## A comprehensive analysis of the fungal degradation of procyanidins as a tool for coffee pulp detoxification

L.J. Valencia-Hernández<sup>1</sup>, J.E. Wong-Paz<sup>2</sup>, M.L. Chávez-González<sup>1</sup>, J.A. Ascacio-Valdes<sup>1</sup>, J.C. Contreras-Esquivel<sup>1</sup>, L. Sepúlveda<sup>1</sup>, R. Rodríguez-Herrera<sup>1</sup> & C.N. Aguilar<sup>2</sup>

 <sup>1</sup>Bioprocesses and Bioproducts Research Group. BBG-UAdeC. Food Research Department. School of Chemistry. Universidad Autónoma de Coahuila, 25280, Saltillo, Coahuila, México.
<sup>2</sup>Engineering Department, Instituto Tecnológico de Ciudad Valles, Tecnológico Nacional de México, Ciudad Valles, SLP, México Keywords: biodegradation, tannins, procyanidins

\*Presenting author email: cristobal.aguilar@uadec.edu.mx

Tannins are natural polyphenolic compounds of variable molecular weight. They differ from other phenols by their ability to precipitate proteins. Tannins are secondary, water-soluble plant metabolism compounds that are widely prevalent in plants. Hydrolysable and condensed tannins are the two main classes of tannins. These compounds have different biological activities, so some tannin-rich plants have been used as astringent, antidiarrheal, antidotes to alkaloid poisoning, anti-inflammatory, antiseptic and hemostatic. The anticancer, antiviral and antioxidant activities of some tannins have been demonstrated, which makes them interesting as possible therapeutic agents or in the preparation of functional foods and beverages.

However, despite the diverse and interesting applications of tannins, their presence in large quantities in water, soil, beverages and food can cause serious problems. These compounds have a range of negative effects on various organisms - from toxic effects on animals, where they are considered antiphysiological agents, to inhibiting the growth of most microbial agents. However, some microorganisms, particularly filamentous fungi, are resistant to tannins and have developed various mechanisms and pathways for their degradation in their natural environment (Lara *et al* 2017).

The microbial degradation of condensed tannins is very poorly understood compared to hydrolysable tannins, regardless of whether it is carried in both aerobic and anaerobic environments therefore its description is seriously limited. At present, there is a need to generate original and relevant knowledge that allows a greater understanding of the biodegradation of condensed tannins, particularly in filamentous fungi. However, scientific knowledge regarding the biological degradation of condensed tannins is limited and scarce (Aguilar *et al* 2007). Scientific knowledge of the biodegradation process of this type of condensed tannins (pro and -anthocyanidins) can have a positive impact on the generation of biocatalysts useful for promoting the health of the human and animal digestive system. Also, to valorize several tannin-rich biomasses.

The main problem in the study on procyanidins lies in the difficulty of obtaining and purifying these compounds due to their great complexity and heterogeneity, to such an extent that currently, procyanidins greater than 3 units of (epi)-catechin are not found commercially. Today it is well known that procyanidins are more resistant to microbial attacks than hydrolysable tannins and can slow or even inhibit the growth of microorganisms. Despite this, several studies have shown that certain microorganisms, especially filamentous fungi, are able to survive in the presence of procyanidins. The use of purified fractions rich in procyanidins of high molecular weight for their study by fungal biotransformation has been explored (Contreras *et al* 2006; Roopesh *et al* 2010; Contreras *et al* 2011). However, these approaches are still very scarce and appear to be distant from the actual direct mechanism involved in the biotransformation of these compounds. A clear example in which these polyphenolic compounds limit the use of an agri-food residue in applications such as cattle feed mainly, is the coffee pulp obtained from the processing of coffee cherry (Jayachandra *et al* 2011). Coffee pulp also poses a potential risk of environmental pollution for producing countries (Mussatto *et al* 2011).

