# Enzymatic hydrolysis of tannin by *Aspergillus niger*

Anna Oberle*, Zuzana Paschová

Mendel University in Brno / Faculty of Forestry and Wood Technology / Department of Wood Science and Technology

* e-mail: anna.oberle@mendelu.cz

## INTRODUCTION:

The fungus of the genus *Aspergillus* is a frequent contaminant of various natural materials. It is active in a wide range of pH and temperatures. *Aspergillus* degrades even condensed tannins that are resistant against many other microorganisms (Scalbert 1991). Thus, the enzymes produced by *Aspergillus* are able to cleave even covalent bonds that are otherwise difficult accessed by simple chemical hydrolysis. The biggest potential of such „green“ approach is the enzymatic selectivity that might be a key parameter in the fixation of extracts in wood of known low natural durability (e.g. common beech).

Cultivation of *Aspergillus niger* was performed in Czapek-Dox Broth (CDB) medium with tannin of starting concentration: 10, 30 and 50 g/L that was dissolved in the medium (Fig. 1). Aliquots were taken in 24hour intervals and collected within 7 days of cultivation. All analyses were measured in triplicate.

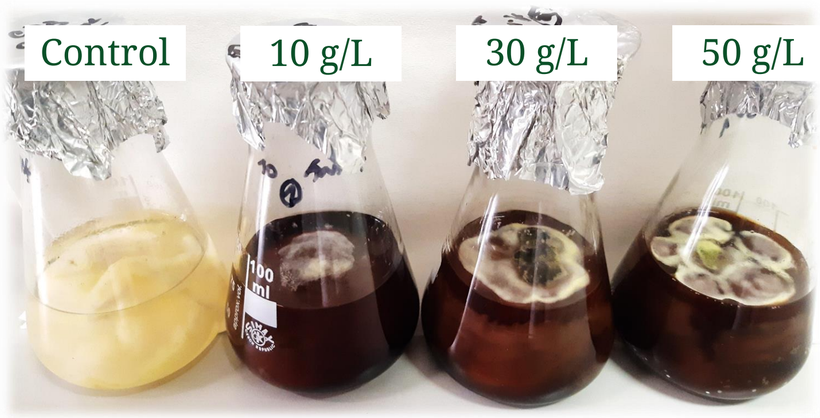


Fig. 1: Cultivation of the *A. niger* in CDB with added tannin (from left: control - pure CDB medium with fungi; medium with tannin of starting concentration 10 g/L, 30 g/L and 50 g/L, respectively)

## SPECTROPHOTOMETRICAL ANALYSIS OF HYDROLYZED TANNIN:

The determination of total phenolic content (Folin-Ciocalteu assay) and catechin content were carried out on the enzymatically treated tannin. The starting tannin concentration revealed to affect the speed of gallic acid and catechin release in time (Fig. 2 and 3).

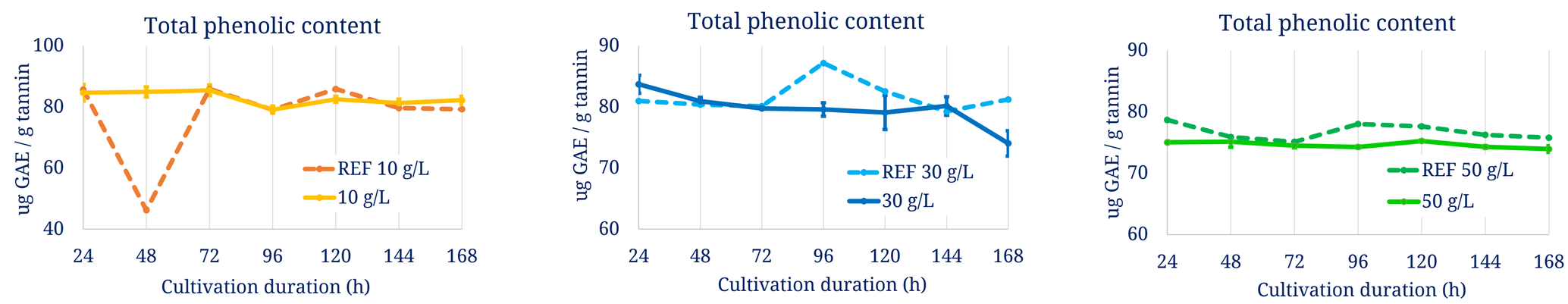


Fig. 2: Result of enzymatic hydrolysis with *A. niger* showing gallic acid released from tannin structure in dependence on cultivation time (expressed in GAE – gallic acid equivalents)

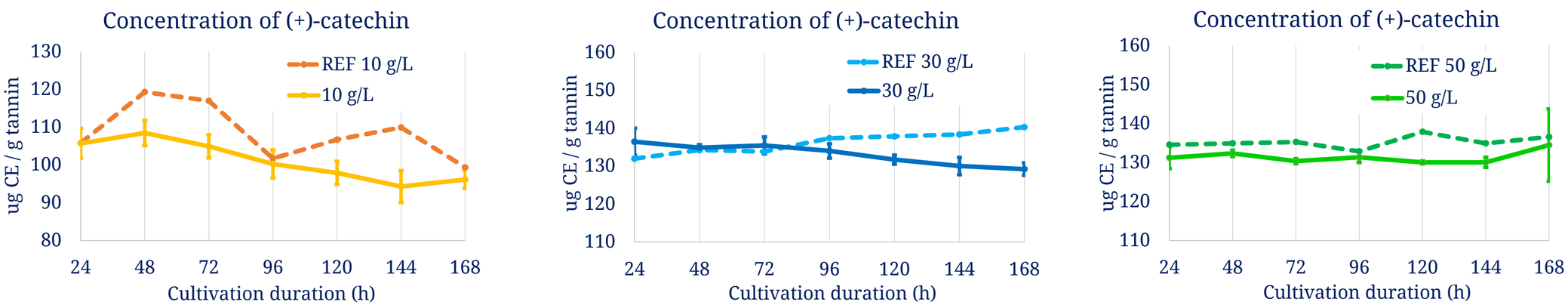


Fig. 3: Result of enzymatic hydrolysis with *A. niger* showing different catechin amounts released from tannin structure in dependence on cultivation time (expressed in catechin equivalents)

## CONCLUSIONS:

- ✚ Following factors of controlled degradation of condensed tannins were studied in presence of filamentous fungi *Aspergillus niger*: 3 tannin concentrations; 24 hours culture sample aliquots; phenolic content determination; catechin estimation
- ✚ The most promising variant for further investigation (i.e. higher catechin concentration) seems to happen **between 24 and 96 hours** of cultivation with **lower tannin concentration**
- ▬ Estimation of the enzyme activity needs to be completed to consider the whole hydrolytical efficacy of this strain for tannin fractionation
- ▬ Even if the desired catechin content might be increased, the stability and feasibility for the implementation into wood via impregnation is questionable whether better diffusion can occur (complex matrix: wood structure/Chemistry and water interaction)

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MENDELU Faculty of Forestry and Wood Technology

ldf.mendelu.cz