**Wood Biochemistry**

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# Degradation of *Pinus sylvestris* and *Populus tremula* by laccate *Ganoderma* species

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**Abstract:** Wood chips and sawdust are used for cultivating *Ganoderma lucidum* mushrooms. In Northern Europe, side-streams of *Pinus sylvestris* are highly abundant, however as cultivation substrate they inhibit the growth of *G. lucidum*. To identify the changes in lignocellulosic composition after fungal degradation, the major lignocellulosic components in *P. sylvestris* and an optimal substrate for *G. lucidum*, *Populus tremula* were analyzed. *Populus tremula* was evenly degraded while the glucan fraction of *P. sylvestris* was not degraded and its lignin fraction was consumed almost completely. Despite not being an optimal substrate, *P. sylvestris* was successfully delignified by *G. lucidum*.

**Keywords:** biomass valorization; fungal decay; mushroom cultivation; mushroom substrate; softwood; wood composition

## 1 Introduction

Forest industries generate significant volumes of wood side-streams worldwide. Sawmills and plywood industries process approximately 50 % of the harvested timber and the remaining mass fraction consist of bark, chips, and sawdust (Lindberg and Tana 2012), which in Europe are mostly used for energy and, to a minor extent, for pulp and panel production (Verkasalo et al. 2019). The European Union is promoting a transition towards more sustainable uses of material resources. The consequential increase of wood side-streams has sparked efforts from industry and research communities to discover innovative applications for these underutilized secondary resources.

The cultivation of mushrooms is one strategy that allows the transformation of wood side-streams into high-value products, such as carbohydrates. Among cultivated fungal species, *Ganoderma lucidum* has been broadly studied in regards of their nutritional values and biological activity. To supply the demand of the bioactive compounds derived from *G. lucidum*, the species has been cultivated in a wide variety of substrates originating from agricultural or forestry side-streams (Atila 2020; Cortina-Escribano et al. 2020a,b).

The commercial cultivation of *G. lucidum* in Northern Europe is still at its infancy. Wood-derived side-streams have been tested for the cultivation of *G. lucidum* with variable results (Cortina-Escribano et al. 2020a,b): *Populus tremula* increased the yield of fruiting bodies, while *Pinus sylvestris* prevented the mycelial growth and probability of fruiting.

The effect of wood structure and composition of *Pinus* sp. and *Populus* sp. on fungal metabolism have been previously studied (Witzell and Martín 2008; Kuhar et al. 2018). For instance, the phenolic compounds present in *P. sylvestris* and *Populus tremuloides* play a role in the defense mechanism against several fungal pathogens and inhibit their growth (Witzell and Martín 2008).

It is expected that some of the wood components of *P. sylvestris* are not degraded by *G. lucidum* and, therefore, the fungus is unable to produce fruiting bodies. In this study, the aim was to identify the changes in lignocellulosic composition of wood side-streams after they have been applied for the cultivation of *G. lucidum*.

## 2 Materials and methods

### 2.1 Lignocellulosic material

The lignocellulosic material was obtained from a previous study (Cortina-Escribano et al. 2020b), in which wood side-

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streams (wood chips and sawdust) from *P. sylvestris* and *P. tremula* were utilized as substrate for the cultivation of *G. lucidum* fruiting bodies. Four wood samples consisting of *P. sylvestris* and *P. tremula* fresh ($pine_{fresh}$ and $poplar_{fresh}$) and degraded wood ($pine_{SMS}$ and $poplar_{SMS}$) were selected for the analysis. In this work, the spent mushroom substrate (SMS) after the mycelium colonization and cultivation of *G. lucidum* (strain MUS192) originating from Finland is referred to as degraded wood (Cortina-Escribano et al. 2020a,b). The $pine_{SMS}$ and $poplar_{SMS}$ substrates were exposed to fungal degradation for approximately six months. The wood samples were kept at −20 °C until the lignocellulosic composition analysis. Each sample was analyzed in duplicate.

