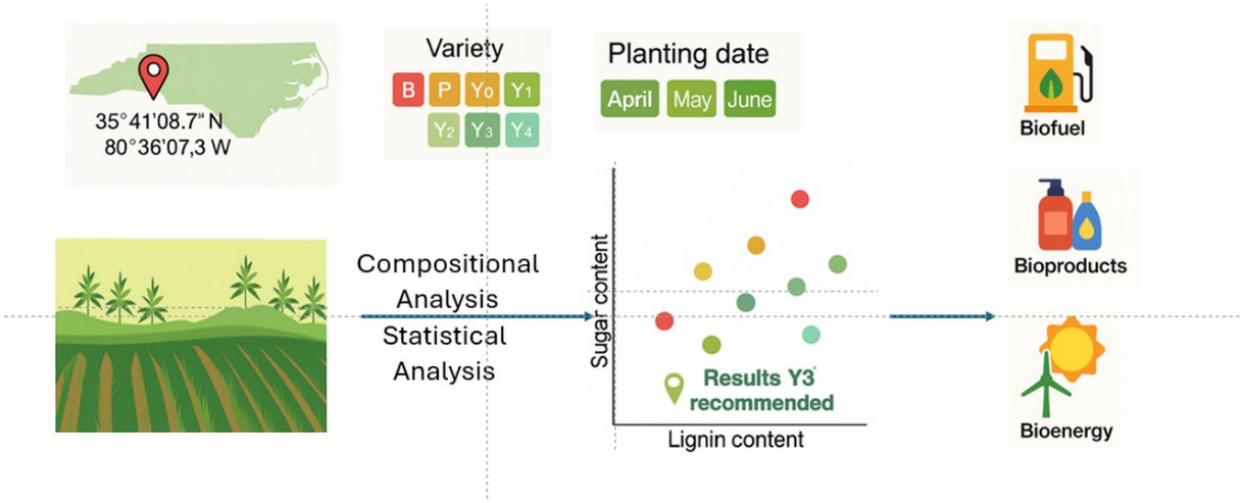
Compositional Analysis of Hemp Hurd Affected by Variety and Planting Season

Jingjing Wang,[a] David Suchoff,[b] Dwi Cahyani,[a] Grant Ellington,[a] and Wenqiao Yuan [a,*]

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GRAPHICAL ABSTRACT



Compositional Analysis of Hemp Hurd Affected by Variety and Planting Season

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Industrial hemp is gaining attention not only for its fiber and cannabidiol (CBD) oil production but also as a promising lignocellulose resource for bioenergy and biochemical development. This study examined seven industrial hemp varieties planted in three seasons and quantified their contents of major cellulosic sugar (glucose), hemicellulosic sugars (xylose, galactose, mannose), and lignin. The results showed that both planting date and variety, as well as their interaction, influenced sugar and lignin accumulation, though the effects varied by case. Variety Y3 exhibited among the highest contents of glucose, xylose, and galactose regardless of planting seasons. Additionally, sugar content (specifically, glucose and xylose) showed a strong negative correlation with lignin content, making it a desirable variety for crop breeding and biorefinery. When actual biomass yield was considered, variety Y1 demonstrated the highest total glucose yield (~6,000 kg-glucose/ha) among the seven hemp varieties studied, making it the choice for glucose-based biofuel or bioproduct development.

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Keywords: Hemp hurd; Hemp variety; Chemical composition; GEI; Biorefinery; Bioenergy; Bioproduct

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INTRODUCTION

Cannabis sativa is a versatile crop with a long history of cultivation worldwide. It is valued for its fiber, seeds, and medicinal properties due to its high levels of Δ9-tetrahydrocannabinol (THC) (Fike 2016). Industrial hemp, a variety of *Cannabis sativa* L. with THC levels below 0.3%, extends its applications beyond traditional uses to cannabidiol (CBD) oil production and also serves as a promising non-food biomass feedstock for biochemical and bioenergy production. In the context of this study, hemp specifically refers to industrial hemp. The global hemp market now includes over 25,000 products spanning nine key industries: agriculture, textiles, recycling, automotive, furniture, food and beverages, paper, construction materials, and personal care (Crini *et al.* 2020).

Hemp is recognized as a low-cost, ecologically sustainable, and multifunctional plant. Over the past three decades, the conversion of hemp biomass into bioenergy and bioproducts has gained significant interest due to the growing demand for sustainable energy solutions (Prade *et al.* 2011). Compared to other lignocellulosic biomass sources such as wheat straw, corn stover, and sorghum, industrial hemp biomass hurd exhibits significantly higher cellulose content, ranging from 40 to 48%. It also contains hemicellulose at 18 to 24% and lignin at 21 to 24%. This composition makes hemp a

promising candidate for biomass-to-bioproducts and bioenergy conversion, particularly when utilizing a biorefinery approach (Zhao *et al.* 2020; Sarker and Wan 2022). As an energy crop, hemp shows great potential for biomass production, making it a viable feedstock for biorefineries. It can be used to produce a range of biofuels, such as bioethanol, biodiesel, and biohydrogen, as well as biogas, organic acids, biomaterials, bio-oil, and various pharmaceutical and nutraceutical compounds (Tripathi *et al.* 2023). Compared to kenaf, switchgrass, and biomass sorghum, hemp yields similar amounts of stem biomass while requiring less inputs. The predicted ethanol yield from hemp stems is approximately 82 gallons per dry ton, which is comparable to that of kenaf, switchgrass, and biomass sorghum. Furthermore, field trials have shown that hemp can produce an additional 1,230 kg/ha of grain, which, when combined with biofuel production from the stem, can lead to higher gross profits per hectare than the other crops (Das *et al.* 2017).

Additionally, hemp is highly adaptable to a range of climatic conditions and is more water-efficient compared to crops such as alfalfa and maize. Its natural resistance to many pests and diseases further reduces the need for chemical biocides, significantly lowering its environmental impact (Parvez *et al.* 2021). In addition, hemp is highly efficient in carbon sequestration, capturing up to 22 tons of CO_2 per hectare, making it one of the most environmentally beneficial crops for reducing atmospheric carbon and utilizing sunlight for photosynthesis (Adesina *et al.* 2020).

Despite many advantages of growing hemp, its production in the United States has historically been restricted due to its association with marijuana. As a result, the U.S. market has long relied on imports of both finished hemp products and raw materials for processing (Johnson 2018). Canada has been the largest supplier, accounting for about 90% of the value of annual hemp imports. According to the Hemp Industries Association (HIA), U.S. retail sales of hemp products reached nearly $700 million in 2016 (Vote Hemp 2017). Following the passage of the 2018 Farm Bill, which reclassified hemp and legalized its cultivation, the U.S. hemp industry has seen a significant growth, presenting new economic opportunities for American farmers (USDA 2020).

