ENZYMATIC HYDROLYSIS OF PRE-GERMINATED BARLEY

Luciane Maria Colla[1*], Gabrielle Fusiger Berwian[1], Ana Luisa Gayger[2], Gabriel Lanza Colvero[3],

[1] *University of Passo Fundo, Graduate Program in Food Science and Technology*
[2] *University of Passo Fundo, Graduate Program in Agronomy*
[3] *University of Passo Fundo, Chemical Engineering undergraduate Course*
[*] *E-mail: lmcolla@upf.br*

ABSTRACT

The study aims to evaluate the enzymatic potential of pre-germinated barley samples for bioethanol production. The methodology consisted of enzymatic hydrolysis of pre-germinated malt and barley samples, testing different pH conditions (5.0, 5.5, and 6.0), temperatures (60, 65, and 70ºC), and solid concentrations (10, 15, and 20%). The results indicate that pre-germinated barley samples had a maximum efficiency of 28%, while the brewing standard malt (standard sample) had a maximum efficiency of 76%. Although hydrolysis efficiency with pre-germinated barley has been limited, research suggests that adding commercial enzymes may provide greater efficiency. The use of brewery waste for the production of bioethanol can bring economic and environmental benefits, as it allows the use of raw materials that would otherwise be discarded, reducing environmental impacts and increasing efficiency in the use of resources.
Keywords: Malt, pre-germinated barley, grain enzymes, saccharification, ethanol.

1 INTRODUCTION

Barley plays a fundamental role in the brewing industry, where it is transformed into malt through a process that is rigorously conducted to ensure optimal humidity and temperature conditions (Pielech-Przybylska *et al*., 2017). However, the challenges faced by producers during cultivation can compromise the quality of the grains, highlighting pre-germination in the field, where the embryo begins its development, only to be interrupted by dehydration before reaching an irreversible stage, damaging the quality of the resulting malt and causing difficulties for producers (Gualano *et al*., 2014). Despite being unsuitable for beer production, pre-germinated grains retain significant starch and maintain the potential for bioethanol production.

During barley germination, amylolytic enzymes are released, such as α-amylase and β-amylase, which promote the breaking of alpha 1-4 bonds, converting starch into fermentable sugars (Carvalho and Beléia, 2019). Therefore, the present study aims to evaluate the enzymatic potential of pre-germinated barley samples for use as a substrate in bioethanol production.

2 MATERIAL & METHODS

For hydrolysis, samples of pre-germinated barley (field germination rate of 95%) and brewing standard malt (reference for hydrolysis) were ground in a knife mill (model MA090/CFT, Marconi, Brazil) and then passed through a 10-mesh sieve to standardize particle size. The hydrolysis process of the raw materials was carried out using only the intrinsic enzymatic potential of the grains, following the 2^3 designs presented in Table 1.

Table 1 - Study of the Influence of pH, Temperature, and Solid Load (SC) on the Total Amylolytic Activity of Malted Grains

Treatment	Temperature (ºC)	pH	Concentration of solids (%)
T1	60	5,0	10
T2	70	5,0	10
T3	60	6,0	10
T4	70	6,0	10
T5	60	5,0	20
T6	70	5,0	20
T7	60	6,0	20
T8	70	6,0	20
T9	65	5,5	15
T10	65	5,5	15
T11	65	5,5	15

Experiments were conducted in duplicate. Aliquots were taken every hour to determine reducing sugars using the 3,5-DNS method (Miller, 1959), continuing until the conversion of reducing sugars stabilized. The efficiency of biomass hydrolysis was calculated using Equation 1. The data were subjected to Analysis of Variance (ANOVA), and the differences between treatments were evaluated by the Tukey test at a 5% significance level, using Excel and Statistica 7 software.

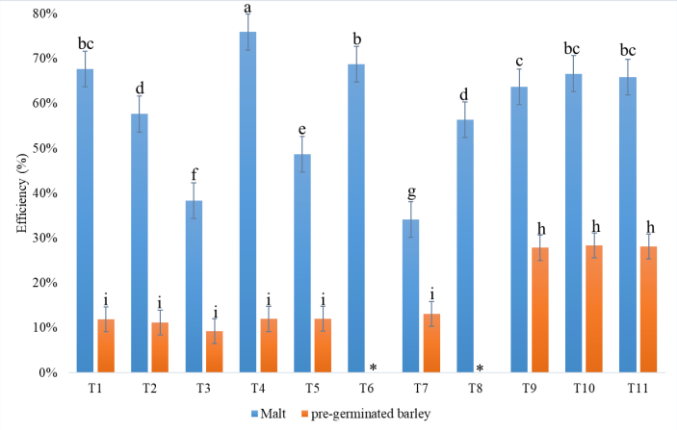
$$EH(\%) = \frac{\mathrm{AR}}{PAi} * 100 \quad (1)$$

Where: EH(%) = hydrolysis efficiency; AR = reducing sugar obtained after hydrolysis; PAi = percentage of initial starch.