## 2.2 Lignocellulosic composition analysis

The wood samples were tested for their chemical composition (extractives, carbohydrates, lignin, and ash contents). Prior to the lignocellulosic composition analysis, approximately 100 g dry weight (dw) samples were oven-dried at 60 ± 2 °C for 24 h and grinded with a centrifugal mill (Retch ZM200, Germany) collecting the wood powder passing 1 mm sieve. The dry weight content of the samples was determined gravimetrically by drying in an oven at 105 °C until constant weight. All compositional data is given as a substrate dry weight-based mass fraction (w-%).

Extractives were prepared (American Society for Testing and Materials - ASTM E1690-08 2016a) using accelerated solvent extraction (Dionex ASE-350). The extraction was conducted using either deionized water, 95 % ethanol or a combined extraction using first deionized water and then 95 % ethanol. The content of extractives was calculated as the mass loss of the biomass sample due to extraction, considering the moisture content of the samples (American Society for Testing and Materials - ASTM D4442-16 2016b). Per each sample, two replicates were extracted with only water (water-soluble extractives), two replicates only with 95 % ethanol (ethanol-soluble extractives), and two replicates were extracted first with water and then with ethanol (extractives). The content of water insoluble but ethanol soluble extractives was determined by subtracting the content of water-soluble extractives from the exhaustive extractives.

Carbohydrates (American Society for Testing and Materials - ASTM E1758-01 2020a) and lignin content (American Society for Testing and Materials - ASTM E1721-01 2020b) of the samples were determined. The sample was hydrolyzed, the acid insoluble residue was filtered out and the sugars and uronic acids were analyzed using ion chromatography (Dionex ICS-3000).

The acid soluble lignin was determined using UV–Vis spectrophotometer (HP 8452A UV–VIS spectrophotometer) at 205 nm. The ash content of the acid insoluble residue was determined for ash correction of the Klason lignin (acid insoluble lignin). The ash content was determined gravimetrically using a muffle furnace (Nabertherm L-240H1SN) at 550 °C according to the European Standard - EN14775 (2010).

# 3 Results and discussion

The chemical composition of fresh ($pine_{fresh}$ and $poplar_{fresh}$) and degraded ($pine_{SMS}$ and $poplar_{SMS}$) substrate is shown in Table 1. The major components of pine $_{fresh}$ and poplar $_{fresh}$ are within the common average range for softwood and hardwood (Sixta 2006; Sjöström 1993). The lignocellulosic composition given in Table 1 does not include the proportion of acetyl groups (Sixta 2006) and methyl esterified pectin (Hafrén et al. 2000), which would explain that the composition does not add up to 100 %.

The component fractions of *P. sylvestris* wood-based substrate changed notably after the fungal degradation compared to *P. tremula* substrate. This indicates that the *G. lucidum* strain degraded evenly the main components of *P. tremula* and, on the contrary, some of the components present in *P. sylvestris* were not consumed by the fungal strain. A determined fungal species within the *Ganoderma* genus may cause different decay processes depending on the hosting wood species and its chemical and physiological characteristics (i.e. Loyd et al. 2018). These results agree with the findings of Baietto and Wilson (2010), where a strain of *G. lucidum* showed lower decay performance when grown on softwood species, including *Pinus* sp., compared to hardwood species such as *Populus* sp.