The most recent studies published by our research group include the enzymatic synthesis, purification and evaluation of the antioxidant capacity in vitro of polyphenolic oxidation products of apple juices (Wong-Paz *et al* 2015), also an analysis of the biotechnological advance and the challenges represented by the tannase study (Chávez-González *et al* 2012) and, the analysis of proanthocyanidins of almonds and walnut shells, where the degree of polymerization of such compounds and the effect they have on the hydrolysis of various nutrients in the human digestive system was evaluated (Vázquez-Flores *et al* 2017).

Based on this, this work analyzes from a comprehensive view the biochemical mechanism involved in the process of fungal degradation of purified coffee pulp procyanidins, which will allow defining the enzyme(s) involved and the intermediate compounds that are generated as a result of the degradation process of condensed tannins, because the understanding of this biochemical phenomenon will facilitate the development of strategies to give added value in this waste of the coffee industry and contribute to the reduction of polluting agents from food industries.

## Acknowledgements.

Authors thank National Council of Science and Technology (CONACYT, Mexico) for the financial support given through the project of basic science/frontiers science FOSEC\_CB 2017-18 A1-S-42515. **References.** 

Aguilar, C. N., Rodríguez, R., Gutiérrez-Sánchez, G., Augur, C., Favela-Torres, E., Prado-Barragan, L. A. & Contreras-Esquivel, J. C. (2007). Microbial tannases: advances and perspectives. *Applied Microbiology and Biotechnology*, 76(1), 47-59.

Chávez-González, M., Rodríguez-Durán, L. V., Balagurusamy, N., Prado-Barragán, A., Rodríguez, R., Contreras, J. C., & Aguilar, C. N. (2012). Biotechnological advances and challenges of tannase: an overview. *Food and Bioprocess Technology*, 5(2), 445-459.

Contreras, M., Guyot, S., Roussos, S. y Augur, C. 2011. Enzimatic degradation of Procyanidin B1 by Aspergillus fumigatus. In: Sabu, A., Roussos, S., Aguilar, C. N. (Eds.), Chemistry and biotechnology of polyphenols, Cibet Publishing House, India, pp 66-74.

Contreras-Domínguez, M., Guyot, S., Marnet, N., Le Petit, J., Perraud-Gaime, I., Roussos, S., & Augur, C. (2006). Degradation of procyanidins by *Aspergillus fumigatus*: Identification of a novel aromatic ring cleavage product. *Biochimie*, 88(12), 1899-1908.

Jayachandra, T., Venugopal, C., & Appaiah, K. A. (2011). Utilization of phytotoxic agro waste—Coffee cherry husk through pretreatment by the ascomycetes fungi Mycotypha for biomethanation. *Energy for Sustainable Development*, 15(1), 104-108.

Lara, F., Veana, F., Hernández-Castillo, F. D., Aguilar, C. N., Reyes-Valdés, M. H., & Rodríguez-Herrera, R. (2017). Variability among strains of *Aspergillus* section *Nigri* with capacity to degrade tannic acid isolated from extreme environments. *Archives of microbiology*, 199(1), 77-84.

Mussatto, S. I., Machado, E. M., Martins, S., & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology*, 4(5), 661.

Roopesh, K., Guyot, S., Sabu, A., Haridas, M., Isabelle, P. G., Roussos, S., & Augur, C. (2010). Biotransformation of procyanidins by a purified fungal dioxygenase: Identification and characterization of the products using mass spectrometry. *Process Biochemistry*, 45(6), 904-913.

Vazquez-Flores, A. A., Wong-Paz, J. E., Lerma-Herrera, M. A., Martinez-Gonzalez, A. I., Olivas-Aguirre, F. J., Aguilar, C. N., ... & de la Rosa, L. A. (2017). Proanthocyanidins from the kernel and shell of pecan (Carya illinoinensis): Average degree of polymerization and effects on carbohydrate, lipid, and peptide hydrolysis in a simulated human digestive system. *Journal of Functional Foods*, 28, 227-234.

Wong-Paz, J. E., Muniz-Márquez, D. B., Aguilar, C. N., Sotin, H., & Guyot, S. (2015). Enzymatic synthesis, purification and in vitro antioxidant capacity of polyphenolic oxidation products from apple juice. *LWT-Food Science and Technology*, 64(2), 1091-1098