ± 0.51[a]
	33		3.61 ± 0.29[b]
	36		9.22 ± 0.52[c]
ChCl/LA	36	0.5	0.75 ± 0.13[a]
		1.75	0.93 ± 0.29[a]
		3	2.18 ± 0.23[b]
ChCl/Gly	36	0.5	2.42 ± 0.20[b]
		1.75	1.08 ± 0.08[a]
		3	0.95 ± 0.62[a]
ChCl/Urea	36	0.5	2.19 ± 0.49[a]
		1.75	2.39 ± 0.44[a]
		3	2.99 ± 0.36[a]

*: Different letters above the numerals represents different subsets of alpha

Table 4. Optimized fermentation conditions for *K. marxianus* and *P. kudriavzevii* for production of 2-PE

Biomass	*K. marxianus*		*P. kudriavzevii*	
	Temperature (°C)	Nitrogen content (%, w/v)	Temperature (°C)	Nitrogen content (%, w/v)
Untreated	30	1.75	30	1.75
ChCl/LA	36	3	36	3
ChCl/Gly	36	3	36	0.50
ChCl/Urea	36	3	36	3

amounts of acetic acid were detected in the pretreatment hydrolysate. Specifically, the pretreatment hydrolysates from ChCl/LA, ChCl/Gly, and ChCl/Urea had acetic acid concentrations of 0.01, 0.10, and 0.04 g/L, respectively. Similarly, trace amounts of acetic acid were found in the fermentation hydrolysates of ChCl/LA and ChCl/Urea when fermented with *K. marxianus*. Both 5-HMF and furfural were absent in the pretreatment and fermentation hydrolysates of the biomasses. This aligns with a study by Kumar et al., (2016), which also reported the absence of furfural and 5-HMF following the DES pretreatment (Kumar et al., 2016). This confirms that DES pretreatment results in minimal or no inhibitor formation.

3.5 Ethanol Analysis

The ability of two non-conventional yeasts to produce bioethanol from untreated and pretreated Napier grass was assessed by spectrophotometry. Fig. 4 depicts the ethanol production by *K. marxianus* and *P. kudriavzevii* at 72 hrs of fermentation. *K. marxianus* was able to produce an ethanol concentration of 4.04% (v/v) whereas *P. kudriavzevii* produced a 3.80% (v/v) ethanol concentration from untreated biomass. A previously reported study on fermentation of acid pretreated wheat straw with two different strains of *K. marxianus* has produced 3.10 and 2.30 g/L of ethanol from *K. marxianus* SLP 1 and *K. marxianus* OFF 1 respectively. Whereas fermentation on acid pretreated sugarcane bagasse with *K. marxianus* SLP 1 and *K. marxianus* OFF 1 produced 9.00 g/L and 5.10 g/L ethanol (Sandoval-Nuñez et al., 2018). Another fermentation study on sweet sorghum biomass pretreated with 0.50% H_2SO_4 and 2% NaOH could produce 19.05 g/L of ethanol using *K. marxianus* CCT 7735 at 37°C (Tinôco et al., 2021).

Simultaneous saccharification and fermentation of alkaline pretreated carnauba straw with *K. marxianus* ATCC-36907 produced 7.53 g/L of ethanol (da Silva et al., 2018). In this study fermentation with *K. marxianus* on acidic and alkaline DES pretreated Napier grass produced 22.29 g/L and 28.70 g/L of ethanol while 33.19 g/L ethanol was produced from neutral DES pretreated biomass. Fermentation with *P. kudriavzevii* RZ8-1 using acid pretreated sugarcane bagasse as substrate yielded 35.50 g/L ethanol at 37°C (Chamnipa et al., 2018). Simultaneous saccharification and fermentation performed with *P. kudriavzevii* NBRC1279 and *P. kudriavzevii* NBRC1664 up on Japanese cedar particles produced the highest ethanol

concentration of 21.90 g/L and 23.80 g/L respectively (Akita et al., 2021). Fermentation with *P. kudriavzevii* in this study has resulted in 27.21 g/L, 30.63 g/L, and 29.34 g/L ethanol from ChCl/LA, ChCl/Gly and ChCl/Urea pretreated biomass respectively. These results are in line with the previously reported ethanol production from *K. marxianus* and *P. kudriavzevii*. However, it could be noted that ethanol production was more from the biomass pretreated with polyol-based DES than the acidic or alkaline pretreatment. This could be possibly due to the acidity or basicity of any DES remnants on the biomass that had a negative impact on yeast growth and metabolism (Zhao et al., 2015). The polyol-based DES which is near neutral may not have a detrimental effect on yeast metabolism, resulting in better ethanol production from it. Additionally, the fermentation conditions employed in this study were specifically optimized for 2-PE production rather than ethanol enhancement. Consequently, this optimization could not increase the ethanol yield despite improved cellulose and sugar content in the pretreated biomass.