The proportion of water-soluble extractives increased in both species after the degradation by *G. lucidum* (Figure 1A). *Ganoderma* spp. exudate enzymes and secondary metabolites during the delignification process (Zhou et al. 2013),

**Table 1:** Lignocellulose composition (% of dry weight) of the primary component groups of *Pinus sylvestris* and *Populus tremula* prior ($pine_{fresh}$ and $poplar_{fresh}$) and after ($pine_{SMS}$ and $poplar_{SMS}$) degradation by *Ganoderma lucidum*.

| Sample | Carbohydrates (%) | Lignin (%) | Extractives (%) | Uronic acids (%) | Ash (%) |
|---|---|---|---|---|---|
| Pine $_{fresh}$ | 59.1 | 28.8 | 5.4 | 2.6 | 0.3 |
| Pine $_{SMS}$ | 73.6 | 6.6 | 10.5 | 0.7 | 0.6 |
| Poplar $_{fresh}$ | 54.6 | 23.4 | 4.3 | 3.0 | 0.2 |
| Poplar $_{SMS}$ | 52.3 | 19.6 | 11.8 | 2.3 | 0.8 |

possibly increasing the extractive fraction in the degraded wood. However, the proportion of ethanol-soluble extractives decreased in pine$_{SMS}$ and increased in poplar$_{SMS}$. Previous studies found that Scots pine sawdust inhibited the mycelium growth and fruiting body production of *G. lucidum* in comparison to other tree species, such as *P. tremula* and *Betula* spp. (Cortina-Escribano et al. 2020a,b). The reason behind the fungal growth inhibition might be the presence of toxic compounds in softwood species (Mata et al. 2019). Earlier biodegradation analyses for wood indicate that fungal inhibition is likely to arise from the resin acids, pinosylvins and polyphenolic extractives from *P. sylvestris* (Harju et al. 2003), such as stilbenes (Venäläinen et al. 2004).

The carbohydrates are the highest fraction per dry matter in both tree species prior and after fungal degradation (Table 1). Surprisingly, the *P. sylvestris* wood-based substrate increased the fraction of carbohydrates markedly after wood degradation from 59.1 to 73.6 w-%, from which most of the fraction corresponds to glucan (Figure 1B).