The chemical composition of biomass is crucial for its potential as a feedstock for biofuels and biochemical production. Key components like cellulose and hemicellulose are vital for producing fermentable sugars used in bioethanol and other fermentation products. Lignin, a significant part of hemp biomass, poses challenges in conversion processes due to its complex structure, which hinders the enzymatic breakdown of carbohydrates and can release toxic compounds that inhibit biofuel production. Therefore, understanding the chemical composition of hemp is essential for optimizing its use in bio-based applications (Ji *et al.* 2021).

Research on Genotype-Environment Interaction (GEI) in relation to hemp's chemical composition is limited, with most studies primarily focusing on traits such as THC and CBD content, grain yield, and plant height (Narh *et al.* 2014). Hemp hurd, a byproduct of industrial hemp fiber processing, has traditionally been underutilized and often discarded. However, it has recently received significant attention as a promising feedstock for biorefining, owing to its high cellulose content and potential for sustainable resource utilization (Bokhari *et al.* 2021). Therefore, there is a critical need for studies exploring the GEI in relation to the chemical composition of hemp hurds, particularly focusing on key components such as cellulose, hemicellulose, and lignin (Campbell *et al.* 2019). Such research that links variations in local environmental and agronomic conditions to existing chemical composition will provide valuable insights into environment-specific cultivation optimization. This will also aid in identifying hemp varieties that are either

more resilient or more susceptible to these environmental conditions. Understanding these dynamics can improve breeding and cultivation strategies, allowing farmers to select varieties that perform optimally in specific local environments, thereby addressing challenges faced by the emerging U.S. hemp sector (Olson *et al.* 2020).

This research examined the content variations in cellulosic and hemicellulosic sugars and lignin across seven different hemp varieties and evaluate changes within each variety over three planting seasons. By doing so, this study sought to fill the knowledge gap regarding how different hemp varieties and planting season impact the main chemical compositions and sugar yields.

EXPERIMENTAL

Hemp Collection

The study evaluated seven hemp varieties: Bama (B), Puma (P), Yuma-0 (Y0), Yuma-1 (Y1), Yuma-2 (Y2), Yuma-3 (Y3), and Yuma-4 (Y4). These hemp varieties originate from southern China, where their bast fibers are traditionally used for textile production. They are currently being assessed for their suitability in textile-grade fiber production, regional adaptability, and the optimization of yield and crop quality in the Southeastern United States. Field experiments were conducted at the Piedmont Research Station in Salisbury, North Carolina (35°41'08.7" N, 80°36'07.3" W), using a split-plot randomized complete block design with four replicates. Planting date served as the main-plot factor and variety as the sub-plot factor, with each variety randomly assigned to 6 m × 1.5 m plots. Three planting dates were evaluated: March, April, and June (Halker *et al.* 2024). March and April represent the recommended planting window for fiber hemp in the region, while June represents a late planting date beyond the optimal window, included to capture the effect of heat stress and shortened vegetative period on hurd chemical composition. Together, these three dates span approximately 90 days and capture meaningful variation in temperature, photoperiod, and accumulated growing days, providing an agronomically relevant representation of seasonal variation for fiber hemp production in the Southeastern United States. For this study, hemp plants were harvested in September 2022 from these trials. For each variety in each planting season, approximately 10 lb (~4.5 kg) of hemp stalk was harvested from the field. The harvested material was sun-dried and stored under a pavilion at ambient temperature, with all processing completed within six months of harvest. Sun-drying is a widely accepted drying method for lignocellulosic biomass in agricultural research. Cellulose and lignin are well known to be stable under dry ambient storage conditions, as demonstrated by studies showing no statistically significant changes in major biomass components during long-term storage (Templeton *et al.* 2016). While hemicellulose may be susceptible to minor degradation over extended periods, moisture content is the primary driver of such degradation, and the dry storage conditions used in this work substantially mitigated this risk (Smith *et al.* 2020). In addition, because all samples were stored under identical conditions, any residual storage effects would be consistent across all variety × season combinations and would not introduce differential bias into the comparative conclusions. Biomass yield was reported in a previous study (Halker *et al.* 2024).

Hemp Hurd Preparation

Decortication of hemp was performed manually to separate the outer fibers from the woody core (hurd). To minimize operator-induced variability, manual decortication was performed by the same trained operators throughout the study. Decortication was applied to all plants harvested from each replicate plot. The hurd from each plot was then combined and homogenized before sub-sampling, effectively integrating variability across all stalks in the plot before any sub-sample was taken for analysis. The hurds were then shredded using a woodchipper (Chipper Shredder Pro-7HP 212CC, Super Handy, CA, USA) and ground into 1.0-mm powder using a microfine grinder (IKA MF10 basic, NC, USA) with a 1-mm screen (Hames *et al*. 2008). Grinding biomass to pass through a 1-mm screen ensures consistent particle size, adequate solvent penetration during Soxhlet extraction, and complete acid hydrolysis during subsequent sugar and lignin determination.

Compositional Analysis of Hemp Hurd

Water- and ethanol-soluble compounds present in the ground samples were extracted using the Soxhlet method according to National Renewable Energy Laboratory's (NREL) analytical procedure (Sluiter 2008). Approximately 5 to 7 g of ground material was used per replicate for Soxhlet extraction, following the protocol without modification. Extractives-free samples (0.3 g per replicate) underwent a two-step acid hydrolysis according to Sluiter *et al.* (2008).

The acid soluble lignin (ASL) in the hydrolysis liquid was analyzed by measuring absorbance at 240 nm using a UV-Visible spectrophotometer (Genesys 10s UV-VIS, Thermo Scientific). The acid insoluble lignin (AIL), collected in a filtering crucible, was dried in an oven at 105 °C for at least 4 h. Then it was placed in a muffle furnace at 575 ± 25 °C for 24 ± 6 h until a constant weight was achieved. Cellulosic and hemicellulosic sugars in the hydrolysis liquid were analyzed using High-Performance Liquid Chromatography (HPLC, Shimadzu) equipped with a Bio-Rad Aminex HPX-87P column and a Refractive Index Detector (RID-10A, Shimadzu) at a column temperature of 85°C. The mobile phase used was water, and the flow rate was 0.5 mL/min.

