

Efficiency and Fermentative Yield of Sweet Sorghum Using Cat-1 And Pedra-2

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Abstract

Background: The increase in demand for fuels has intensified the search for renewable energy sources, mainly biomass, with sugarcane being the most important factor in the production of bioethanol. In addition, sorghum cultivation presents high potential mainly for using the same industrial production complex and being processed in the off-season of sugarcane. However, information on the chemical and technological characteristics of sweet sorghum in the national literature is scarce. These are of extreme importance considering the preparation and adaptation stages of musts and yeasts, preceding the process of ethanolic fermentation. The objective of this research was to evaluate the fermentation process of the CVSW80007 genotypes; CVWS80147 and BRS610 in laboratory scale, evaluating the performance of CAT-1 and PEDRA-2 yeasts by determining yields and fermentative efficiencies and alcohol production ($L \cdot ha^{-1}$ and $L \cdot t^{-1}$).

Results: The experimental design was the randomized blocks in a 3x2x6 Factorial model in the fermentation process (for each of the yeasts studied). The results indicated that CAT-1 and PEDRA-2 yeasts demonstrate similar behavior, being efficient in the fermentation process. The fermentation of must was prepared from stalks with leaves and without leaves (135 days after sowing) of the three genotypes, results in 47.0 and 39.8 $L \cdot t^{-1}$ ethanol, respectively, using sweet sorghum.

Conclusions: The conditions studied on a laboratory scale demonstrate that the different sweet sorghum genotypes have potential for ethanol production, with the stalks with leaves and panicles increasing the alcohol content and fermentative efficiency.

Background

The growing global concerns about environmental pollution from the use of non-renewable energy sources coupled with future dependence of energy on petroleum and the release of greenhouse gases have stimulated the global community to find alternative fuels, for the increase and efficiency of energy [1].

Moreover, it is growing the demand for reducing costs and sustainability about the environment, this results in the importance of the use of biofuels to supply the global energy demand. In addition to being produced from biomass, they emit lower amounts of carbon dioxide and other pollutants particulates and have a great advantage as they are renewable fuels [2].

In this context, sugarcane has highlighted as raw material, utilized for ethanol production in Brazil. Similarly, other countries have adopted this method, based on sugarcane, which has excellent results and bringing in the paradigm changes in this new time.

Evaluating the quantities of fuels produced and consumed, both with the future statistical forecasts, it is assumed that the use of a single source of raw material may not be enough for all expected demand. Among the most economically promising alternatives, sweet sorghum highlight as a raw material for the ethanol production, both from the agronomic and industrial process. Sweet sorghum has stalks rich in fermentable sugars, similar to sugarcane. [2]

For obtaining good industrial yields it is essential that the raw material to be processed presents high levels of fermentable sugars. However, there is not a lot of information about the chemical and technological

characteristics of sweet sorghum in the literature. These parameters are extreme importance considering the preparation and adequation stages of broth and for the fermentation process by the yeasts.

In this context, the goals of this research were to evaluate the fermentation process of the genotypes CVSW80007, CVWS80147 and BRS610 in laboratory scale, evaluating the performance of the industrials yeasts CAT-1 and PEDRA-2 (PE-2) determining the yields, fermentative efficiencies, and ethanol production ($L \cdot ha^{-1}$ and $L \cdot t^{-1}$).

Material And Methods

Sweet Sorghum

Sweet sorghum was grown in the experimental area of the Vegetable Production Department of FCAV/UNESP Jaboticabal - SP, Brazil. The sorghum seeds of the CVSW80007 and CVWS80147 genotypes were donated by the company Canavialis (Monsanto do Brasil) and the BRS610 genotype donated by the company Embrapa. The Planting was done with a combined spacing of 90x70 cm between the lines. Excess seeds were used at sowing and at 15 days after sowing, thinning was done leaving 10 plants per meter, to obtain a final stand of 100.000 plants per hectare.

Experiment design

The experimental design was in random blocks at a factorial of 3x2x6 model. Each plot consisted of 10 lines of 10 meters in length, with a combined spacing of 90 and 70 centimeters between the lines. The treatments used were: Factor A: three genotypes (CVSW80007, CVWS80147, and BRS610); Factor B: two harvests (whole stalks and stalks without leaves and panicle), and Factor C: six different days for the process (100, 105, 110, 118, 135 and 160 days after the planting).

The theoretical yield was calculated considering that 1g of Total Reducing Sugar (TRS) produces 0.6475 mL of ethanol (stoichiometric reaction). The practical yield (considering the ethanol content of the broth after fermentation), Fermentation Efficiency and Ethanol Production ($L \cdot ha^{-1}$ and $L \cdot t^{-1}$) were calculated according to Fernandes (2011).

The results were submitted to variance analysis (F test), comparison of averages test (Tukey 5%) and polynomial regression analysis using the System for Statistical Analyzes of Agronomic Tests - AgroEstat [4].

Broth preparation, fermentation process and broth after fermentation

In each day of the process, there were collected 25 whole stalks (stem + leaves) and 25 clean stalks (without leaves). These were sent to the Technology of Sugar and Alcohol and Microbiology of Fermentations Laboratory at the FCAV/UNESP, being weighed and submitted to the extraction of the juice using a laboratory scale mill. The obtained juice was used for the accomplishment of the chemical and technological analyzes. The extracted juice was subjected to a clarification process to remove impurities.