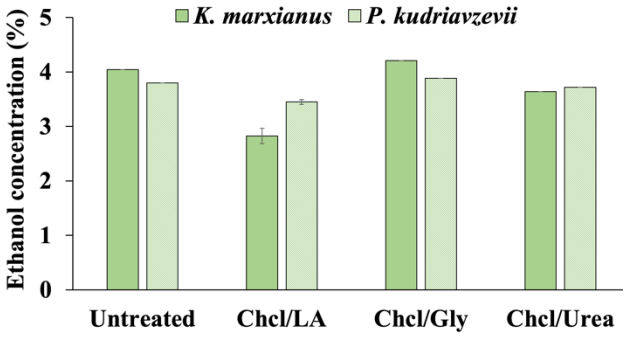

Fig. 4. Ethanol concentration at 72 hrs obtained by fermentation with *K. marxianus* and *P. kudriavzevii*

3.6 2-PE Production from Yeasts

The optimum fermentation conditions for each DES pretreated sample as shown in Table 4 were repeated in triplicates after the addition of L-Phe (0.50% w/v) to enhance the 2-PE production. The 2-PE produced at optimum fermentation conditions was quantified using the standard curve of 2-PE. Fig. 5 depicts the 2-PE production from untreated and pretreated biomass when fermented with *K. marxianus* and *P. kudriavzevii*. It could be noted that 2-PE production is better from the biomass fermented with *P. kudriavzevii* than *K. marxianus*. This might be attributed to the concentration of L-Phe used in this study. It has been previously reported that each yeast strain requires a different L-Phe concentration for enhanced production of 2-PE (Fan et al., 2020). In a study to evaluate the bio flavor production from tomato pomace and pepper pomace by *K. marxianus* in shake-flask conditions produced 2- PE at a concentration of 0.20 ppm and 0.07 ppm from tomato pomace and pepper pomace respectively (Güneşer et al., 2015). Another study on the production of alcohols and esters from tomato pomace hydrolysate by fermentation with *K. marxianus* reported a relative abundance of 2-PE as 828 µg/L (equivalent to 0.82 ppm) in a shake flask experiment (Kılmanoğlu et al., 2021). Fermentation by *K. marxianus* in a bioreactor to determine the flavor compounds resulted in a 2-PE concentration of 1.37 ppm within 72 hrs of fermentation (Hoşoğlu, 2018). Another study on the biosynthesis of 2-PE from corn stover, sugarcane straw, and corn syrup using *Pseudomonas putida* resulted in 2-PE production between 82100 ppm (Godoy et al., 2024). In this study, 2-PE production from pretreated biomass varied between 82–117 ppm and 235–511 ppm when fermented with *K. marxianus* and *P. kudriavzevii* respectively. It was obvious from the study that ChCl/Gly pretreated biomass was more efficient in 2-PE production with the yeasts which may be due to its neutral nature (Elgharbawy et al., 2020). Any remnants of ChCl/LA and ChCl/Urea DES in the biomass may have interfered with the fermentation ability of yeasts resulting in reduced 2-PE yield that ChCl/Gly pretreatment (Elgharbawy et al., 2020). Table 5 depicts the 2-PE yield reported from a few other previous studies. It can be noted that the 2-PE yield was higher from DES pretreated Napier grass. Nonetheless, the 2-PE yield observed in this study underscores the significant potential of lignocellulosic biomass as a valuable feedstock for 2-PE production.

