



Determination of Sugar Present before and after Fermentation from *Dovyalis Caffra* and *Musa* Species peels Mixture

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Abstract

The ever-increasing depletion and rising cost of fossil fuel reserves and the environmental consequences associated with their use have intensified search for sustainable and renewable energy alternatives such as bioethanol. Fruit waste and agricultural residues are particularly attractive feedstocks because they are abundant, inexpensive, and do not compete directly with food resources. Among these potential raw materials, *Dovyalis caffra* (Kei apple) and *Musa* species peels are of interest because they contain carbohydrates and reducing sugars that can be converted into ethanol through fermentation. This study evaluated the potential of a mixture of *Dovyalis caffra* and *Musa* species peels as a low-cost substrate for bioethanol production by assessing fermentable sugar content before and after fermentation. This study employed an experimental approach in which ripe fruits of *Dovyalis caffra* were sourced from different farms while *Musa* species peels were collected from different markets in Nairobi. The fruit extracts and pretreated peels were then mixed in ratios of 5:5, 6:4, and 7:3 and subjected to fermentation using *Saccharomyces cerevisiae*. Key parameters optimized were temperature (25°C, 30°C, and 35°C) and pH (4.5, 5.0, and 5.5). Sugar content was determined using the Brix method. Data collected were analyzed using ANOVA and considered statistically significant at $p \leq 0.05$. Results indicated a significant reduction in sugar content ranging between 31.23 g/L and 80.91 g/L after fermentation, consistent with microbial utilization of fermentable sugars. Temperature had a pronounced effect, with the highest sugar reduction observed at 35°C, followed by 30°C, while the lowest occurred at 25°C. Optimal pH for fermentation was 5.0, which resulted in greater sugar consumption compared to pH 4.5 and 5.5, although variations were relatively small. Among the mixture ratios, 6:4 (*Dovyalis caffra*: *Musa* species peels) showed slightly higher sugar reduction, though the effect of ratio was less significant compared to temperature and pH. The highest overall sugar reduction was achieved at 35°C, pH 5.0, and a 6:4 mixture ratio. Statistical analysis confirmed that temperature, pH, mixture ratio, and their interactions significantly influenced sugar conversion ($p < 0.05$). The findings indicate that *Dovyalis caffra* and *Musa* species peels contain substantial fermentable sugars, suggesting potential as a substrate for bioethanol production; direct quantification of ethanol yield is required to confirm this potential. This study highlights the potential of utilizing fruit waste for renewable energy generation, contributing to waste management and reduced reliance on fossil fuels. Further research is recommended to quantify actual ethanol yield, optimize fermentation conditions, evaluate production efficiency at larger scales, and explore practical applications in sustainable energy systems.

Keywords: ANOVA, Bioethanol, *Dovyalis caffra*, Fermentation, *Musa* species

Introduction

Global reliance on fossil fuels for transportation energy cannot continue indefinitely, since known reserves are being drawn down at a pace that outstrips the discovery of new ones (Mathews *et al.*, 2015). Concern over this trajectory, together with the environmental costs of continued fossil fuel combustion, has driven a growing body of research into cleaner, renewable alternatives (Jahid *et al.*, 2018). Bioethanol is among the most widely studied of these alternatives: it is renewable, biodegradable, and can be manufactured from biomass rich in fermentable sugars (Danmaliki *et al.*, 2016). Even so, current bioethanol output falls well short of the volumes that would be needed to meaningfully offset rising energy demand (Abdulkareem *et al.*, 2015), which keeps the search for viable, low-cost feedstocks an active area of research.

One promising direction is the use of fruit waste and agricultural residues as fermentation feedstock, since these materials are abundant, cheap to source, and do not draw on land or crops needed for food production. *Dovyalis caffra* (Kei apple) and *Musa* species peels are two such materials that merit closer attention. *Dovyalis caffra* is rarely eaten fresh because of its high acidity, leaving much of the fruit produced with little commercial use, while *Musa* species account for roughly 16% of global fruit production (Bhatia & Paliwal, 2010), generating correspondingly large

volumes of peel waste. Both retain appreciable carbohydrate and reducing-sugar content that can, in principle, be converted to ethanol through microbial fermentation, and prior work on fruit peels and over-ripened fruit has already shown that such substrates can support bioethanol production, with sugar levels falling measurably as fermentation proceeds and microorganisms consume the available sugars (Guo *et al.*, 2018; Janani *et al.*, 2019). Combining *Dovyalis caffra* and *Musa* species peels therefore offers a plausible, and previously under-examined, substrate for studying sugar conversion during fermentation.