The broth for fermentation was prepared with the clarified juice. It was adjusted the soluble solids content (Brix) ($16 \pm 0,1$), the pH (corrected with sulfuric acid to 4.5 ± 0.3), and the temperature ($32^{\circ}C$). That were determined

the Total Reducing Sugars (TRS) and Total Acidity [5].

At the fermentation process were evaluated the industrial yeasts CAT-1 and PE-2, which have been shown to be persistent in the fermentation processes carried out in the State of São Paulo and are presented as excellent fermenters. The broths were inoculated at a concentration recommended by Amorim (2005) and kept at $32^{\circ} \pm 1^{\circ}\text{C}$ throughout the process.

During the fermentation process, after 50 minutes of the inoculation of the yeast and at the end of the processes, there were evaluated viability of yeast cells, bud rate and viability [7].

The fermentation process was carried out in batch with the recovery of yeast by centrifugation. The process was monitored by densimetry, and the end of the fermentation was established when the Brix was less than or equal to 1, or when the stabilized within 30 minutes. At the end of each fermentation, the broth after fermentation was centrifuged at 1650g, 25°C for 5 minutes (HIMAC CR 21G centrifuge) to separate yeast and from the broth. In the broth after fermentation was determined the pH, Sulfuric Acidity, Glycerol [8], Total Residual Reducing Sugars (TRRS) [9].

Results

The results observed for the Total Residual Reducing Sugar (TRRS) content of the broth after fermentation process are at the Table 01 indicate it was no significant difference between the genotypes when the yeast CAT-1 was used in the fermentation process.

Table 1

Results of the analysis of variance (F test) and Tukey test comparison of means (5% probability) for the chemical-technological characteristics of the broth after fermentation process. Jaboticabal-SP.

| | TRRS (%) | | Total Acidity (g.L ⁻¹ H ₂ SO ₄ .L ⁻¹) | | pH | | Glycerol (mg.100mL ⁻¹) | |
|----------------------------|-------------|--------|---|---------|---------|---------|---------------------------------------|---------|
| Genotypes (A) | CAT-1 | PE-2 | CAT-1 | PE-2 | CAT-1 | PE-2 | CAT-1 | PE-2 |
| CVSW80007 | 1.99A | 2.60A | 3.52A | 3.57A | 3.67C | 3.80C | 9.01A | 8.78A |
| CVWS80147 | 1.62A | 1.82AB | 3.89A | 3.74A | 3.79B | 3.94B | 8.60A | 7.72B |
| BRS610 | 0.79A | 0.73B | 3.61A | 3.49A | 3.94A | 4.07A | 6.90B | 5.92C |
| F Test | 6.14ns | 15.37* | 5.77ns | 1.33ns | 40.79** | 53.48** | 13.27* | 95.86** |
| MSD | 1.25 | 1.20 | 0.40 | 0.55 | 0.10 | 0.09 | 1.54 | 0.74 |
| CV | 101.17 | 83.19 | 13.22 | 18.39 | 3.28 | 2.77 | 22.48 | 11.82 |
| Leaves (B) | | | | | | | | |
| With leaves | 1.20A | 1.50B | 3.68A | 3.60A | 3.85A | 3.97A | 7.77B | 7.15A |
| Without leaves | 1.73A | 1.94A | 3.67A | 3.61A | 3.75B | 3.90B | 8.58A | 7.79A |
| F Test | 5.12ns | 6.87* | 0.03ns | 0.01ns | 18.74** | 10.11* | 9.05* | 2.69ns |
| MSD | 0.57 | 0.40 | 0.17 | 0.36 | 0.05 | 0.05 | 0.65 | 0.95 |
| CV | 83.12 | 50.29 | 10.28 | 21.31 | 3.01 | 2.97 | 17.09 | 27.21 |
| Harvesting time (C) | | | | | | | | |
| 100 | 1.23AB | 1.54A | 4.03A | 4.48A | 3.86AB | 3.89B | 7.15B | 7.16A |
| 105 | 1.78A | 1.44A | 3.94AB | 3.47BCD | 3.65C | 4.05A | 9.72A | 7.12A |
| 110 | 1.92A | 2.15A | 3.93ABC | 3.85B | 3.62C | 3.69C | 8.09AB | 8.04A |
| 118 | 1.44AB | 1.87A | 3.47CD | 3.02D | 3.79B | 4.01AB | 8.27AB | 7.19A |
| 135 | 1.72A | 1.90A | 3.17D | 3.12CD | 3.92A | 4.06A | 7.55B | 7.21A |
| 160 | 0.70B | 1.40A | 3.49BCD | 3.67BC | 3.96A | 3.92AB | 8.25AB | 8.13A |
| F Test | 3.49** | 1.87ns | 9.37** | 13.77** | 22.86** | 12.62** | 4.95** | 2.39* |
| MSD | 1.00 | 0.91 | 0.46 | 0.59 | 0.12 | 0.16 | 1.64 | 1.27 |
| CV | 69.41 | 53.98 | 12.94 | 16.85 | 3.28 | 4.17 | 20.50 | 17.39 |
| Inter. AxB | 0.26ns | 1.34ns | 0.02ns | 2.12ns | 1.86ns | 1.02ns | 1.16ns | 1.38ns |
| Inter. AxC | 1.58ns | 1.31ns | 1.44ns | 1.01ns | 1.31ns | 1.35ns | 1.31ns | 2.36* |