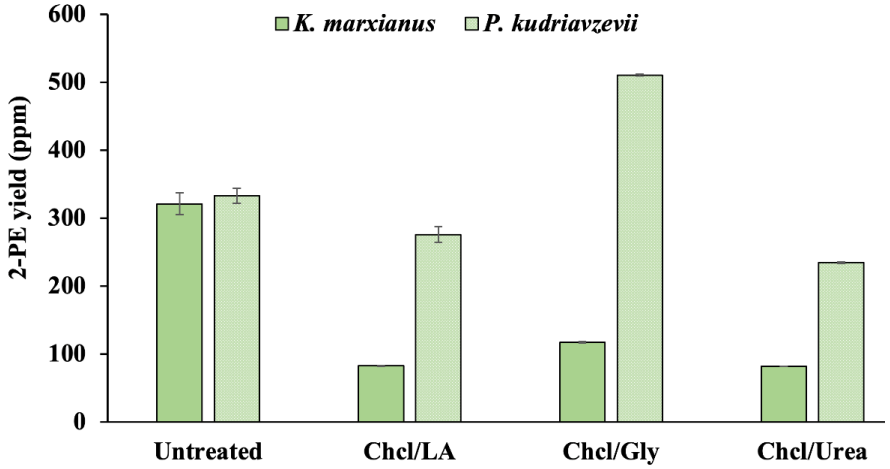

Fig. 5. 2-PE yield from *K. marxianus* and *P. kudriavzevii*

Table 5. 2-PE yield from previous studies using different microbes

Microbe	Fermentation medium	Concentration of L-Phe (g/L)	Fermentation condition		2-PE yield (ppm)	Reference
			Temperature (ºC)	Time (hrs)		
Pseudomonas putida PG2E	Corn syrup hydrolysate	0.45	30	24	110	Godoy et al. (2024)
Pseudomonas putida PG2E	Sugarcane straw hydrolysate	0.45	30	24	93	Godoy et al. (2024)
Candida utilis	Tequila vinasse	7	30	96	242.65	Rodríguez Romero et al. (2020)
Kluveromyces marxianus	Tomato pomace	-	28	48	1.51	Kılmanoğlu et al. (2021)
Debaryomyces hansenii	Tomato pomace	-	30	72	0.29	Güneşer et al. (2015)
P. kudriavzevii	ChCl/Gly pretreated Napier grass	5	36	120	510.51	This study

4. CONCLUSION

This present study investigated the potential of Napier grass pretreated with three different DES for enhanced production of the flavor compound 2-PE via fermentation using two non-conventional yeasts. DES pretreatment significantly increased cellulose content, around 50.88% more than the native biomass with ChCl/Gly. The enzymatic digestibility of ChCl/LA pretreatment resulted in 1.24-fold more glucose yield than untreated biomass. Fermentation optimization revealed strain-specific peptone requirements for maximal 2-PE production at an optimum temperature of 36ºC. Notably, *P. kudriavzevii* achieved a maximum 2-PE yield of 510.51 ppm under optimized conditions using ChCl/Gly pretreatment. While this study was conducted at a small scale, it demonstrates the promise of Napier grass, a readily available lignocellulosic biomass, for sustainable 2-PE production through DES pretreatment and fermentation with *P. kudriavzevii*. Future research focusing on process scale-up, economic feasibility, and optimization of fermentation parameters for even higher 2-PE yields holds significant promise for the development of a commercially viable route to produce this valuable flavor compound.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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REFERENCES

Agrawal, R.., Verma, A., Singhania, R. R., Varjani, S., Di Dong, C., Patel, A.K. 2021. Current understanding of the inhibition factors and their mechanism of action for the lignocellulosic biomass hydrolysis. Bioresource Technology, 332, 125042.

Akbarian, A., Andooz, A., Kowsari, E., Ramakrishna, S., Asgari, S., Cheshmeh, Z.A 2022. Challenges and opportunities of lignocellulosic biomass gasification in the path of circular bioeconomy. Bioresource Technology, 362, 127774.

Akita, H., Goshima, T., Suzuki, T., Itoiri, Y., Kimura, Z.I., Matsushika, A. 2021. Application of Pichia Kudriavzevii NBRC1279 and NBRC1664 to simultaneous saccharification and fermentation for bioethanol production. Fermentation, 7, 83.

Ao, T.J., Li, K., Mehmood, M.A., Zhao, X.Q., Bai, F.W., Boopathy, R., Liu, C.G. 2024. Process optimization for acidic deep eutectic solvent pretreatment of Corn Stover to enhance enzymatic saccharification. Biomass Conversion and Biorefinery, 14, 6215–6228.

Ashokkumar, V., Venkatkarthick, R., Jayashree, S., Chuetor, S., Dharmaraj, S., Kumar, G., Ngamcharussrivichai., C. 2022. Recent advances in lignocellulosic biomass for biofuels and value-added bioproducts - A critical review. Bioresource Technology, 344, 126195.