Kenya continues to face rising energy demand, high fuel prices, and dependence on imported petroleum products, while fossil fuels remain a major source of greenhouse gas emissions and environmental degradation. At the same time, large quantities of fruit peels and other organic wastes are discarded, creating disposal problems despite their potential value as biofuel feedstock. These wastes have become a severe problem for municipalities in developing countries due to reduction of landfill capacity (Shah *et al.*, 2019). Although *Dovyalis caffra* and *Musa* species peels are believed to contain fermentable sugars, there is limited local evidence on the actual amount of sugars present before fermentation and the extent to which these sugars are reduced after fermentation when the peels are mixed. Without this information, it is difficult to determine the suitability of the mixture for bioethanol production or to compare fermentation potentials with other waste substrates. Therefore, a clear assessment of sugar content before and after fermentation is necessary to establish the value of these peels as raw materials for renewable energy sources.

The aim of this study was to quantify the sugars present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture. This was to provide baseline data on fermentable sugar content, which is essential for evaluating generation potential in bioethanol production. Using agricultural and fruit wastes for energy production supports waste reduction, resource recovery, and sustainable energy development, while also reducing competition with food crops since it grows naturally in waste lands (Ahmed *et al.*, 2019). The study is also relevant to Kenya's energy and environmental goals because bioethanol offers a cleaner alternative to fossil fuels and can contribute to reduced emissions and improved energy security. In addition, since fruit wastes are cheap, readily available and underutilized, the findings support future development of low-cost bioethanol production systems and provide useful information for further research on renewable energy from local biomass.

Materials and Methods

Study Area and Design

This study employed an experimental approach in which ripe fruits of *Dovyalis caffra* were sourced from different farms in Nandi and Uasin Gishu while *Musa* species peels were collected from different markets in Nairobi. The two were then mixed in specific ratios and subjected to fermentation using *Saccharomyces cerevisiae*. Temperature, pH, and mixture ratio were the key parameters considered in the experiment, giving a 3 x 3 x 3 factorial arrangement of three temperatures (25°C, 30°C, 35°C), three pH levels (4.5, 5.0, 5.5), and three mixture ratios (5:5, 6:4, 7:3), for 27 treatment combinations in total. This experiment was arranged in a Completely Randomized Design (CRD) with three replicates ($n = 3$) per treatment combination, giving $27 \times 3 = 81$ total experimental units, and data were collected and recorded for statistical analysis.

Sample Collection and Processing of *Musa* species peels

5 kilograms of *Musa* species peels were collected from different markets in Nairobi County. They were washed with distilled water and sun dried at 30°C for five days to lower the moisture content from approximately 85% to 14%. They were further oven dried at 60°C for 4 hours to further reduce the moisture content to 8%. The dry peels were then ground to powder using a grinder, sieved and weighed to 100g masses. Pre-treatment followed, where 100g of ground peels were mixed with 500ml of 0.1M sulphuric (VI) acid and cooked at 120°C for six hours. The mixture was then neutralized with 0.1M sodium hydroxide followed by filtration. The residue obtained was rinsed using distilled water and oven dried at 45°C. The amount of reducing sugars in the product was determined using a Brix refractometer. Following the pretreatment procedure, the resulting sample was hydrolyzed at a temperature of 110°C for 30 minutes then neutralized with 0.1M sodium hydroxide until pH reached 7.0, as described by Danmaliki *et al.*, (2016).

Sample Collection and Processing of *Dovyalis caffra* fruits

5 kilograms of ripe *Dovyalis caffra* fruits were collected from farms and put in plastic containers. They were washed with potassium permanganate to remove food-borne pathogens and rinsed with distilled water. The fruits were then covered for ten days to allow them to rot. This deliberate over-ripening step was included because pectin disassembly during over-ripening softens fruit tissue and has been shown to improve juice and sugar extractability from the pulp (Paniagua *et al.*, 2014). The rotten fruits were weighed, mashed, mixed with distilled water, and finally blended. The liquid extract was filtered using muslin cloth, with the filtrate then diluted with 5 liters of water. For pretreatment, 200ml of *Dovyalis caffra* juice were mixed with 500ml of 0.1M sulphuric acid and the mixture was then neutralized with 0.1M sodium hydroxide, and the amount of reducing sugars was determined using a Brix refractometer.