| | | | | | | | | |
|--|--------|--------|--------|--------|--------|--------|--------|--------|
| Inter. BxC | 0.62ns | 0.69ns | 0.79ns | 0.54ns | 0.33ns | 0.25ns | 0.60ns | 0.62ns |
| Inter. AxBxC | 0.85ns | 0.91ns | 2.72** | 2.93** | 0.16ns | 1.03ns | 1.14ns | 0.91ns |
| The averages followed by the same letter do not differ statistically from each other (Tukey test 5%). The letters compare averages in the column. ** significant at the 1% probability level ($p < 0.1$) * significant at the 5% probability level ($0.1 = p < 0.5$). MSD – Minimum Significant Difference. CV - Coefficient of Variation. | | | | | | | | |

When used PE-2 yeast as inoculum these differences were significant. Considering the genotypes, it was verified that BRS610 presented a significantly lower value than CVSW80007, which presented higher mean values. The presence of leaves resulted in a significantly lower value of total residual reducing sugar for PE-2, whereas for CAT-1. Although it has no significant effect, the highest average was for stalks without leaves. About the harvesting time, it has no significant effect for PE-2, and at 160 days after sowing a significant effect was observed for CAT-1, presenting lower averages. These values can be considered high when compared to those obtained for broth fermented from sugarcane.

Considering the total acidity of the broth after fermentation process, it was verified that there was no significant effect for the genotypes and the processes with or without leaves, independently of the yeast used (Table 01). There was a significant result, at the 1% level, only for the interaction AxBxC, that is, different behavior for the different genotypes, submitted to the harvest systems in the studied harvest time.

The pH of the fermented broth showed the same behavior for both yeasts (Table 01), for both genotypes and systems process. BRS610 presented a significantly higher mean than CVWS80147 and CVSW80007, which had the lowest pH. Considering the presence of leaves and panicles, it was verified a higher pH value in the broth after fermentation process, and for the last sampling seasons these were higher for the two yeasts.

In this study, it has evaluations carried out for the fermented broth at 135 days after sowing, which was considered one of the indicated seasons for the processing of the stalks for ethanol production. From the results obtained, the fermentation efficiency are presented in Figs. 01, 02, and 03.

The results indicated that the process without leaves was more adequate for all the genotypes studied, considering the fermentation efficiency. It can be verified that for the genotype CVSW80007, when the stalks was processed with and without leaves, using yeast CAT-1 showed higher efficiency than PE-2 (Fig. 01), of the order of 6 to 8%.

However, CVWS80147 (Fig. 02) for without leaves and the yeast PE-2 provided the best efficiency, but similar to CAT-1. However, stalks with leaves in CVWS80147 showed no difference for both yeasts.

BRS610 genotype is considered forage, with different characteristics, mainly the high potential of biomass production. However, when planning the trial was defined as including this treatment and comparing it to the other sweet sorghum genotypes, it was observed that and stalks processed with and without leaves resulted in higher efficiency when PE-2 yeast was used as inoculum in the fermentation process (Fig. 03). When the yeast was CAT-1, results were lower than those found for PE-2 and did not differ from each other.

In order to compare the performance of the studied genotypes using CAT-1 and PE-2 yeast as inoculum of the fermentation process, the yields for theoretical and produced alcohol and were calculated in $L \cdot ha^{-1}$ and $L \cdot t^{-1}$, are in Figs. 4 and 5. Using sorghum stalks without the presence of leaves and panicles, the average production

of 1059 L.ha⁻¹ of theoretical ethanol, varying from 980 to 1062 L.ha⁻¹, it was observed for the genotypes CVWS80147 and BRS610, respectively (Fig. 04).

When processing included leaves and panicles a variation of 1522 to 1905 L.ha⁻¹ of theoretical ethanol was observed, respectively for CVSW80007 and CVWS80147. That can be verified that the processing of leaves and stalks with resulted in an increase of 58% in the ethanol production (L.ha⁻¹) compared to the process without leaves. These results demonstrate the harvesting time of sorghum stalks, without leaves, does not impact the raw material for the preparation of the broth and fermentation process. From the analysis of the results obtained for the Alcohol Produced (L.ha⁻¹), it was verified that the processing of leaves less stalks resulted in an average production of 996 L.ha⁻¹, where the presence of leaves and panicles was of the order of 1530 L.ha⁻¹. Considering the two yeasts used in the fermentation process, they presented similar behaviors. During the fermentation process carried out using with leaves stalks, genotype CVWS80147 presented the highest yields (Fig. 04).