Statistical Analysis

All samples were tested in triplicate. A two-way ANOVA with a post hoc Tukey HSD test was conducted to examine the effects of hemp variety, planting season, and their interaction on sugar and lignin yields. A significance level of 0.05 ($p < 0.05$) was established for the analysis. Normality of residuals was assessed using the Shapiro-Wilk test. For glucose ($W = 0.991$, $p = 0.591$) and xylose ($W = 0.987$, $p = 0.259$), residuals were normally distributed ($p > 0.05$). For galactose, mannose, ASL, and AIL, the Shapiro-Wilk test indicated departures from normality ($p < 0.05$), largely attributable to the pronounced variety × season interaction effects. Statistical analyses were performed using SAS OnDemand for Academics (SAS Institute Inc.) and Origin (2024b Academic, OriginLab Corporation, USA).

RESULTS AND DISCUSSION

To ensure a uniform material suitable for compositional analysis, all hemp samples were prepared in accordance with the specifications outlined in the Laboratory Analytical Procedure (Hames *et al.* 2008). Figure 1 illustrates the key steps involved in the preparation

of hemp for compositional analysis, which included collecting hemp from the field, drying the material, separating the bast fiber from the hurd, and milling the hemp hurd to a particle size appropriate for subsequent analyses.

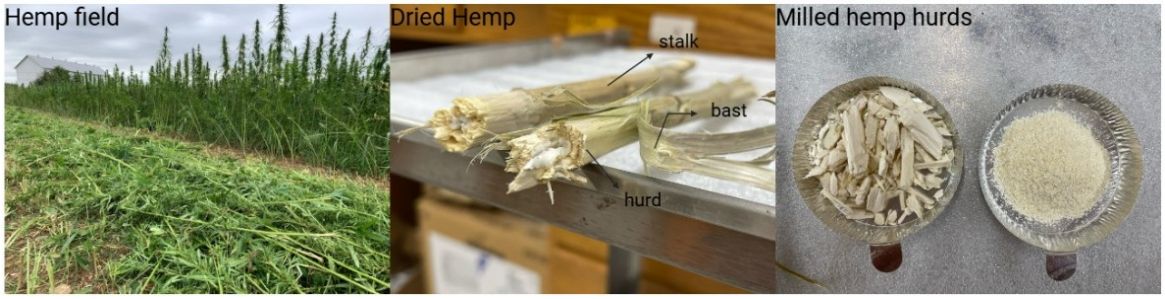


Fig. 1. The process of preparation of hemp for compositional analysis

Glucose Content

The glucose content of hemp hurd, a critical factor influencing its suitability for bio-based applications, is known to vary across different varieties that have evolved through targeted plant breeding and selection programs (Mishra *et al.* 2022). For instance, "grain-forward" varieties are optimized for seed production, while "fiber-forward" varieties are bred to maximize fiber yield.

In this context, genetic variations significantly influenced glucose content in hemp hurd (Fig. 2a). Among the seven varieties analyzed, Y1 and Y3 exhibited high overall glucose content (47.5%), while P showed the lowest (45.1%). Although the numerical differences were relatively narrow (range: 2.4%), a one-way ANOVA (Supplementary Table 1) confirmed statistically significant distinctions among the varieties ($p < 0.05$). This systematic variability suggests that genetic differences play a key role in driving glucose accumulation. Such variations may have practical implications in industrial applications, where even marginal improvements in feedstock quality can directly enhance biorefinery output.

In addition to the variation among varieties, environmental factors—such as soil type, sowing and harvest timing, fertilization practices, and plant density—also play a crucial role in hemp growth, biomass production, and biomass chemical composition (Zhao *et al.* 2020). Studies have shown that variety type and planting year, as the main treatments, significantly affect key growth parameters such as flowering time, plant height, stem and seed yield, and seed composition. These environmental factors influence how well the plant adapts to its surroundings, ultimately affecting both the yield and quality of hemp as a raw material for industrial uses (Baldini *et al.* 2020). Seasonal conditions, defined by planting date or season (April, June, March) with fixed harvest dates, profoundly affected glucose yield (Fig. 2b). March planting yielded the highest average glucose content (47.4%). June (46.8%) and April (45.8%) followed. These trends underscore the role of temperature and photoperiod in modulating metabolic pathways critical to glucose synthesis.