Fermentation of fruit extract

Before fermentation was done, the prepared samples were mixed in the species ratios of 5:5, 6:4 and 7:3 of *Dovyalis caffra* to *Musa* (v/w). 10g of yeast were added to each sample and fermented at a given pH value.

Optimization of pH and temperature

To stabilize the pH, buffer solutions were added. To optimize the pH value for the fermentation process, pH values ranging from 4.5 to 5.5 were used. Different samples of 200ml mixture were kept at pH 4.5, 5.0 and 5.5 for 3 days of incubation at the same temperature.

To optimize the temperature, 200ml of the mixture were fermented each at 25°C, 30°C and 35°C respectively for three days. This was achieved using a water bath. The final sugar content was determined using the Brix method.

Data analysis

Data was recorded and tabulated in a Microsoft Excel spreadsheet. The results were expressed as mean $\pm$ standard error of the mean (SEM). ANOVA was used to determine the statistical significance of the difference between the groups at the 95% confidence level ($p \leq 0.05$).

Results and Discussion

Effects of temperature on amount of sugar present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture

Three temperatures were used to assess the effects on the amount of sugar present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture: 25°C, 30°C and 35°C. The mean initial sugar level was 87.33 ± 0.9 g/L, while the final sugar level after fermentation at 25°C was 56.10 ± 1.3 g/L, with a difference of 31.23 ± 1.5 g/L (Table 1). After fermentation at 30°C, the difference between the initial (136.51 ± 0.8 g/L) and the final (71.37 ± 0.8 g/L) was 65.14 ± 1.0 g/L, while fermentation at 35°C resulted in a sugar difference of 80.91 ± 1.8 g/L from an initial sugar level of 139.96 ± 1.8 g/L and a final sugar level of 59.05 ± 1.5 g/L, as indicated in Table 1 below.

Table 1: Effects of temperature on the amount of sugar present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture

Temperature (°C)	Initial Sugars	Final Sugars	Sugars difference
25	87.33 ± 0.9	56.10 ± 1.3	31.23 ± 1.5
30	136.51 ± 0.8	71.37 ± 0.8	65.14 ± 1.0
35	139.96 ± 1.8	59.05 ± 1.5	80.91 ± 1.8

The initial sugars less the final sugars after fermentation indicated the amount of sugars consumed during fermentation. Fermentation at 35°C resulted in relatively higher sugar consumption compared to 25°C, while fermentation at 30°C resulted in moderate sugar consumption, as indicated in Table 2 below. This trend strongly aligns with established biochemical and microbiological principles governing fermentation by *Saccharomyces cerevisiae*.

Table 2: Effects of temperature, pH and mixture ratio on the amount of sugars consumed (sugar difference) during fermentation of *Dovyalis caffra* and *Musa* species peels mixture

Temperature	Mixture Ratio	pH 4.5	pH 5.0	pH5.5
25°C	5:5	28.33 ± 0.7	40.00 ± 1.5	20.00 ± 3.5
	6:4	27.33 ± 1.5	40.33 ± 0.3	41.67 ± 0.3
	7:3	30.00 ± 0.6	23.00 ± 0.0	29.67 ± 0.3
30°C	5:5	61.00 ± 1.7	74.00 ± 0.0	61.33 ± 0.3
	6:4	67.00 ± 1.2	63.33 ± 1.2	59.33 ± 2.0
	7:3	63.00 ± 0.0	69.00 ± 2.3	67.33 ± 1.5
35°C	5:5	91.00 ± 6.4	88.67 ± 2.6	73.33 ± 1.5
	6:4	82.00 ± 1.7	92.67 ± 0.7	73.00 ± 0.6
	7:3	71.00 ± 0.6	86.33 ± 0.9	71.00 ± 0.0

Temperature is a critical environmental factor influencing yeast metabolism, primarily through its effect on enzyme kinetics and membrane transport processes (Lin *et al.*, 2022). According to the Arrhenius principle, increasing temperature enhances enzymatic reaction rates up to an optimum point. In the case of *S. cerevisiae*, enzymes involved in glycolysis (e.g., hexokinase, phosphofructokinase and pyruvate decarboxylase) exhibit peak activity between 30°C and 35°C (Lin & Tanaka, 2006). This explains the significantly higher sugar consumption observed at 35°C in this study.