Calculation of the expected yield of ethanol per processed ton (Fig. 05) ranges from 40 to 49 L.t⁻¹ when processed stalks with leaves and 47 to 52 L.t⁻¹ when processed stalks without leaves. The general, the yield for the processed with leaves was 10.75% less. For the genotype CVWS80147, with the average yield of ethanol (Fig. 05), it can be used without leaves it an average yield of 47.0 L.t⁻¹, whereas for with leaves was 39.8 L.t⁻¹, that results in a reduction of about 15%. The yeasts used showed similar behavior for genotype CVWS80147. However, for genotypes CVSW80007 and BRS610, it was found that CAT-1 and PE-2 showed better behavior, respectively. Although processing stalks with leaves results in lower ethanol yields (Fig. 05), it should be considered that the higher yield (L.ha⁻¹) it was a consequence of the total biomass is 77.7% higher than that quantified for the treatment without leaves and panicles.

Discussion

After the end of the fermentation process, the broth became the raw material for studies and evaluations. Thus, the sugars that initially composed the broth now are in the form of ethanol, acids, alcohols, glycerol, among other molecules, which were transformed by yeast action [6]. In this context, the evaluation of the components that make up the broth and/or distillate is of fundamental importance when it comes to proposing a new technology.

This behavior due to the absence of the enzymatic treatment in order to favor the unfolding of starch compounds present after the extraction of the broths. It is evident the necessity of its use when the sorghum participates in the composition of the raw material for the preparation of the musts.

Glycerol is a secondary compound that forms in the auxiliary route of the ethanol forming pathway, using the same structures, and is therefore inversely proportional to its production [10]. Considering the central objective is the greater production of ethanol, it is verified that the ideal is that the obtained values are the smallest ones possible [6]. The analysis of Table 01, it is shown that the glycerol contents in the wine indicate that CVSW80007 was the one that presented the highest levels, and BRS610 the lowest for the two yeasts studied. By evaluating the harvesting system with leaves or removed, it was found that they resulted in significantly

higher glycerol values for CAT-1 yeast. For epochs no significant effect was observed for PE-2, while for CAT-1 at 100 and 135 days after sowing the glycerol contents were lower.

The results of fermentation efficiency observed in this study (Fig. 1) are similar those reported by Ribeiro Filho [11], who obtained yields of the sorghum broth, working with a clean stem of 32.91%, while in stem with leaves obtained values of 27.20%. The clean stalk showed an increase in broth yield of 5.71%, which explains the fact that many distilleries use the process called "clean cane", which in addition to a higher yield preserves the equipment that makes up the industrial unit [11].

In general, the observed efficiencies were similar to those obtained by Mancilha [12] and Ratnavathi [13], who obtained conversion of sugars present in the sorghum broth, with efficiencies higher than 90% regardless of the strains used and the genotypes considered.

Conclusions

The conditions studied on a laboratory scale demonstrate that the different sweet sorghum genotypes have potential for ethanol production, with the stalks with leaves and panicles increasing the alcohol content and fermentative efficiency.

Declarations

Ethics approval and consent to participate:

'Not applicable' for that section.

Consent for publication:

'Not applicable' for that section.

Availability of data and materials:

'Not applicable' for that section.

Competing Interests:

'Not applicable' for that section.

Funding:

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Authors Contributions:

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Lidyane Aline de Freitas, Cristhyane Millena de Freitas, Juliana Pelegrini Roviero, Gustavo Henrique Gravatim Costa, Osania Emerenciano Ferreira, Aline Ferreira Silva, Vitor Teixeira, Letícia Fernanda Tralli, Natália Novais Ribeiro and Márcia Justino Rossini Mutton. The first draft of the manuscript was written by Lidyane Aline de Freitas and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

1. Freitas L, Costa G, Masson I, Ferreira O, Mutton M, Mutton M. Chemico-technological parameters and maturation curves of sweet sorghum genotypes for bioethanol production. *African Journal of Agricultural Research*. 2014;9 (50) January:3638–44.
2. dos Santos Masson I, Costa GHG, Roviero JP, de Freitas LA, Mutton MA, Mutton MJR. Produção de bioetanol a partir da fermentação de caldo de sorgo sacarino e cana-de-açúcar. *Ciencia Rural*. 2015;45:1695–700.
3. FERNANDES AC. Cálculos na agroindústria da cana-de-açúcar. 3. ed. Piracicaba-SP; 2011.
4. BARBOSA, J. C.; MALDONADO JR. W. *AgroEstat - Sistema para análises estatísticas de ensaios agrônômicos*. 2011.
5. ICUMSA. International Commission for Uniform Methods of Sugar Analysis. 2015.
6. AMORIM H V. *Fermentação alcoólica: ciência & tecnologia*. Fermentec; 2005.
7. Ceccato-Antonini SR. *Microbiologia da fermentação alcoólica: a importância do monitoramento microbiológico em destilarias*. 2010.
8. COPERSUCAR. *Manual de controle químico da fabricação de açúcar*. Piracicaba-SP; 2001.
9. Miller GL. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*. 1959;31:426–8.
10. David L. Nelson MMC. *Princípios de Bioquímica de Lehninger*. 7th edition. Artmed; 2018.
11. Ribeiro Filho NM, Florêncio IM, Rocha AS, Dantas JP, Florentino ER, Silva FLH. Aproveitamento Do Caldo Do Sorgo Sacarino Para Produção De Aguardente. *Revista Brasileira de Produtos Agroindustriais*. 2008;10:9–16.
12. deMancilha IM, Pearson AM, Momose H, Pestka JJ. Increasing alcohol yield by selected yeast fermentation of sweet sorghum. II. Isolation and evaluation of mutants and wild types for ethanol production. *Food Chemistry*. 1984;14:313–8.
13. Ratnavathi C V., Suresh K, Kumar BSV, Pallavi M, Komala V V., Seetharama N. Study on genotypic variation for ethanol production from sweet sorghum juice. *Biomass and Bioenergy*. 2010;34:947–52.