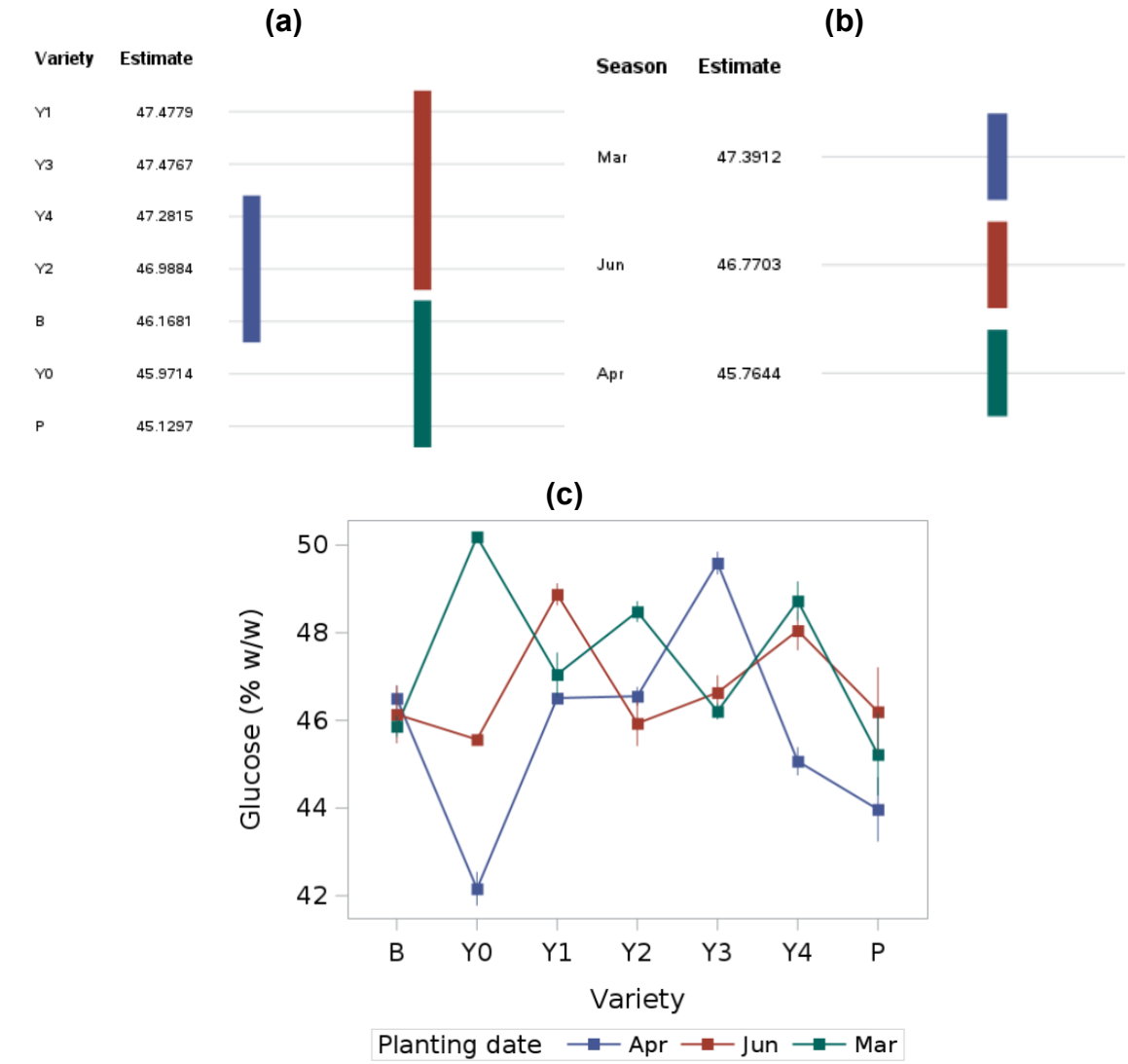


Fig. 2. Impact of variety (a), planting season (b), and their interaction (c) on glucose content (% w/w) of hemp hurd. In panels (a) and (b), bars sharing the same letter are not significantly different (Tukey HSD, $p < 0.05$). Panel (c) shows the variety × planting season interaction, with varieties on the x-axis and glucose content on the y-axis. Color-coded lines represent the three planting dates (green = March, blue = April, red = June), and error bars represent ± standard error of the mean. Parallel lines indicate consistent variety performance across planting dates, while diverging or crossing lines indicate variety-specific responses to planting season.

Genotype × Environment (G×E) interactions revealed variety-specific responses to seasonal conditions (Fig. 2c), with some varieties exhibiting pronounced seasonal variability. Y0 demonstrated the most dramatic seasonal fluctuation, reaching its highest glucose level in March (50.20%) but dropping sharply to 42.16% in April. In contrast, B and P display relatively stable glucose levels with only minor fluctuations across seasons.

Xylose Content

Xylose, as the second most abundant sugar in lignocellulosic biomass, enhances biorefinery economics and sustainability by enabling value-added product development from waste streams while supporting zero-waste goals through complete biomass valorization (Lee *et al.* 2021). Therefore, understanding its content in hemp hurd biomass

is essential for optimizing its potential in industrial processes. Analysis of xylose content across multiple hemp varieties and planting seasons reveals significant varietal and seasonal effects, as well as interactions between these factors.

Varieties Y3 (24.37%), P (24.31%), and B (24.00%) exhibited high xylose content with no significant differences among them as shown in Fig. 3. In contrast, Y0 (22.44%) had the lowest xylose content that differed significantly from all other varieties. This pattern highlights the superior performance of Y3, P and B in xylose accumulation, suggesting that these varieties may possess genetic advantages or favorable growth responses that enhance xylose synthesis, regardless of the planting season.

Analysis of the impact of planting seasons revealed that xylose content was generally highest in March and lowest in June, suggesting that a longer growth period may enhance xylose accumulation. Xylose, a major component of hemicellulose in plant cell walls, tends to increase as plants mature and their secondary cell walls thicken. This aligns with findings from similar studies, which have shown that delayed harvest is associated with increased cell wall biomass, further supporting the idea that extended growth periods favor the accumulation of structural carbohydrates like xylose (Le Ngoc Huyen *et al.* 2010).

Further analysis of the interaction between variety and planting season revealed that the influence of planting season on xylose content varied by variety. For example, Y0, Y1 and Y2 exhibited strong seasonal fluctuations, indicating heightened sensitivity to environmental changes. In contrast, the other varieties maintained relatively stable xylose levels across seasons, suggesting greater resilience to seasonal variability.

Statistical analysis using Tukey's Grouping confirmed that variety had a stronger influence on xylose content than season. While early-season planting in March may marginally enhanced xylose levels, the effect was minimal compared to the benefits of selecting high-performing varieties such as Y3 and P. These findings highlight the importance of prioritizing superior varieties over seasonal adjustments for optimizing xylose content in hemp hurd biomass. This approach not only maximizes xylose yield but also supports the development of efficient and sustainable biorefinery processes.