At 25°C, the reduced sugar utilization (31.23 ± 1.5 g/L) can be attributed to slower metabolic rates, reduced enzyme activity, and decreased membrane fluidity, which collectively limit glucose uptake and fermentation efficiency. Similar findings were reported by Bai *et al.*, (2008), who observed that fermentation rates decline significantly below 30°C due to reduced yeast growth kinetics. At 35°C, the enhanced sugar consumption observed in this study is consistent with findings by Almeida *et al.*, (2007), who reported that elevated temperatures improve sugar transport across yeast membranes and increase ATP turnover, thereby accelerating fermentation. However, it is important to note that temperatures beyond 37°C may lead to protein denaturation, membrane damage, and ethanol toxicity, which inhibit yeast activity. The fact that 35°C yielded the highest sugar reduction observed in this study suggests that it lies within the optimal physiological tolerance range of the yeast.

Furthermore, temperature also affects substrate hydrolysis, particularly in fruit-based fermentations. Breakdown of complex carbohydrates into fermentable sugars is enhanced at higher temperatures, increasing substrate availability (Woo *et al.*, 2024). This may partly explain the higher initial sugar values observed at 30°C and 35°C compared to 25°C.

Effects of pH on amount of sugar present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture

The effects of three distinct pH levels on sugar content were assessed before and after fermenting *Dovyalis caffra* and *Musa* species peels mixture. These were pH 4.5, 5.0 and 5.5. The mean initial sugars before fermentation at pH 4.5 was 122.26 ± 5.5 g/L while the final sugars after fermentation was 64.35 ± 1.9 g/L, translating to a mean sugar difference of 57.91 ± 4.4 g/L. At pH 5.0, the initial sugars were 123.67 ± 5.1 g/L while the final sugars after fermentation were 59.48 ± 1.7 g/L, indicating a reduction of 64.19 ± 4.6 g/L. pH 5.5 resulted in final sugars of 62.69 ± 1.6 g/L from initial sugars of 117.88 ± 3.8 g/L prior to fermentation, indicating a mean sugar difference of 55.19 ± 3.7 g/L, as illustrated in Table 3.

Table 3: Effects of pH on the amount of sugar present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture

pH	Initial Sugars	Final Sugars	Sugars difference
4.5	122.26±5.5	64.35±1.9	57.91±4.4
5.0	123.67±5.1	59.48±1.7	64.19±4.6
5.5	117.88±3.8	62.69±1.6	55.19±3.7

Sugars consumed during fermentation were also compared at pH 4.5, 5.0 and 5.5. pH 5.0 resulted in relatively higher sugar consumption compared to pH 4.5 and 5.5, as shown in Table 2 above. However, the variation was minimal, indicating that pH was not the main factor influencing the amount of sugars consumed during fermentation.

The result from this study suggests that pH within this range (4.5-5.5) is not a major limiting factor. The influence of pH on fermentation is primarily related to its effects on enzyme structure, nutrient solubility, and microbial cell integrity. *S. cerevisiae* is known to thrive in slightly acidic environments, with an optimal pH range of 4.0-5.5 (Black & Walker, 2023). Within this range, enzymes involved in glycolysis and ethanol production maintain their structural conformation and catalytic efficiency. The slightly higher sugar consumption observed at pH 5.0 can be attributed to optimal intracellular pH balance and membrane transport efficiency. At this pH, proton gradients across the cell membrane are ideal for nutrient uptake, particularly glucose transport via facilitated diffusion.