Figures

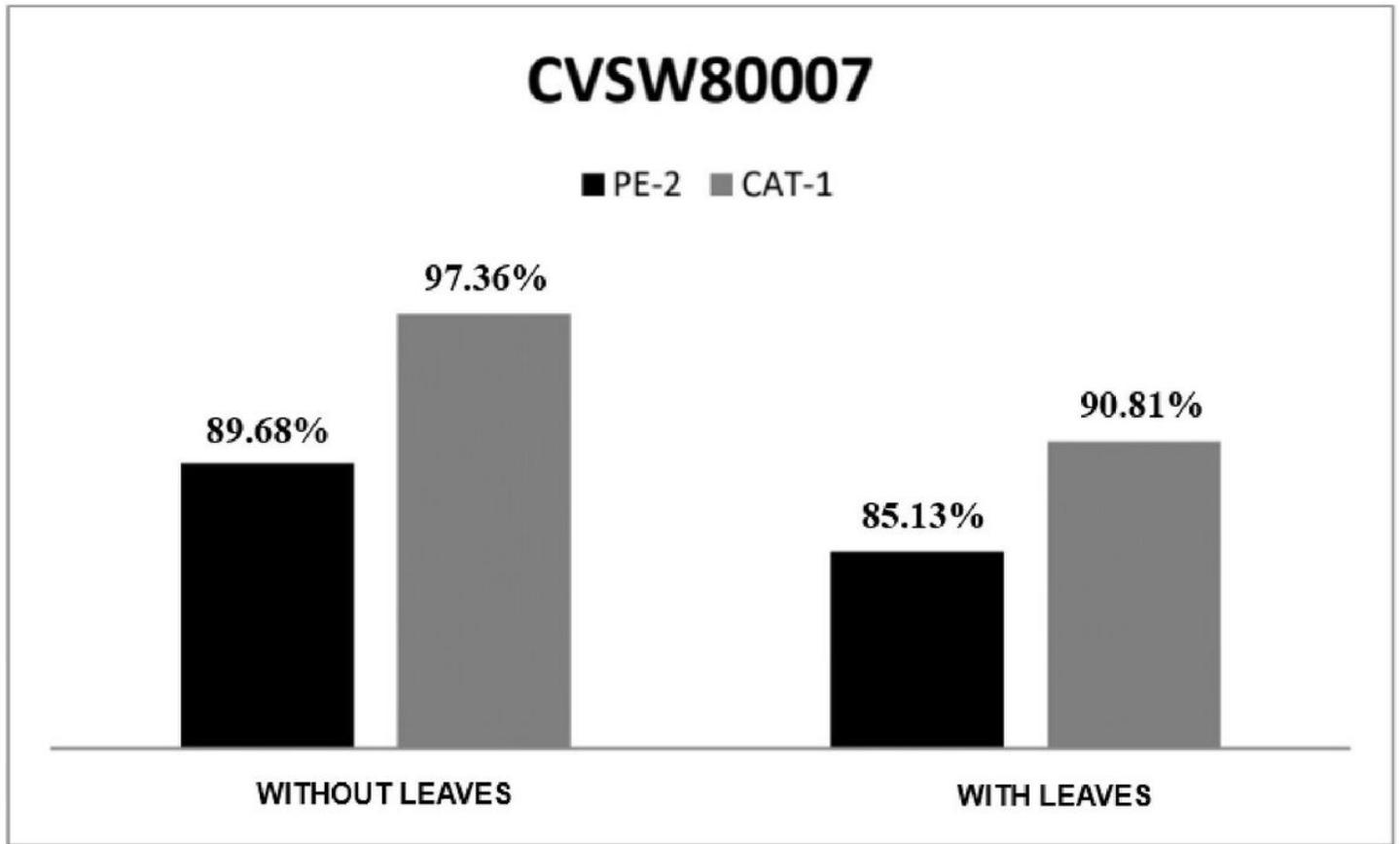


Figure 1

Fermentation efficiency (%) for genotype CVSW80007 at 135 days after sowing.