Baksi, S., Saha, D., Saha, S., Sarkar, U., Basu, D., Kuniyal, J.C. 2023. Pre-treatment of lignocellulosic biomass: Review of various physico-chemical and biological methods influencing the extent of biomass depolymerization. International Journal of Environmental Science and Technology, 20, 13895–13922.

Baptista, M., Domingues, L. 2022. Kluyveromyces Marxianus as a microbial cell factory for lignocellulosic biomass valorisation. Biotechnology Advances, 60, 108027.

Chamnipa, N., Thanonkeo, S., Klanrit, P., Thanonkeo, P. 2018. The Potential of the newly isolated thermotolerant yeast Pichia kudriavzevii RZ8-1 for high-temperature ethanol production. Brazilian Journal of Microbiology, 49,

378–391.

Chinwatpaiboon, P., Saejao, C., Boonsombuti, A., Luengnaruemitchai, A. 2021. Optimization of ionic liquid 1-ethyl-3-methylimidazolium acetate pretreatment of Napier grass combined with acetic acid and ferric chloride under microwave radiation by response surface methodology. Biomass Conversion and Biorefinery, 13, 1–12.

Chreptowicz, K., Sternicka, M.K., Kowalska, P.D., Mierzejewska, J. 2018. Screening of yeasts for the production of 2-phenylethanol (rose aroma) in organic waste-based media. Letters in Applied Microbiology, 66, 153–160.

Chung Jr, H., Lee, S.L., Chou, C.C. 2000. Production and molar yield of 2-Phenylethanol by Pichia fermentans L-5 as affected by some medium components. Journal of Bioscience and Bioengineering, 90, 142–147.

Conde-Báez, L., Castro-Rosas, J., Villagómez-Ibarra, J.R. Páez-Lerma, J.B. Gómez-Aldapa, C. 2017. Evaluation of waste of the cheese industry for the production of aroma of roses (phenylethyl alcohol). Waste and Biomass Valorization, 8, 1343–1350.

Conde-Báez, L., López-Molina, A., Gómez-Aldapa, C., Pineda-Muñoz, C., Conde-Mejía, C. 2019. Economic projection of 2-phenylethanol production from whey. Food and Bioproducts Processing, 115, 10–16.

Cordero-Soto, I.N., Huerta-Ochoa, S., Moussa, M., Ochoa-Martinez, L.A., Soto-Cruz, N.O., and Rutiaga-Quinones, O.M. 2020. Recent advances in the bioconversion of 2-phenylethanol through biotechnological processes for using as a natural food additive. Food Microbiology and Biotechnology, 231–247.

Elgharbawy, A.A., Hayyan, M., Hayyan, A., Basirun, W.J., Salleh, H.M., Mirghani, M.E. 2020. A grand avenue to integrate deep eutectic solvents into biomass processing. Biomass and Bioenergy, 137, 105550.

Etschmann, M.M.W., Sell, D. Schrader, J. 2003. Screening of yeasts for the production of the aroma compound 2-phenylethanol in a molasses-based medium. Biotechnology Letters, 25, 531–536.

Fakayode, O.A., Akpabli-Tsigbe, N.D.K., Wahia, H., Tu, S., Ren, M., Zhou, C., Ma, H. 2021. Integrated bioprocess for bio-ethanol production from watermelon rind biomass: Ultrasound-assisted deep eutectic solvent pretreatment, enzymatic hydrolysis and fermentation. Renewable Energy, 180, 258–270.

Fan, G., Cheng, L., Fu, Z., Sun, B., Teng, C., Jiang, X., Li, X. 2020. Screening of yeasts isolated from Baijiu environments for 2-Phenylethanol production and optimization of production conditions. 3 Biotech, 10, 1–17.

Fisher, C., Scott, T.R. 2007. Introduction–problems in flavour research.

Garavaglia, J., Flôres, S.H., Pizzolato, T.M., Peralba, M.D.C., Ayub, M.A.Z. 2007. Bioconversion of L-phenylalanine into 2-phenylethanol by Kluyveromyces marxianus in grape must cultures. World Journal of Microbiology and Biotechnology 23, 1273–1279.