At pH 4.5, although still within the optimal range, increased acidity may impose mild stress on yeast cells, leading to energy expenditure for maintaining intracellular pH homeostasis. This can slightly reduce fermentation efficiency. Conversely, at pH 5.5, enzyme activity may begin to decline due to reduced proton availability, affecting metabolic reactions. These observations are consistent with findings by Sarkar *et al.*, (2023), who reported that maximum sugar utilization and ethanol production occur at pH 4.5-5.0 in fruit waste fermentations. Similarly, Banjo *et al.*, (2019) observed minimal differences in sugar consumption within this pH range, supporting the conclusion that pH was not the primary determinant of fermentation efficiency.

Effects of the ratio used in mixing *Dovyalis caffra* and *Musa* species peels on amount of sugar present before and after fermentation

Three different ratios (5:5, 6:4 and 7:3) of *Dovyalis caffra* and *Musa* species peels mixtures were used. The initial sugar before fermentation with the ratio 5:5 of *Dovyalis caffra* and *Musa* species peels mixture was 120.51 ± 4.9 g/L while after fermentation was 60.78 ± 2.2 g/L, with a mean sugar difference of 59.73 ± 4.8 g/L (Table 4). The mixture ratio of 6:4 for *Dovyalis caffra* and *Musa* species peels resulted in a mean sugar difference of 60.80 ± 3.9 g/L after fermentation, from initial sugars (122.11 ± 4.7 g/L) and final sugars (61.31 ± 1.7 g/L) (Table 4). For the mixture ratio of 7:3, the initial sugar before fermentation was 121.19 ± 5.1 g/L while after fermentation was 64.43 ± 1.2 g/L, translating to a mean sugar difference of 56.75 ± 4.2 g/L, as portrayed in Table 4.

Table 4: Effects of mixture ratio on the amount of sugar present before and after fermentation of *Dovyalis caffra* and *Musa* species peels mixture

Ratio	Initial Sugars	Final Sugars	Sugars difference
5:5	120.51±4.9	60.78±2.2	59.73±4.8
6:4	122.11±4.7	61.31±1.7	60.80±3.9
7:3	121.19±5.1	64.43±1.2	56.75±4.2

Fermentation at the ratio of 6:4 resulted in relatively higher sugar consumption compared to the ratios 5:5 and 7:3, as shown in Table 4 above. However, the variation among ratios was minimal, indicating that mixture ratio was not the main factor influencing the amount of sugars consumed during fermentation.

Substrate composition plays a crucial role in fermentation, particularly in determining the availability of fermentable sugars, micronutrients, and inhibitory compounds. Mixed substrates often provide a more balanced nutrient profile, enhancing yeast growth and metabolism (Ferdes *et al.*, 2023). For instance, banana peels are rich in carbohydrates and potassium, while *Dovyalis caffra* contributes organic acids and simple sugars.

The slightly improved performance at the 6:4 ratio may reflect an optimal balance between sugar availability and nutrient composition. However, the minimal differences observed suggest that all ratios provided sufficient fermentable substrates, consistent with findings by Mota (2023), who reported that substrate ratio has limited impact when sugar concentration is not limiting. Additionally, the presence of inhibitory compounds such as phenolics or organic acids in higher proportions (e.g., in 7:3 mixtures) may have slightly reduced fermentation efficiency, explaining the lower sugar consumption in that ratio.

Effects of temperature, pH and ratios of mixtures of *Dovyalis caffra* and *Musa* species peels on amount of sugar present before and after fermentation

The highest sugar reduction after fermenting the mixture of *Dovyalis caffra* and *Musa* species peels was recorded in the 6:4 ratio of *Dovyalis caffra* to *Musa* species peels at a temperature of 35°C and a pH of 5.0 (92.78 ± 1.1 g/L), the 5:5 ratio at 35°C and pH 4.5 (90.78 ± 11.01 g/L), the 5:5 ratio at 35°C and pH 5.0 (88.56 ± 4.5 g/L), and the 7:3 ratio at 35°C and pH 5.0 (86.22 ± 1.6 g/L), while the lowest was recorded at 25°C and pH 5.5 (19.89 ± 6.0 g/L) and in the 7:3 ratio at 25°C and pH 5.0 (22.89 ± 0.2 g/L). Figure 1 below illustrates how temperature, pH, and the mixture ratios of *Dovyalis caffra* and *Musa* species peels influence sugar content before and after fermentation; the full set of combination-level sugar-difference values, with their associated SEMs, is given in Table 2.