3 RESULTS & DISCUSSION

Figure 1 presents the data on hydrolysis efficiency at the optimal saccharification times for both samples, with pre-germinated barley taking 8 hours and malt taking 1 hour.

Figure 1 - Enzymatic hydrolysis efficiency at the optimal saccharification times: 1 hour for malt and 8 hours for pre-germinated barley



*DNS analysis could not be performed on pre-germinated barley in treatments T6 and T8 due to the inability to achieve solid-liquid separation. Means were evaluated using analysis of variance followed by Tukey's comparison of means. Equal letters on each graph indicate that the means do not differ from each other (p>0.05).

Pre-germinated barley showed inferior results, with optimal efficiencies achieved only in treatments T9, T10, and T11, where an efficiency of around 28% was observed. Pre-germinated barley is characterized by its germination under field conditions (Laus *et al*., 2022) being subject to environmental variations that can negatively affect the activation of the necessary enzymes. This suggests that the enzymes present in pre-germinated barley may not be activated as efficiently as those in controlled malt, which is reflected in the reduced hydrolysis efficiency of microbial origin to the residue, increasing its efficiency in the production of sugars.

About malt, which was subjected to controlled germination, they demonstrated greater enzymatic efficiency. Notably, the T4 treatment stands out as the optimum point for these samples, reaching a maximum efficiency of 76%. This result is by the hydrolysis time intervals practiced in the brewing industry (Langenaeken *et al*., 2019) and highlights the efficiency of the controlled process in activating the enzymes necessary for the effective hydrolysis of the substrates present in the malt. Given the excellent results obtained in the study, investment in controlled germination techniques can be highly advantageous, increasing the conversion of substrates into fermentable sugars and enhancing efficiency when combined with commercial enzymes in ethanol production.

This not only increases ethanol yield but also ensures greater consistency and quality in the production process, which is crucial for sustainability and economic viability in an increasingly competitive biofuels market, while also reducing food waste.

4 CONCLUSION

The effective utilization of these biomasses for bioethanol production not only supports the search for renewable energy alternatives but also underscores the importance of optimizing preparation and germination processes to maximize enzyme availability and activity. In summary, while malt stands out as an efficient substrate for bioethanol production, pre-germinated barley, despite its limitations, can still be utilized, providing a noble purpose for the residue. However, due to the low hydrolysis efficiency of the grain's own enzymes, pre-germinated barley requires the addition of commercial enzymes or mixing with non-standard brewer's malt to be used in bioethanol production.

REFERENCES

CARVALHO, P. T.; BELÉIA, A. D. P. 2019. Malting of preharvest sprouted wheat. Agricultural Science Magazine, v. 50, n. 4, p. 571-577.

GUALANO, N. A.; DEL FUEYO, P. A.; BENECH-ARNOLD, R. L. 2014. The potential longevity (Ki) of batches of malting barley grains (Hordeum vulgare L.) is related to their degree of pre-germination evaluated through different industrial quality parameters. Cereal Science Magazine, v. 1, p. 222-228.

LANGENAEKEN, N. A.; DE SCHEPPER, C. F.; DE SCHUTTER, D. P.; COURTIN, C. M. 2019. Different gelatinization characteristics of small and large barley starch granules impact their enzymatic hydrolysis and sugar production during mashing. Food Chemistry, v. 295, p. 138-146.

LAUS, A.; ENDRES, F.; HUTZLER, M.; ZARNKOW, M.; JACOB, F. 2022. Isothermal Mashing of Barley Malt: New Insights into Wort Composition and Enzyme Temperature Ranges. Food and Bioprocess Technology, v. 15, n. 10, p. 2294-2312.

MILLER, G.L. 1959. Modified DNS method for reducing sugars. Anal. Chem., 31: 426-428.

PIELECH-PRZYBYLSKA, K.; BALCEREK, M.; NOWAK, A.; WOJTCZAK, M.; CZYŻOWSKA, A.; DZIEKOŃSKA-KUBCZAK, U.; PATELSKI, P. 2017. The Effect of Different Starch Liberation and Saccharification Methods on the Microbial Contaminations of Distillery Mashes, Fermentation Efficiency, and Spirits Quality. Molecules, v. 22, n. 10, p. 1647.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the National Council for Scientific and Technological Development (CNPq) for the financial support provided for the Universal Pproject 403786/2021-5, and to the Rio Grande do Sul State Research Support Foundation (FAPERGS) - process number 21/2551-000223-7. Additionally, we acknowledge the Coordination for the Improvement of Higher Education Personnel (CAPES) for funding source 001, which was essential for this work and valid from March 1, 2023, to February 28, 2025. Furthermore, we extend our appreciation to the University of Passo Fundo (UPF) for providing the infrastructure and to our colleagues at the Biochemistry and Bioprocesses Laboratory (Labio) for their collaboration.