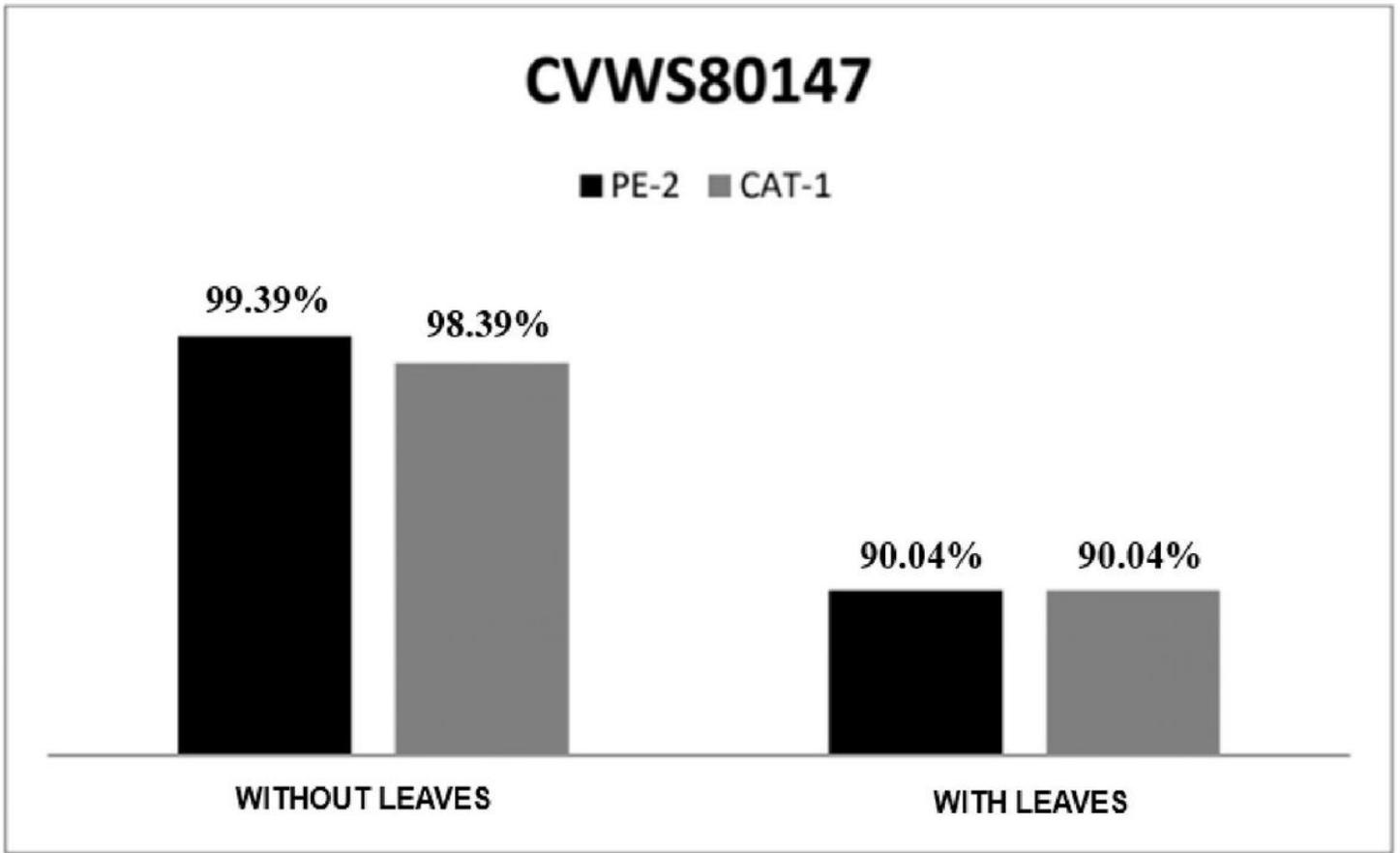


Figure 2

Fermentation efficiency (%) for genotype CVSW80147 at 135 days after sowing.