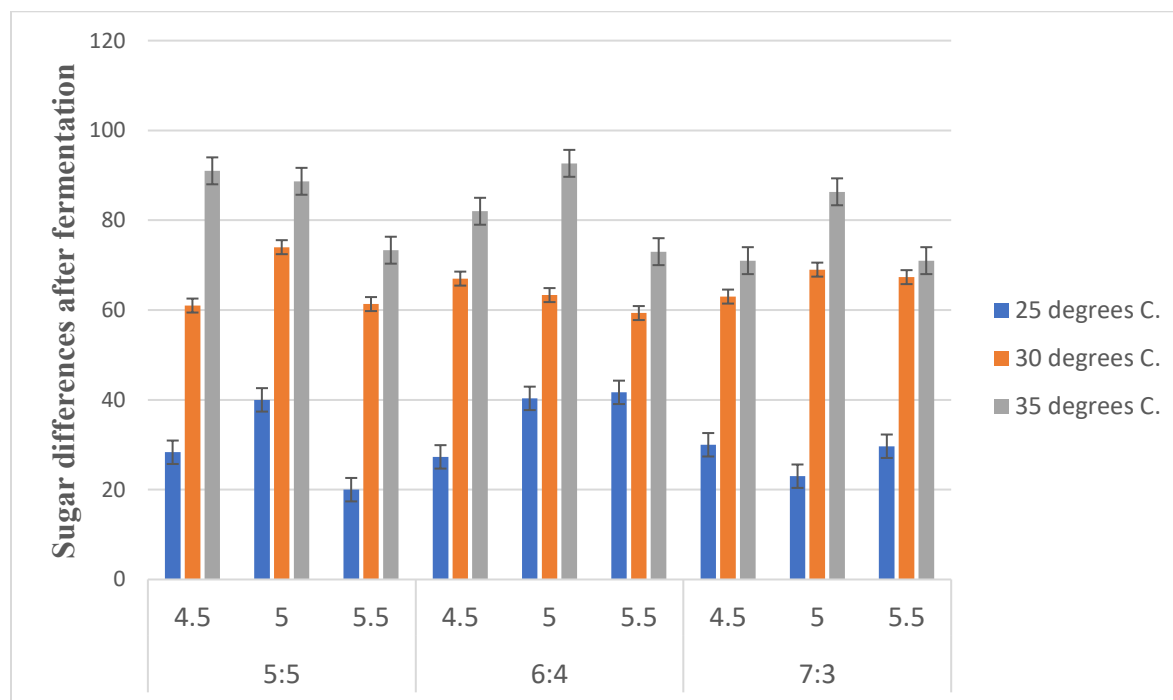


Figure 1: Effects of temperature, pH and ratios of mixtures of *Dovyalis caffra* and *Musa* species peels on differences of the sugars before and after fermentation

The Shapiro-Wilk test on the raw sugar difference values indicated that the data has no evidence of unequal variances, $W = 0.923$, $p = 0.271$. 3-way ANOVA was then used to determine if the different factors (temperature, pH and ratio) as well as their interactions had a statistically significant effect on the sugar differences obtained after fermentation at 95% confidence level. ANOVA determined that all the main factors as well as their interactions significantly influenced the sugar levels during fermentation at 95% confidence level ($p \leq 0.05$) as indicated in Table 5 below. This highlights the complex and interdependent nature of fermentation processes. That every two-way interaction and the three-way interaction among temperature, pH, and ratio also were significant (Table 5) confirms that these factors do not act additively on sugar consumption, and this finding retrospectively validates the decision to examine all three factors within a single factorial design rather than through sequential, one-factor-at-a-time optimization, since a sequential approach could not have detected the AC and ABC interaction terms.