Gethins, L., Guneser, O., Demirkol, A., Rea, M.C., Stanton, C., Ross, R.P., Yuceer, Y., Morrissey., J.P. 2015. Influence of carbon and nitrogen source on production of volatile fragrance and flavour metabolites by the yeast Kluyveromyces marxianus. Yeast, 32, 67–76.

Godoy, P., Udaondo, Z., Duque, E., Ramos, J.L. 2024. Biosynthesis of fragrance 2-Phenylethanol from sugars by Pseudomonas putida. Biotechnology for Biofuels and Bioproducts, 17, 51.

Güneşer, O., Demirkol, A., Karagül Yüceer, Y., Özmen Toğay, S., İşleten Hoşoğlu, M., Elibol, M. 2015. Bioflavour production from tomato and pepper pomaces by Kluyveromyces marxianus and Debaryomyces hansenii. Bioprocess and Biosystems Engineering, 38, 1143–1155.

Hassan, E.S.R., Mutelet, F. 2022. Evaluation of miscanthus pretreatment effect by Choline chloride based deep eutectic solvents on bioethanol production. Bioresource Technology, 345, 126460.

Hoşoğlu, M.I. 2018. Study of increasing the production of volatile flavor compounds by the yeast Kluyveromyces marxianus through optimization of carbon and nitrogen sources. Food and Health, 4, 112–123.

Kılmanoğlu, H., Hoşoğlu, M.İ., Güneşer, O., Yüceer, Y.K. 2021. Optimization of pretreatment and enzymatic hydrolysis conditions of tomato pomace for production of alcohols and esters by Kluyveromyces marxianus. LWT, 138, 110728.

Kongkeitkajorn M.B., Sae-Kuay C., Reungsang, A. 2020. Evaluation of napier grass for bioethanol production through a fermentation process. Processes, 8, 567.

Kumar, A.K., Parikh, B.S., Pravakar, M. 2016. Natural deep eutectic solvent mediated pretreatment of rice straw: Bioanalytical characterization of lignin extract and enzymatic hydrolysis of pretreated biomass residue. Environmental Science and Pollution Research, 23, 9265–9275.

Kumar, N., Gautam, R., Stallings, J.D., Coty, G.G., Lynam, J.G. 2021. Secondary agriculture residues pretreatment using deep eutectic solvents. Waste and Biomass Valorization, 12, 2259–2269.

Lee, K.M., Quek, J.D., Tey, W.Y., Lim, S., Kang, H.S., Quen, L.K., Mahmood, W.A.W., Jamaludin, S.I.S., Teng, K.H., Khoo, K.S. 2022. Biomass valorization by integrating ultrasonication and deep eutectic solvents: delignification, cellulose digestibility and solvent reuse. Biochemical Engineering Journal, 187, 108587.

Li, C., Huang, C., Zhao, Y., Zheng, C., Su, H., Zhang, L., Luo, W., Zhao, H., Wang, S., Huang L.J. 2021. Effect of choline-based deep eutectic solvent pretreatment on the structure of cellulose and lignin in Bagasse. Processes, 9, 384.

Li, P., Yang, C., Jiang, Z., Jin, Y., Wu, W. 2023. Lignocellulose pretreatment by deep eutectic solvents and related technologies: A review. Journal of Bioresources and Bioproducts, 8, 33–44.

Liu, Y., WeiZhuo, X., Wei, X. 2022. A review on lipase-catalyzed synthesis of geranyl esters as flavor additives for food, pharmaceutical and cosmetic applications. Food Chemistry Advances, 1, 100052.

Manokhoon, P., Rangseesuriyachai, T. 2020. Effect of two-stage sodium hydroxide pretreatment on the composition and structure of Napier Grass (Pakchong 1) (Pennisetum purpureum). International Journal of Green Energy, 17, 864–871.

Martínez, O., A. Sánchez, A., Font, X., Barrena, R. 2018. Bioproduction of 2-henyplethanol and 2-phenethyl acetate by Kluyveromyces marxianus through the solid-state fermentation of sugarcane bagasse. Applied Microbiology and Biotechnology, 102, 4703–4716.

Martínez-Avilla, O., Sánchez, A., Font, X., Barrena, R. 2020. 2-phenylethanol (rose aroma) production potential of an isolated Pichia kudriavzevii through solid-state fermentation. Process Biochemistry, 93, 94–103.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, 426–428.

