PECTINASE PRODUCTION BY SOLID STATE FERMENTATION FOR REMOVAL OF COFFEE MUCILAGE

Lucas L. Dos Santos[1], Lorena T. Barbosa[2] & Tatiana S. Porto[3*]

[1] *Department of Rural Technology, Federal Rural University of Pernambuco, Recife, Brazil.*
[2]*Postgraduate degree in Food Science and Technology, Federal Rural University of Pernambuco, Recife, Brazil.*
[2]*Department of Animal Physiology and Morphology, Federal Rural University of Pernambuco, Recife, Brazil.*
** Corresponding author's email address: tatiana.porto@ufrpe.br*

ABSTRACT

The production and characterization of pectinases from *Aspergillus phoenicis* URM 4924 and *Aspergillus niger* URM 5741 produced during the coffee fermentation process were investigated. Pectinase is an enzyme widely used in the clarification of juices, enzymatic peeling, among others. In the production of specialty coffees, this enzyme is used to remove mucilage from coffee cherries. Fungal pectinases showed optimal pH in acidic conditions, pH 5 (*Aspergillus phoenicis* URM4924) and pH 6 (*Aspergillus niger* URM5741). Therefore, it can be observed that both enzymes produced demonstrated an acidic character for their activities, this behavior being very characteristic of fungal enzymes. However, the pectinase from *Aspergillus niger* URM 5741 showed an optimum temperature of 50°C, a high value than the pectinase from *Aspergillus phoenicis* URM4924, which had an optimum temperature of 40°C. Thus, we can conclude that pectinases are produced during the coffee solid-state fermentation process, thus carrying out the demucilage process.

Keywords: Coffee mucilage. Pectinase. Aspergillus. Enzymatic production.

1 INTRODUCTION

Brazil is considered the largest producer and exporter of coffee in the world, exporting around 2.2 million tons, equivalent to 39.4 million bags of coffee in 2022, reaching a total of US$ 9.2 billion dollars collected. Coffee is the second most consumed beverage in Brazil, only behind water [1]. Understanding the action of microbial enzymes as the mechanism responsible for the degradation of coffee mucilage, the application of enzymes can contribute to the development of the bioprocess and represents a little explored technology. The removal of coffee mucilage through enzymatic treatment reduces fermentation time by modulating microbial growth and preventing the development of undesirable microorganisms [2]. Pectic enzymes are applied in the process of coffee benefit, specifically in the stage of fermentation of the grain [3]. Pectic enzymes are also applied in the food industry for processes related to the clarification and extraction of fruit juices and enological treatments [4]. There is still need for research in alternative and profitable uses of coffee pulp [5]. (Oliveira and Franca, 2015) and, for that reason, the use of the pulp for the production of pectic enzymes by solid-state fermentation requires further research [6]. In this sense, the objective of this work was the evaluation of the coffee pulp as substrate for polygalacturonase production in solid state fermentation through a study of the characteristics of the coffee demucilage, together with the establishment of the biochemical characteristics of the produced polygalacturonase by Aspergillus strains.

2 MATERIAL & METHODS

Microorganism

The microorganisms used for pectinase production were the fungi *Aspergillus phoenicis* URM 4924 and Aspergillus niger URM 5741 obtained from the URM fungal collection of the Federal University of Pernambuco (UFPE). The fungal strains were preserved in Czapek Dox Agar and mineral oil medium, reactivated in glucose broth and subsequently transferred to Czapek Dox Agar solid medium for 7 days of cultivation at 30°C in a bacteriological incubator, until sporulation occurred. The spores were suspended with the addition of a sterile saline solution of NaCl (0.9% w/v) and Tween 80 (0.01% v/v).

Coffee

Samples of Arabica coffee cherries of the Typica variety were collected at Florentina farm, located in Taquaritinga do Norte - PE, where the coffee plantations were cultivated using organic management practices and in high-altitude swamps. The ripe cherries were collected manually and at a perfect stage of maturity.