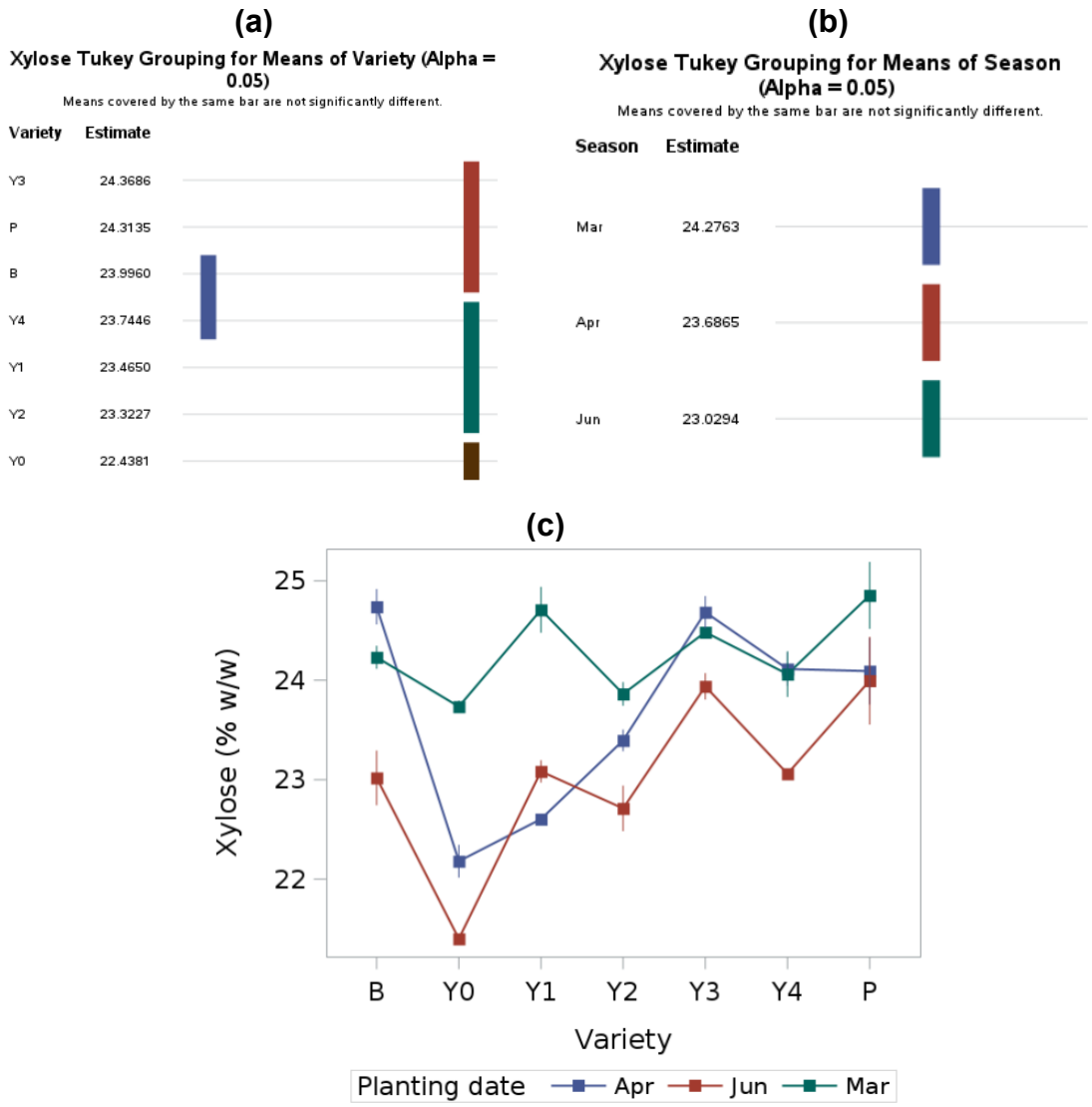


Fig. 3. Impact of variety (a), planting season (b), and their interaction (c) on xylose content (% w/w) of hemp hurd. In panels (a) and (b), bars sharing the same letter are not significantly different (Tukey HSD, $p < 0.05$). Panel (c) shows the variety × planting season interaction, with varieties on the x-axis and xylose content on the y-axis. Color-coded lines represent the three planting dates (green = March, blue = April, red = June), and error bars represent ± standard error of the mean. Parallel lines indicate consistent variety performance across planting dates, while diverging or crossing lines indicate variety-specific responses to planting season.

Galactose Content

Galactose, a minor monosaccharide in hemp hurds compared to glucose and xylose, showed significant variation across varieties and planting seasons. Varieties Y3, Y4, and P exhibited the highest mean galactose content, with estimates of 3.87%, 3.83%, and 3.82%, respectively, as shown in Fig. 4. These varieties were statistically similar, indicating no significant differences in galactose level. In contrast, Y2 had the lowest galactose content (2.76%) and differed significantly from the others.

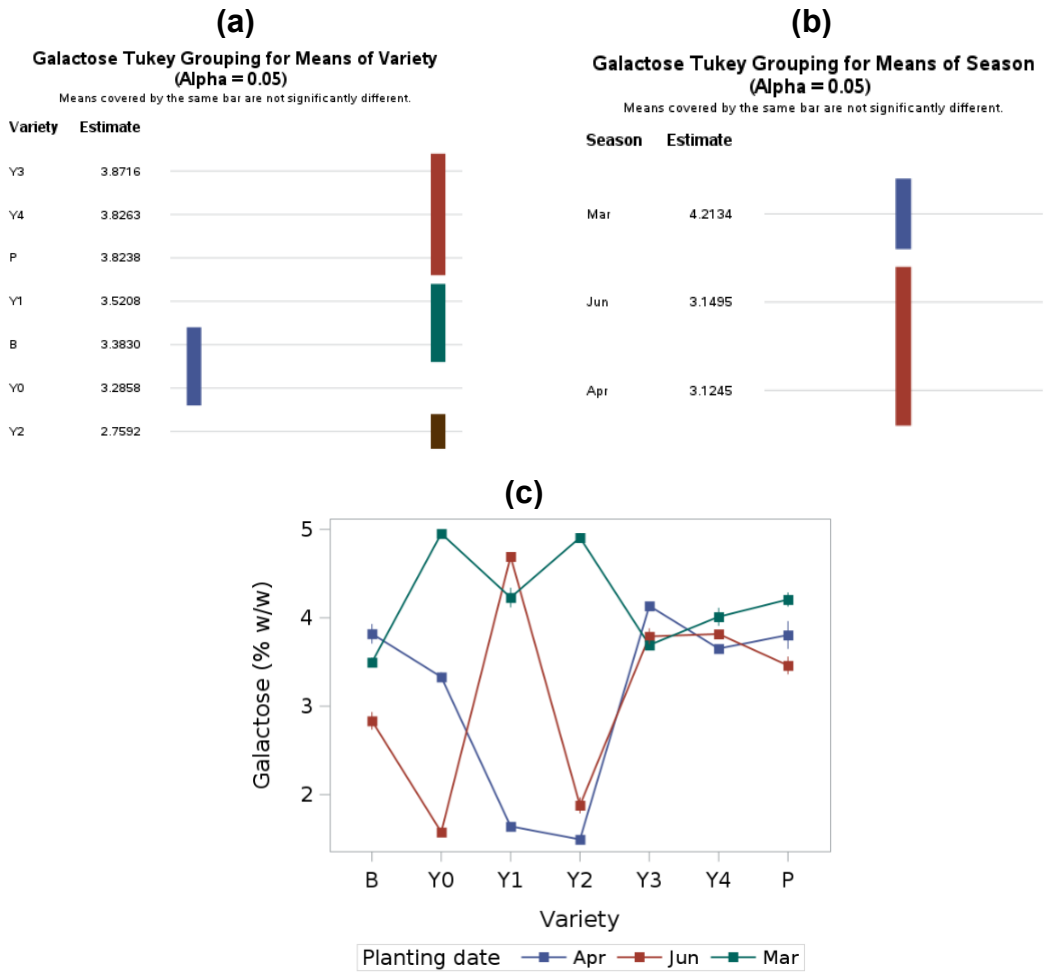


Fig. 4. Impact of variety (a), planting season (b), and their interaction (c) on galactose content (% w/w) of hemp hurd. In panels (a) and (b), bars sharing the same letter are not significantly different (Tukey HSD, $p < 0.05$). Panel (c) shows the variety × planting season interaction, with varieties on the x-axis and galactose content on the y-axis. Color-coded lines represent the three planting dates (green = March, blue = April, red = June), and error bars represent ± standard error of the mean. Parallel lines indicate consistent variety performance across planting dates, while diverging or crossing lines indicate variety-specific responses to planting season.

Analysis of the impact of planting seasons indicated that March was the most favorable for galactose accumulation, with a mean content of 4.21%. This is likely attributable to optimal environmental conditions or a longer growth period that promotes galactose synthesis. In contrast, planting in April and June showed no significant impact on galactose accumulation, suggesting that these seasons may not provide the same conducive conditions for enhancing galactose levels.