Mitri, S., Koubaa, M., Maroun, R.G., Rossignol, T., Nicaud, J.M., Louka, N. 2022. Bioproduction of 2-Phenylethanol through yeast fermentation on synthetic media and on agro-industrial waste and by-products: A review. Foods, 11, 109.

Mortzfeld, F.B., Hashem, C., Vranková, K., Winkler, M., Rudroff, F. 2020. Pyrazines: Synthesis and industrial application of these valuable flavor and fragrance compounds. Biotechnology Journal, 15, 2000064.

Nie, K., Li, B., Song, Y., Qiao, X., Zhao, T., Zhang, Y., Ben, H., Han, G. 2023. A promising degumming method to prepare kenaf fibers by using recyclable deep eutectic solvents. Industrial Crops and Products, 191, 115990.

Nie, K., Li, B., Wang, P., Song, Y., Ben, H., Han, G., Jiang, W., Ragauskas, A. 2024. Efficient fractionation of kenaf chemical components by using recyclable acidic DES. Industrial Crops and Products, 211, 118239.

Oh, Y., Park, S., Jung, D., Oh, K.K., Lee, S.H. 2020. Effect of hydrogen bond donor on the choline chloride-based deep eutectic solvent-mediated extraction of lignin from pine wood. International Journal of Biological Macromolecules, 165, 187–197.

Pakchamni, P., Afedzi, A.E.K., Parakulsuksatid, P. 2022. Optimization of alkaline-assisted organosolv pretreatment of sugarcane trash for the production of succinic acid using response surface methodology. Biocatalysis and Agricultural Biotechnology, 43, 102374.

Pan, M., Zhao, G., Ding, C., Wu, B., Lian, Z., Lian, H. 2017. Physicochemical transformation of rice straw after pretreatment with a deep eutectic solvent of choline chloride/urea. Carbohydrate Polymers, 176, 307–314.

Panakkal, E.J., Cheenkachorn, K., Chuetor, S., Dasari, S., Katam, K., Phusantisampan, T.S, Cheng, Y.S., Sriariyanun, M. 2024. A comparative study on effectiveness and recyclability of three different deep eutectic solvents for biomass fractionation. Biomass Conversion and Biorefinery, 1–15.

Panakkal, E.J., Cheenkachorn, K., S. Chuetor, S., Tantayotai, S., Raina, P., Cheng, Y.S., Sriariyanun, M. 2022. Optimization of deep eutectic solvent pretreatment for bioethanol production from Napier Grass. Sustainable Energy Technologies and Assessments, 54, 102856.

Panakkal, E.J., Cheenkachorn, K., Gundupalli, M.P., Kitiborwornkul, N., Sriariyanun, M. 2021a. Impact of sulfuric acid pretreatment of durian Peel on the production of fermentable sugar and ethanol. Journal of the Indian Chemical Society, 98, 100264.

Panakkal, E.J., Kitiborwornkul, N., Sriariyanun, M., Ratanapoompinyo, J., Yasurin, P., Asavasanti, S., Rodiahwati, W., Tantayotai, P. 2021b. Production of food flavouring agents by enzymatic reaction and microbial fermentation. Applied Science and Engineering Progress, 14, 297–312.

Procentese, A., Raganati, F., Olivieri, G., Russo, M.E., Rehmann, L., Marzocchella, A. 2017. Low-energy biomass pretreatment with deep eutectic solvents for bio-butanol production. Bioresource Technology, 243, 464–473.

Raj, T., Singh, V. 2024. Natural deep eutectic solvents (NADES) assisted deconstruction of oilcane bagasse for high lipid and sugar recovery. Industrial Crops and Products, 210, 118127.

Research Dive. (2022). Packaged food products market. Retrieved July 17, 2024, from https://*www.researchdive.com/8397/packaged-food-products-market.*

de Jesús Rodríguez-Romero, J., Aceves-Lara, C.A., Silva, C.F., Gschaedler, A., Amaya-Delgado, L., Arrizon, J. 2020. 2-Phenylethanol and 2-phenylethylacetate production by nonconventional yeasts using Tequila vinasses as a substrate. Biotechnology Reports, 25, e00420.

Sai, Y.W., Lee, K.M. 2019. Enhanced cellulase accessibility using acid-based deep eutectic solvent in pretreatment of empty fruit bunches. Cellulose, 26, 9517–9528.