Solid-state fermentation

To perform solid-state fermentation, 125 mL Erlenmeyer flasks containing 15 g of substrate (coffee cherries) were used. The spore solutions from the cultures *of Aspergillus phoenicis* URM 4924 and *Aspergillus niger* URM5741 were removed using sterilized saline containing NaCl (0.9% w/v) and Tween 80 (0.01% v/v), and then inoculated into the substrates at a concentration of 10^7 spores/mL to reach a moisture content of 55%. The inoculated substrates were incubated in a bacteriological incubator at 30°C for 10 days. Ten days after the end of the fermentation process, the demucilaged coffee cherries were removed from the bacteriological incubator, and an acetate buffer solution at pH 5.0 was added. The Erlenmeyer flasks were then placed in an orbital shaker at 120 rpm for 1 hour and 30 minutes at 30°C. The Erlenmeyer flasks were then placed in a properly sterilized

laminar flow chamber to separate the husk from the coffee bean. After separation, the husk was subjected to maceration and filtration, and the crude extract obtained was stored at -22°C for later analysis.

Analytical determinations of pectinase

Pectinase activity was determined by preparing a reaction mixture containing 0.5 mL of the enzyme extracted from the coffee husk with 0.5 mL of pectin solution (1% prepared in sodium acetate buffer, 0.1 M, pH 5.0). It was vortexed and kept in a water bath at 40°C for 40 min. Subsequently, the reaction was stopped by an ice bath for 5 min. Then, 0.1 mL of the mixture was removed and mixed with 1 mL of the DNSA reagent [7]. The reducing sugars in the hydrolysate were analyzed in triplicate by the 3,5 dinitrosalicylic method [8]. The absorbance was determined at 540 nm with a UV/VIS spectrophotometer. One pectin unit was defined as the amount of enzyme that produces one micromole of galacturonic acid per minute, under standard test conditions. The activity was calculated according to the following equation:

Determination of the optimum temperature and pH.

The effect of the optimum temperature for pectinase activity was determined by incubating the enzymes and substrate (1% pectin in acetate buffer, pH 5.0) for 40 min at temperatures of 30, 40, 50, 60, 70, 80° and 90° C and the reducing sugars released, as well as the enzymatic activity were determined in the same way as described in previous section.

The effect of the optimum pH for maximum enzyme activity was evaluated by incubating the enzyme with the substrate (1% pectin) prepared in buffer solutions with different pH values: Citrate (pH 3.0 and 4.0), Sodium acetate (pH 4.0 and 5.0), citrate-phosphate (pH 5.0 and 6.0), Phosphate (pH 6.0–8.0) and Tris-HCl (pH 8.0 and 9.0). The reaction took place at 40 °C for 40 minutes. The reducing sugars released as well as the enzymatic activity were determined in the same way as described in previous section.

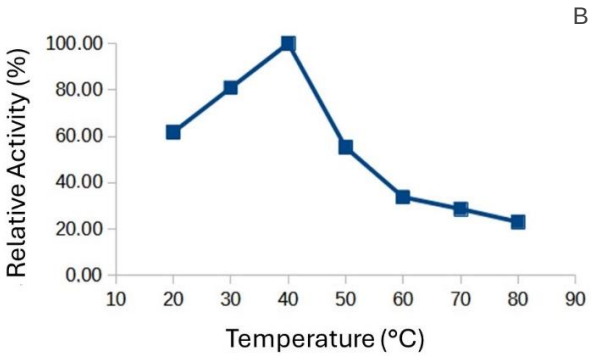
3 RESULTS & DISCUSSION

The fermentation process was carried out with the two strains of *Aspergillus phoenicis* URM 4924 and *Aspergillus niger* URM 5741, providing pectinase low activities of 1.088 U/mL and 0.416 U/mL, respectively. Thus, it was found that the strain of *Aspergillus phoenicis* URM4924 was the most promising for coffee demucilage (Figure 1A).

The incubation temperature varied between 30 and 90°C for the enzymes of the two microorganisms. The results showed that the highest activity of the enzyme produced by *Aspergillus phoenicis* URM 4924 was at 40°C. The activity showed a decrease after its maximum temperature, as observed in Figure 1 B. The pectinase from *Aspergillus niger* URM 5741 showed an optimum temperature at 50ºC.

Figure 1. Demucilage process using solid-state fermentation(A) and optimum temperature of the pectinase (B) produced by *Aspergillus phoenicis* URM 4924 in solid-state fermentation using coffee cherry as substrate.