The interaction between variety and planting season further highlights that B, P, Y3, and Y4 maintained stable galactose levels across all seasons, indicating resilience to seasonal variations. In contrast, Y0 showed strong sensitivity to planting season, with a significant decline in galactose content from March to April and June.

Mannose Content

The analysis of mannose content reveals significant impacts of variety, planting season, and their interaction. Y2 exhibited the highest mannose content (5.42%), followed

by Y1 (4.25%) and Y0 (3.39%), all of which significantly outperformed the other varieties. When evaluating the effect of planting season alone, April was the optimal season for mannose accumulation, while March showed the lowest performance.

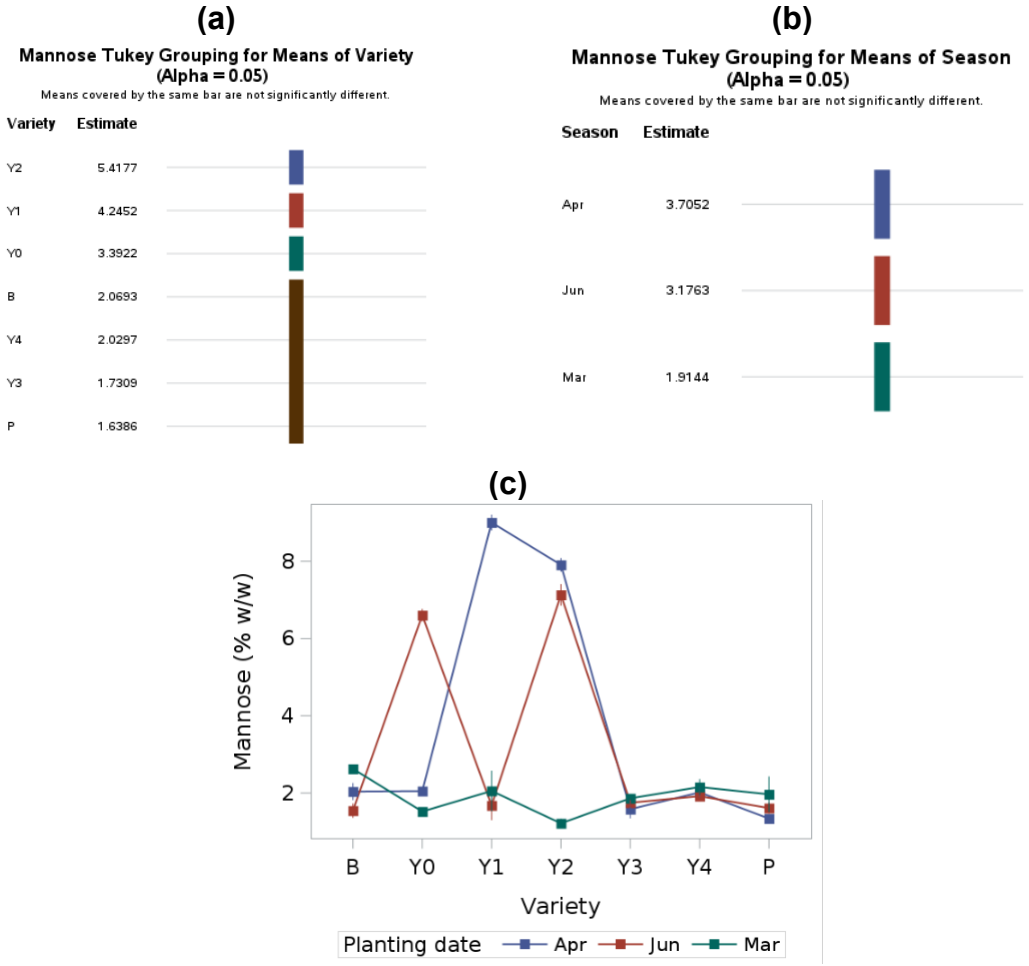


Fig. 5. Impact of variety (a), planting season (b), and their interaction (c) on mannose content (% w/w) of hemp hurd. In panels (a) and (b), bars sharing the same letter are not significantly different (Tukey HSD, $p < 0.05$). Panel (c) shows the variety × planting season interaction, with varieties on the x-axis and mannose content on the y-axis. Color-coded lines represent the three planting dates (green = March, blue = April, red = June), and error bars represent ± standard error of the mean. Parallel lines indicate consistent variety performance across planting dates, while diverging or crossing lines indicate variety-specific responses to planting season.

The interaction between variety and planting season had a more profound influence on mannose content. Y1 planted in April achieved the highest mannose content (9.00%), significantly exceeding its performance in March (2.05%) and June (1.68%). Similarly, Y2 planted in April reached a content of 7.91% but dropped to its lowest level (1.22%) when planted in March. Variety Y0 exhibited significant differences in mannose accumulation across the three planting seasons, highlighting its sensitivity to seasonal variations. These trends highlight the high sensitivity of some varieties to planting season, demonstrating that evaluating variety performance alone is insufficient. Planting season plays a critical role in mannose accumulation, and optimizing both factors is essential for maximizing yields.

Lignin Content

Varieties Y4 (20.9%) and B (20.6%) exhibited the highest lignin content compared to other varieties, but there were no significant differences between them. In contrast, Y0 (19.1%) had the lowest lignin content and differed significantly from the other varieties.