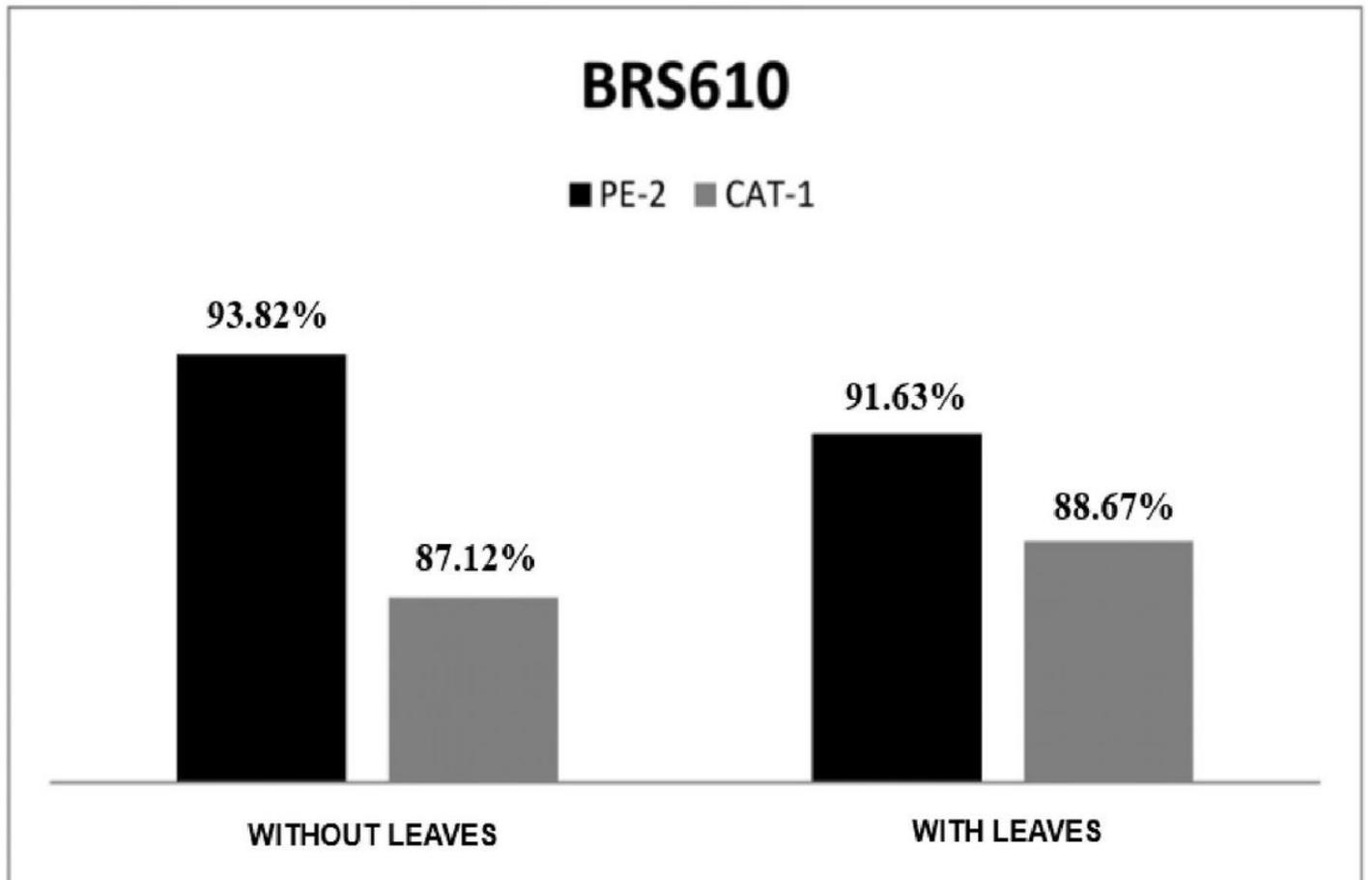


Figure 3

Fermentation efficiency (%) for genotype BRS610 at 135 days after sowing.