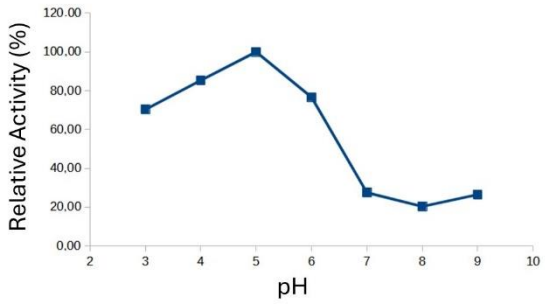
A



The effect of pH on the activities of the enzymes produced by the two microorganisms was evaluated by incubating the enzymes in different pH ranges (3.0 - 9.0), observing the activity of the enzyme of *Aspergillus phoenicis* URM4924. The maximum activity value was observed at pH 5.0 for the citrate-phosphate buffer, as can be seen in Figure 2. For *Aspergillus niger* URM 5741, the maximum activity value reached was at pH 6.0 for the citrate-phosphate and phosphate-phosphate buffers. Therefore, it can be concluded that both enzymes produced demonstrated an acidic character for their activities, this behavior being very characteristic of fungal enzymes.

Studies carried out with solid-state fermentation showed greater activity than that found in the present study. However, the biochemical characteristics of pectinase are similar. A strain of *Aspergillus niger* [6], called van Thiegem, has been selected as a good producer of polygalacturonases (60 U/ml) using the coffee pulp as the growth substrate. During fermentation, reducing sugars, caffeine and phenolic compounds were consumed until almost exhaustion. A partial characterization of the polygalacturonase using high methoxyl pectin as substrate indicates an optimal pH of 4.0 and 45°C as optimal temperature, which are good values for the use of the enzyme in vegetable processing, including coffee processing.

Figure 2. Optimal pH of the pectinase produced by *Aspergillus phoenicis* URM 4924 in solid-state fermentation using coffee cherry as substrate.



These results are similar to those found in polygalacturonases from *Aspergillus niger* (pH 4.5, 40ºC) [9] and *Aspergillus niger* (pH 4.8, 45ºC[10]). Other interesting enzymatic characteristics require further research.

4 CONCLUSION

From the production of enzymatic extracts with fermentation on coffee cherries, it was possible to characterize the pectinases of both *Aspergillus phoenicis* URM 4924 and *Aspergillus niger* URM 5741. Although both enzymes presented different optimum pHs, the pectinases demonstrated maximum activity in acidic conditions. However, the pectinase of *Aspergillus phoenicis* URM 4924 presented greater activity at 20 °C, which corresponds to 20 °C less than the pectinase of Aspergillus niger URM 5741. Low temperatures are desirable because they reduce the degradation of volatile compounds important in the formation of the organoleptic characteristics of specialty coffees.

REFERENCES

1 BRASIL. Brasil é o maior produtor mundial e o segundo maior consumidor de café. Disponível em: <https://www.gov.br/agricultura/pt-br/assuntos/noticias/brasil-e-o-maior-produtor-mundial-e-o-segundo-maior-consumidor-de-cafe>. Acesso em: 08 abr. 2024.

2 TAI, E-S.; HSIEH, P-C.; SHEU, S-C. Process Biochem., v. 49, p. 1274-1280, 2014.

3 Murthy, P. S. and M. M. Naidu. 2011.. Eur. J. Appl. Sci. 3: 130-139.

4 APOLINAR-VALIENTE, R., I. ROMERO-CASCALES, E. GÓMEZ-PLAZA AND J. M. ROS-GARCÍA. 2017. Eur. Food Res. Technol. 243: 1933-1942..

5 OLIVEIRA, L. S., FRANCA A. S.. 2015. Ch. 31. Elsevier Inc., Amsterdam, Netherlands, pp. 283-291..

6 FRÓMETA, R, AMARILIS, O., SANCHEZ, J.S, GARCÍA, J.M.R. (2020) Emirates J. of Food and Agriculture. 32(2): 117-124

7 ROS, J. M., J. M. NÚÑEZ, D. SAURA, M. C. SALMERÓN and J. LAENCINA.1991. Biotechnol.Lett. 13: 287-290..

8 MILLER, G. L. (1959).. Analytical Chemistry. 31: 426-428.

9 BUGA, M. L., S. IBRAHIM AND A. J. NOK. 2010. Afr. J. Biotechnol. 9: 8944-8954.

10 Kant, S., A. Vohra and R. Gupta. 2013. Protein Expr. Purif. 87: 11-16.

ACKNOWLEDGEMENTS

The authors are grateful to FACEPE and National Council for Scientific and Technological Development, Brazil (CNPq) (financial code no APQ-1560-5.03/22) for financial funding and Federal Rural University of Pernambuco for the NUBIOTEC laboratory infrastructure.