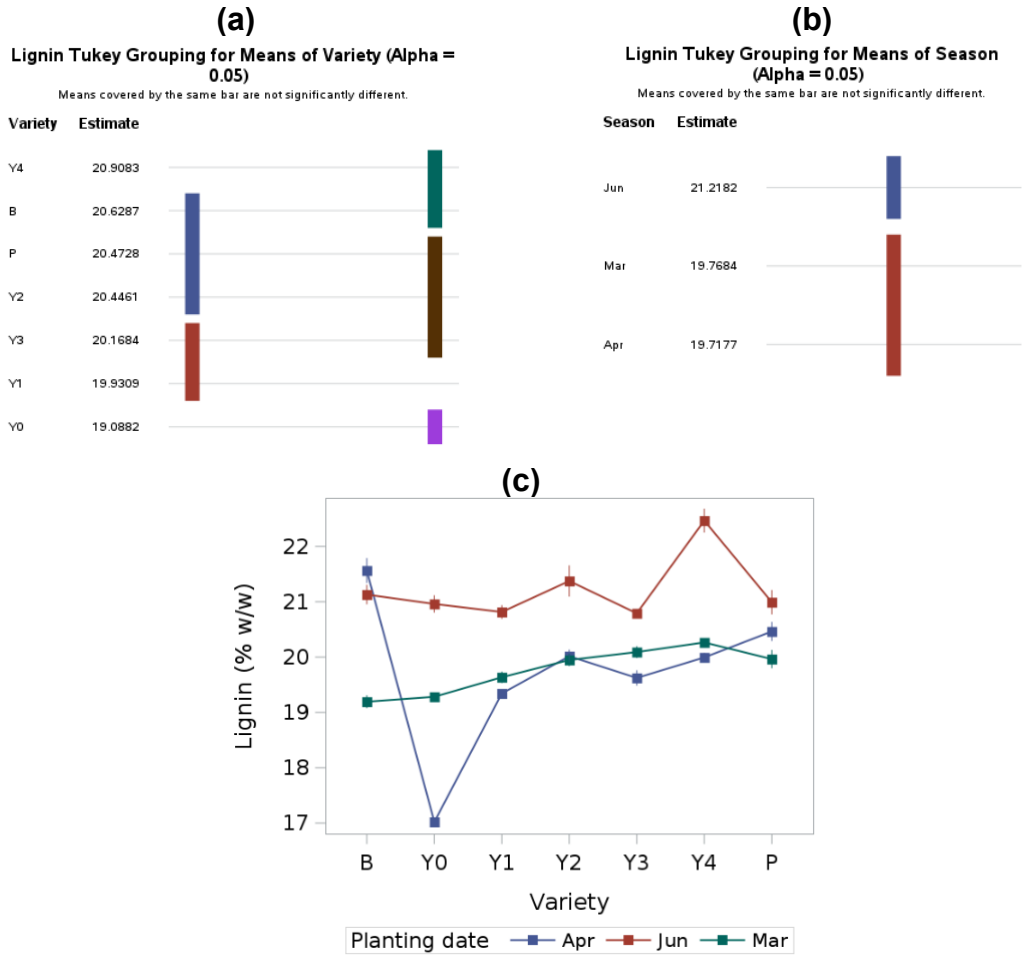


Fig. 6. Impact of variety (a), planting season (b), and their interaction (c) on lignin content (% w/w) of hemp hurd. In panels (a) and (b), bars sharing the same letter were not significantly different (Tukey HSD, $p < 0.05$). Panel (c) shows the variety × planting season interaction, with varieties on the x-axis and lignin content on the y-axis. Color-coded lines represent the three planting dates (green = March, blue = April, red = June), and error bars represent ± standard error of the mean. Parallel lines indicate consistent variety performance across planting dates, while diverging or crossing lines indicate variety-specific responses to planting season.

June was the most favorable planting season for lignin accumulation, with the highest mean lignin content of 21.2%. This season consistently supports higher lignin levels across most varieties. In contrast, April showed the lowest lignin content, particularly for Y0, which exhibited strong seasonal fluctuations. Y0 experienced significant drops in lignin content in April compared to June and March, highlighting its sensitivity to planting seasons.

Correlation among Major Fermentable Sugar (Glucose and Xylose) and Lignin Content

For efficient and economically viable biorefinery applications, high fermentable sugar content (glucose and xylose) is critical, as it directly increases the amounts of substrate available for bioethanol or biochemical production, while low lignin content reduces biomass recalcitrance, enabling more efficient pretreatment and enzymatic hydrolysis. Therefore, investigating the correlation between fermentable sugars (glucose and xylose) and lignin content is crucial for biorefinery applications. In this study, the Y3 variety demonstrated a strong negative correlation between these components (Fig. 7), with Pearson's *r* values of -0.7 for glucose-lignin and -0.8 for xylose-lignin. This inverse relationship suggests that Y3 possesses favorable traits for crop breeding or biomass processing, as higher sugar content coupled with lower lignin may enhance saccharification efficiency and lower pretreatment severity. Due to the small sample size (n = 9), these results should be interpreted with caution. This analysis was intended as exploratory, aimed at identifying Y3 as a variety of interest rather than making definitive mechanistic claims. While the observed correlations (glucose–lignin: $r = -0.708$, $p < 0.001$; xylose–lignin: $r = -0.838$, $p < 0.001$) were statistically significant and directionally consistent with known biological relationships in lignocellulosic biomass, a larger dataset would be needed to draw more robust conclusions.

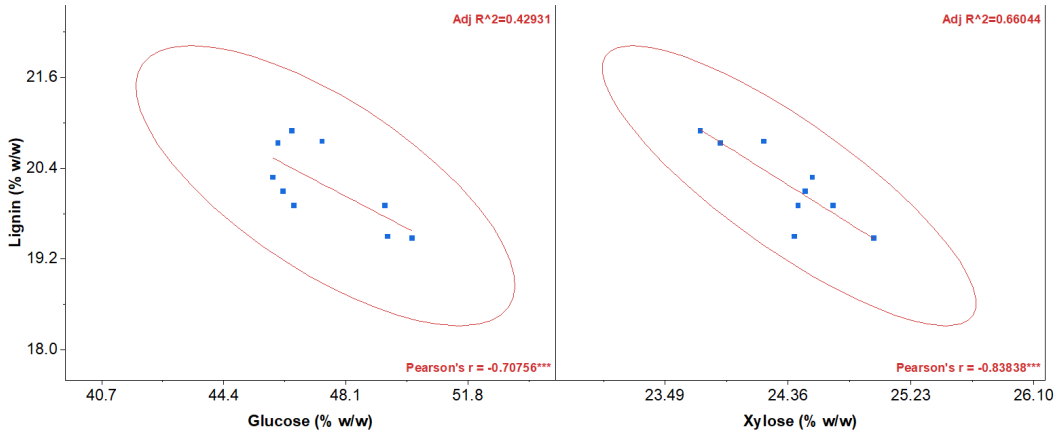


Fig. 7. Correlation between fermentable sugars (glucose/xylose) and lignin content. Correlation analysis was conducted on a small sample (n=9). While strong correlations (|r| > 0.7) may be detected, results should be interpreted with caution due to limited statistical power and wider confidence intervals. Adj. R^2 values suggest biological relevance. *** denotes a significant correlation at p <0.001.

Evaluating Sugar Yield Potential from Hemp Varieties

Sugar yield from hemp biomass was estimated by combining the dry-retted straw yield with the sugar content of the hemp hurd, as shown in Fig. 8. As the biomass yield reflects the total dry weight of harvested hemp straw—and the hurd constitutes a substantial portion of that biomass—the sugar yield can be calculated by determining the amount of sugar within the hurd. This is typically done by multiplying the biomass yield per unit area (kg/ha) by the proportion of hurd in the stem and its sugar content, providing a reliable estimate of sugar per hectare. Because the straw yield was significantly affected by planting season, with the June planting producing a markedly lower yield (4,824 kg/ha) compared

to March (18,648 kg/ha) and April (17,636 kg/ha) (Halker *et al.* 2024), only the March and April plantings were considered for calculating sugar and lignin yields. This approach ensures that yield estimates reflect realistic biomass productivity. For these calculations, the hurd portion was assumed to constitute 60% of the straw weight, based on findings from Ramesh and Bhoopathi (2022).