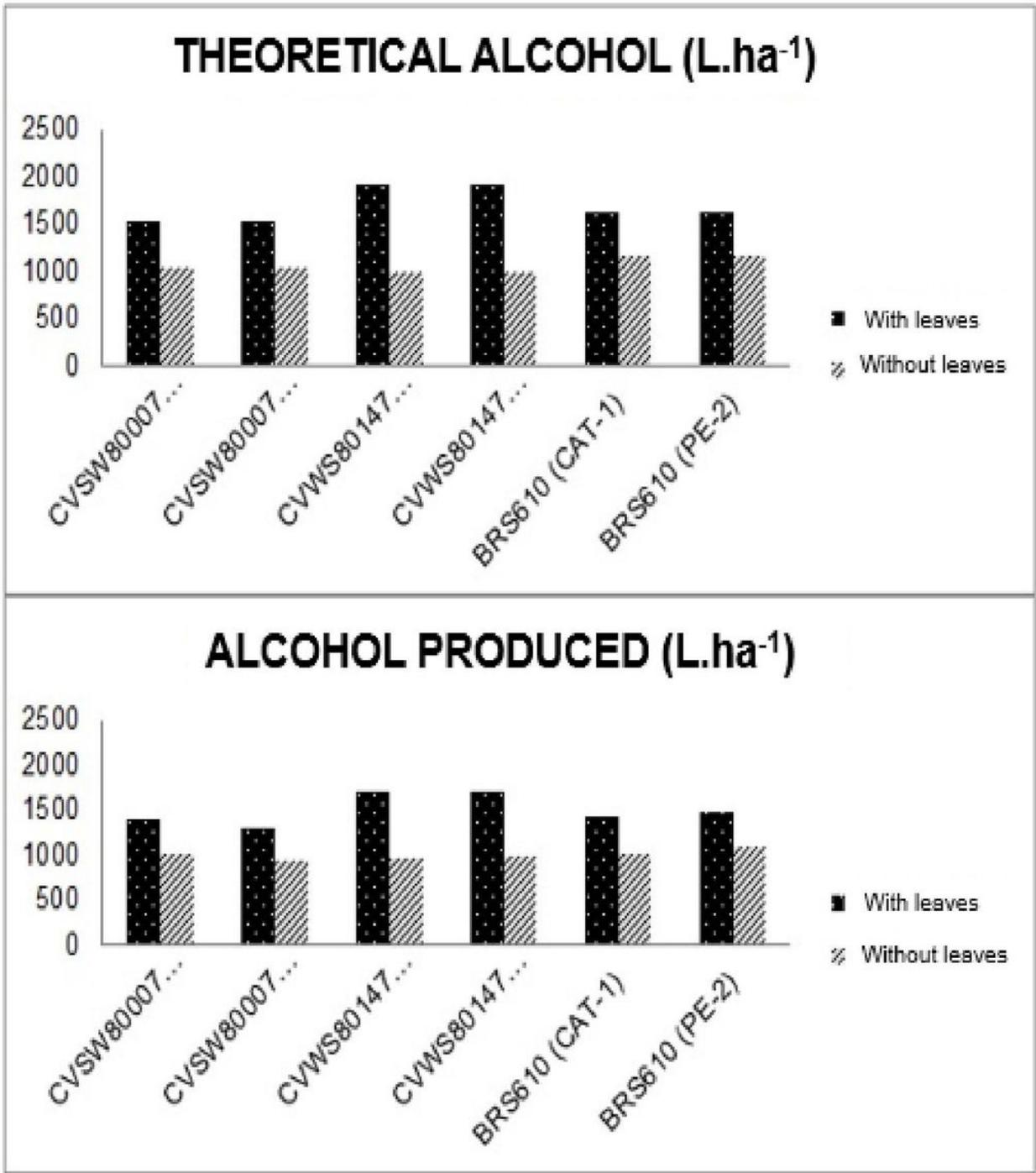


Figure 4

Results of Calculated Theoretical Alcohol and Alcohol Produced (L.ha⁻¹) for the genotypes CVSW80007, CVWS80147 and BRS610, and CAT-1 and PE-2 yeasts at 135 days after sowing.

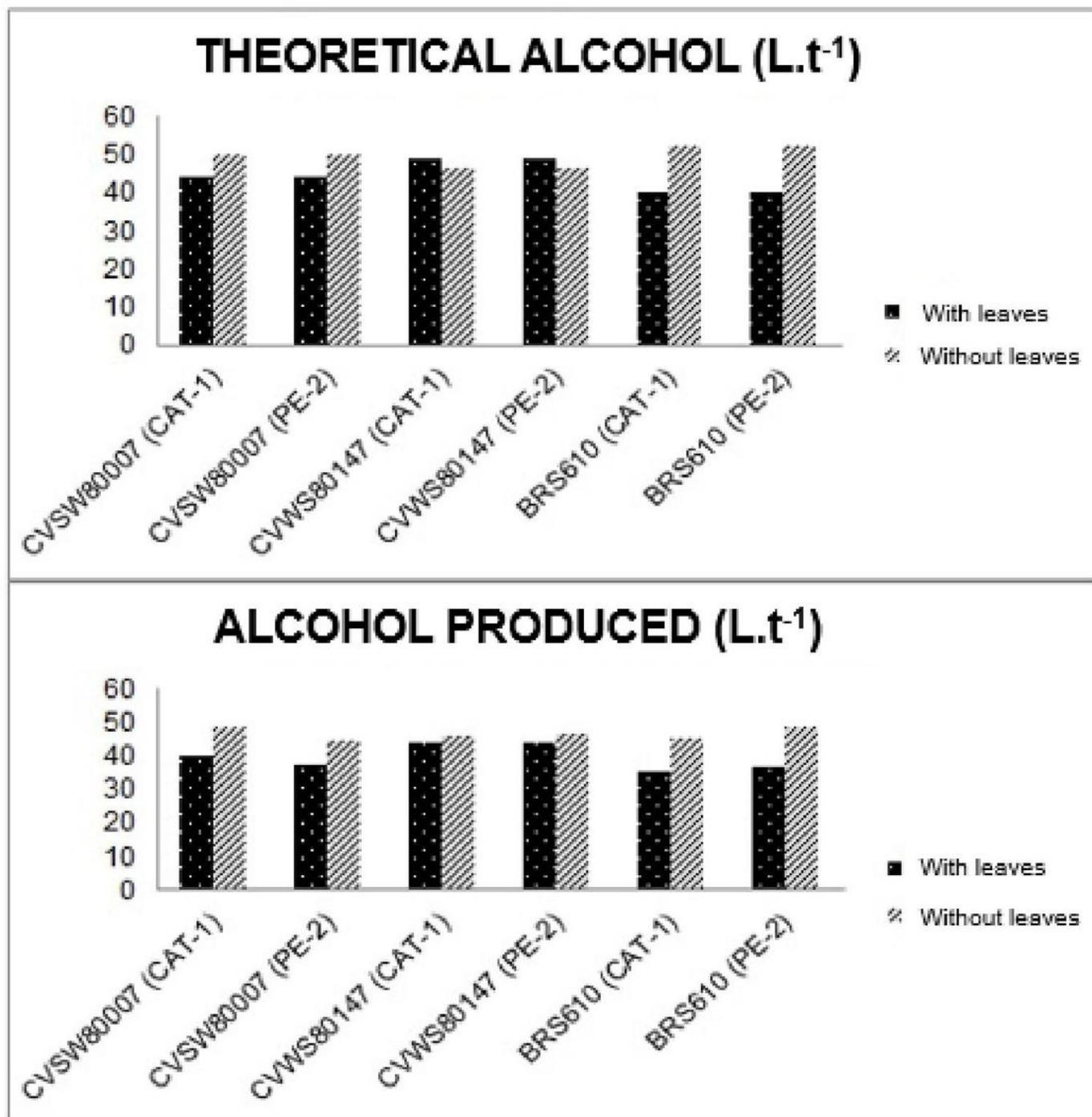


Figure 5

Results of Calculated Theoretical Alcohol and Alcohol Produced (L.t⁻¹) for the genotypes CVSW80007, CVWS80147 and BRS610, and CAT-1 and PE-2 yeasts at 135 days after sowing.

Supplementary Files

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