Table 5: Analysis of Variance for sugar differences after fermentation of the mixture of *Dovyalis caffra* and *Musa* species peels

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value	Effect
A: PH	1143.8	2	571.901	57.33	< 0.001	Significant
B: Ratio	238.691	2	119.346	11.96	< 0.001	Significant
C: TEMP	34996.3	2	17498.2	1754.15	< 0.001	Significant
AB	420.864	4	105.216	10.55	< 0.001	Significant
AC	480.568	4	120.142	12.04	< 0.001	Significant
BC	541.901	4	135.475	13.58	< 0.001	Significant
ABC	1109.88	8	138.735	13.91	< 0.001	Significant
RESIDUAL	538.667	54	9.9753			
TOTAL	39470.7	80				

Post hoc analysis attributes essentially all of the resolvable treatment effect on sugar difference to temperature, with the sharpest gain occurring between 25°C and 30°C and a further, smaller gain between 30°C and 35°C. The highest sugar reduction observed at 35°C, pH 5.0, and ratio 6:4 demonstrates a synergistic interaction among factors. Temperature optimized enzyme activity, pH then maintained enzyme stability and membrane transport while substrate ratio(s) ensured nutrient balance. Such interactions are well documented in fermentation optimization studies using response surface methodology (RSM). Arora *et al.*, (2015) reported that temperature-pH interactions significantly influence fermentation efficiency by affecting enzyme conformation and metabolic flux. This study findings confirm that optimal fermentation conditions cannot be determined by evaluating individual factors in isolation, but rather through their combined effects.

Variability among replicates

Several cells in Table 2 show comparatively large standard errors of the mean relative to the rest of the dataset, most notably the 5:5 ratio at 35°C and pH 4.5, where the sugar difference was 91.00 ± 6.4 g/L, and the corresponding combination-level estimate reported above for the same treatment (90.78 ± 11.01 g/L), which is the widest SEM recorded in this study. Most other treatment combinations show SEMs in the region of ± 0.3 to ± 2.0 g/L, so these wider values stand out. Because only three replicates were used per treatment combination, a single divergent replicate reading can noticeably inflate the SEM for that specific cell. These larger SEMs most likely reflect genuine biological

and procedural variability among replicates, for example small differences in fruit ripeness, extraction efficiency, or yeast activity between fermentation runs carried out at the same nominal temperature, pH, and ratio, rather than a systematic effect of the treatment itself.

Limitations of the fruit rotting step

This study did not include explicit monitoring of spoilage or microbial contamination during the ten-day rotting step used to prepare *Dovyalis caffra* fruit for extraction. The rotting step was included deliberately, since pectin disassembly during over-ripening softens fruit tissue and has been shown to improve juice and sugar extractability (Paniagua *et al.*, 2014). However, the fruit was not monitored for microbial load, pathogen growth, mycotoxin formation, or biogenic amine accumulation during this extended, uncontrolled ripening period. Spontaneous or uncontrolled fermentation and spoilage processes of this kind are known to carry these risks (Capozzi *et al.*, 2017), and their presence could confound sugar measurements or, in a food or beverage application, raise safety concerns. Future work on this substrate should incorporate microbial load counts and other quality indicators throughout extended ripening or rotting steps to rule out this source of uncertainty.

Conclusions and Recommendations

The study found that the mixture of *Dovyalis caffra* and *Musa* species peels contains fermentable sugars that are measurably reduced during fermentation, indicating that the substrate can support microbial sugar utilization and suggesting potential for bioethanol production; because ethanol itself was not quantified in this study, this potential remains to be confirmed through direct measurement of ethanol yield. The amount of sugar consumed during fermentation varied significantly with temperature, pH, and mixing ratio, showing that fermentation conditions strongly influence sugar conversion.

Among the tested conditions, the highest sugar reduction was recorded at 35°C and pH 5.0, particularly in the 6:4 mixture ratio, indicating that this combination provided the most favorable fermentation environment for sugar utilization. Lower sugar reduction was observed at 25°C and at less favorable pH conditions, suggesting that insufficient temperature or suboptimal acidity limits fermentation efficiency. Overall, the findings suggest that *Dovyalis caffra* and *Musa* species peels are promising low-cost feedstocks for renewable energy production and waste valorization, pending confirmation of actual ethanol yield.

It is recommended that further studies be carried out to optimize fermentation conditions for maximum ethanol yield from the peel mixture. Future research should directly measure and quantify actual ethanol concentration produced, investigate different yeast concentrations and fermentation periods, and conduct pilot-scale experiments to assess feasibility for practical application. Future work should also monitor microbial load and other quality indicators during extended ripening or rotting steps used in substrate preparation. In addition, the use of fruit peel waste for biofuel production should be promoted as a sustainable strategy for waste reduction and renewable energy development, once ethanol yield has been directly confirmed.

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