Sandoval-Nuñez, D., Arellano-Plaza, M., Gschaedler, A. Arrizon, J. Amaya-Delgado, L. 2018. A comparative study of lignocellulosic ethanol productivities by Kluyveromyces marxianus and Saccharomyces cerevisiae. Clean Technologies and Environmental Policy, 20, 1491–1499.

Scognamiglio, J., Jones, L., Letizia, C.S., Api, A.M. 2012. Fragrance material review on phenylethyl alcohol. Food and Chemical Toxicology, 50, S224–S239.

da Silva, F.L., de Oliveira Campos, A., dos Santos, D.A., Magalhães, E.R.B., de Macedo, G.R., dos Santos, E.S. 2018. Valorization of an agroextractive residue—Carnauba Straw—for the production of bioethanol by simultaneous saccharification and fermentation (SSF). Renewable Energy, 127, 661–669.

Sriariyanun, M., Mutrakulcharoen, P.A.R.I.T.A., Tepaamorndech, Dech, S.U.R.A.P.U.N., Cheenkachorn, K., Rattanaporn, K. 2019. A rapid spectrophotometric

method for quantitative determination of ethanol in fermentation products. Oriental Journal of Chemistry, 35, 744–750.

Tian, D., Guo, Y., Hu, J., Yang, G., Zhang, J., Luo, L., Xiao, Y., Shen, F. 2020. Acidic deep eutectic solvents pretreatment for selective lignocellulosic biomass fractionation with enhanced cellulose reactivity. International Journal of Biological Macromolecules, 142, 288–297.

Tinôco, D., Genier, H.L.A., da Silveira, W.B. 2021. Technology valuation of cellulosic ethanol production by Kluyveromyces marxianus CCT 7735 from sweet Sorghum Bagasse at elevated temperatures. Renewable Energy, 173, 188–196.

Wang, Z., Bai, X., Guo, X. He, X. 2017. Regulation of crucial enzymes and transcription factors on 2-phenylethanol biosynthesis via Ehrlich pathway in Saccharomyces cerevisiae. Journal of Industrial Microbiology and Biotechnology, 44, 129–139.

Wei, H.L., Wang, Y.T., Hong, Y.Y., Zhu, M.J. 2021. Pretreatment of rice straw with recycled ionic liquids by phase-separation process for low-cost biorefinery. Biotechnology and Applied Biochemistry, 68, 871–880.

Yan, D., Ji, Q., Yu, X., Li, M., Fakayode, O.A., Yagoub, A.E.A., Chen, L., Zhou, C. 2021. Multimode-ultrasound and microwave assisted natural ternary deep eutectic solvent sequential pretreatments for corn straw biomass deconstruction under mild conditions. Ultrasonics Sonochemistry, 72, 105414.

Semelsberger, T.A., Li, C., Tanjore, D. 2020. Characterizing variability in lignocellulosic biomass: A review. ACS Sustainable Chemistry and Engineering, 8, 8059–8085.

Zhao, B.Y., Xu, P., Yang, F.X., Wu, H., Zong, M.H., Lou, W.Y. 2015. Biocompatible deep eutectic solvents based on choline chloride: Characterization and application to the extraction of rutin from Sophora japonica. ACS Sustainable Chemistry & Engineering, 3, 2746–2755.

Zhou, R., Song, Q., Xia, H., Song, N., Yang, Q., Zhang, X. Yao, L., Yang, S., Dai, J., Chen, X. 2023. Isolation and identification of non-saccharomyces yeast producing 2-Phenylethanol and study of the ehrlich pathway and shikimate pathway. Journal of Fungi, 9, 878.

SUPPLEMENTARY FILE

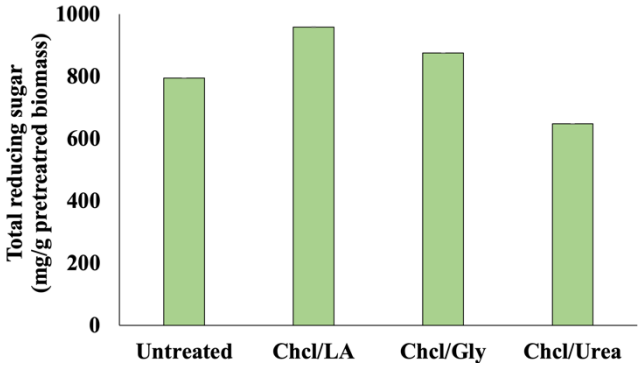

Fig. S1. Total reducing sugar yield from untreated and pretreated biomass