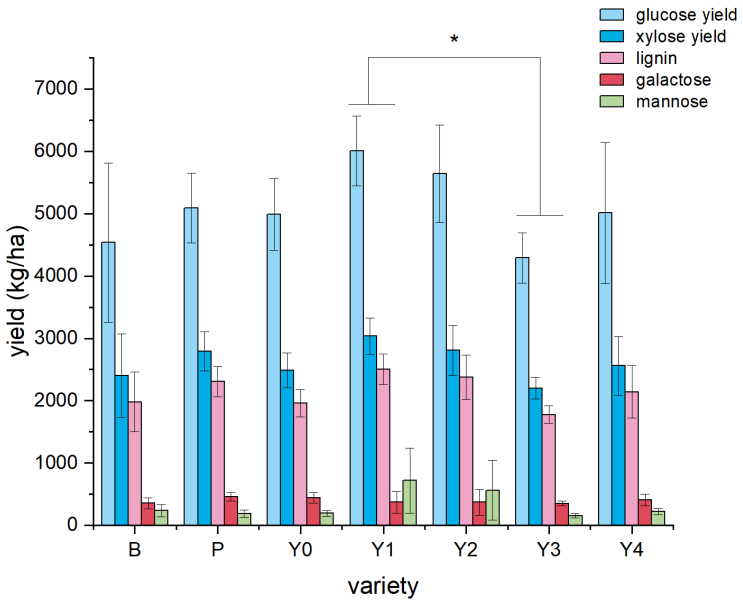


Fig. 8. Yields of sugar and lignin across seven hemp varieties. Yields of glucose, xylose, lignin, galactose, and mannose (kg/ha) across seven hemp varieties (B, P, Y0, Y1, Y2, Y3, Y4). Each bar represents the mean yield with standard deviation. Variety Y1 exhibited the highest sugar and lignin yields, which were significantly different from those of variety Y3 ($p < 0.05$), as indicated by the asterisk (*).

Among the seven hemp varieties evaluated, notable differences were observed in both biomass yield and chemical composition. Variety Y1 demonstrated the highest biomass yield, indicating strong growth performance. It also had the highest glucose content in the hurd, resulting in a total glucose yield exceeding 6,000 kg/ha. In contrast, variety Y3 had the highest xylose content in the hurd but the lowest biomass yield, resulting in a relatively low total xylose yield. This underscores the importance of considering both biomass yield and chemical composition when selecting cultivars for biorefinery applications. For large-scale biorefinery applications where total fermentable sugar output per hectare is the primary objective, Y1 is the preferred choice given its combination of high biomass yield and competitive sugar content. Y3, despite its lower biomass yield, showed consistently high glucose and xylose content with low lignin, a favorable compositional profile that reduces pretreatment severity and improves sugar release efficiency during bioconversion. Y3 may therefore be better suited for high-value biochemical applications where feedstock quality is prioritized over bulk yield, or as a genetic resource for breeding programs targeting improved composition. Ideally, future variety development should aim to combine the high biomass yield of Y1 with the favorable sugar-lignin profile of Y3.

Although June planting produced substantially lower biomass yield, hurd chemical composition expressed on a dry-weight basis was independent of yield and remained

informative regardless of productivity. Therefore, June data in the compositional analysis was still retained because it captured how variety × season interactions influenced hurd composition under late-season stress conditions, which was relevant for identifying varieties with stable compositional profiles across diverse growing environments. However, to avoid misleading conclusions about productivity, June planting was excluded from the per-hectare sugar and lignin yield calculations (Fig. 8), where biomass yield was the primary driver. This allows compositional and productivity data to be interpreted separately, providing a more reasonable basis for variety selection for biorefinery applications.

While the absolute differences in sugar content among varieties may appear small in this study, they become economically significant at commercial biorefinery scale. The standard reference scale used in NREL techno-economic analyses of lignocellulosic biorefineries is 2,000 dry tonnes of biomass per day — equivalent to approximately 730,000 dry tonnes per year (Davis *et al*. 2025). Hemp hurd has a similar lignocellulosic composition to corn stover, the primary feedstock in NREL design cases. At this processing scale, a 2% absolute difference in glucose content among hemp varieties translates to approximately 14,600 additional tonnes of fermentable glucose per year — representing a substantial additional bioethanol output and revenue. Furthermore, lower lignin content reduces pretreatment severity and associated costs. These considerations highlight the practical importance of variety selection based on compositional quality for future hemp hurd biorefinery development.

CONCLUSIONS

1. This study provided baseline data for genotype × environment interaction (GEI) effects in industrial hemp, demonstrating that variety and planting season significantly impacted fermentable sugar profiles and lignin content.
2. For some varieties (*e.g.*, Y0), planting season had a strong influence on their chemical composition, while for others (*e.g.*, P), inherent genetic traits appeared to play a more dominant role.
3. The combined influence of variety and planting season on sugar and lignin accumulation highlights the complex interplay between genetic and environmental factors in shaping hemp hurd composition.
4. Some varieties (*e.g.*, Y3) had the highest glucose and xylose contents and exhibited a strong negative correlation between fermentable sugars (glucose and xylose) and lignin content, which is favorable for biorefinery applications. Higher sugar content combined with lower lignin levels can enhance the efficiency and yield of bioproduct generation.
5. Sugar content and lignin composition alone do not determine overall suitability. For a hemp variety to be truly effective in biorefinery settings, it must also achieve high biomass yield. High sugar content is insufficient if the total biomass is low, as this limits the amount of fermentable material available. Variety Y1 illustrated this well, combining high biomass yield with elevated glucose content, resulting in a high total glucose yield and demonstrating strong potential for bioproduct conversion.

6. While this study focused on evaluating the effects of hemp variety, planting season, and biomass yield on sugar yield, future research should also consider fiber yield and quality to support the development of dual-purpose hemp varieties suitable for both biorefinery and fiber applications. Incorporating both hurd composition and fiber characteristics into variety selection will enable a more comprehensive assessment of hemp's value for multiple industries.
7. By evaluating these traits across different environmental conditions, region-specific recommendations can be developed to guide farmers in choosing high-performing varieties tailored to local climates, soils, and market demands. This approach will help maximize economic returns and promote the adoption of hemp as a resilient, sustainable, and versatile crop.

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