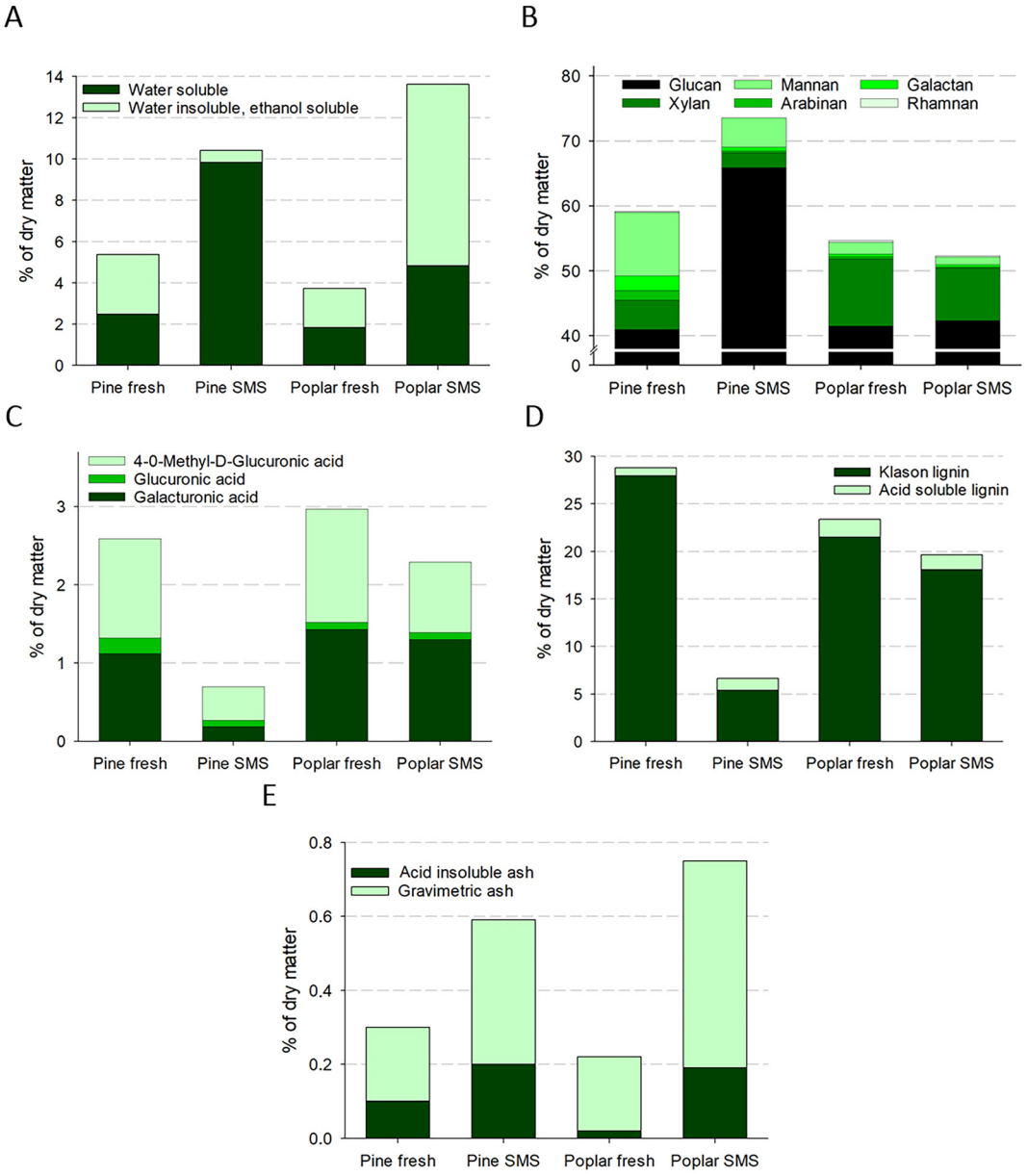


**Figure 1:** Chemical composition of *Pinus sylvestris* and *Populus tremula* fresh wood (pine$_{fresh}$ and poplar$_{fresh}$) and degraded wood samples (pine$_{SMS}$ and poplar$_{SMS}$). (A) Water and ethanol soluble (water soluble extractives subtracted) extractives. (B) Content of carbohydrates. (C) Content of uronic acids. (D) Content of Klason lignin and acid soluble lignin. (E) Content of acid insoluble ash and gravimetric ash.

The glucan fraction in *P. tremula* wood-based substrate remained around 40 % prior and after fungal degradation. A possible explanation of these results is the selective degradation ability of fungal species towards wood components. For example, *Pleurotus pulmonarius* consumed a lower proportion of glucan on *Picea abies* than in *Alnus glutinosa* (Chen et al. 2020).

The differences in glucan content in the substrates can also be explained by the formation and harvest of fruiting bodies. In a previous study, higher fruiting body yields were recorded from poplar$_{SMS}$ than from pine$_{SMS}$ (Cortina-Escribano et al. 2020b), therefore, it is possible that a fraction of the poplar glucans is transformed to *G. lucidum* fruiting bodies. *G. lucidum* produces branched polysaccharides, (1→3, 1→6)-β-glucans that could be seen in carbohydrate analyses (Chang and Lu 2004).

Pine$_{SMS}$ presented lower content of uronic acids as compared to poplar$_{SMS}$ (Figure 1C). Galacturonic and 4-0-methyl-D-glucuronic acids, which are part of pectin and xylan respectively, represent most of the uronic acid fraction prior and after wood degradation for both species. A small fraction of glucuronic acid has been also detected in all substrates. Mannuronic and guluronic acids were not present in any of the samples analyzed.

As reflected in the results, softwood contains higher content of lignin and galactan than hardwood (Figure 1D). This can be explained by the reaction of the tree to strain forces; compression wood is formed in softwood, whereas tension wood is formed in hardwood (Kandhola et al. 2017).

After fungal degradation, the substrate containing wood side-streams of *P. tremula* maintained the fraction of lignin at 19.6 %. On the contrary, the fungi degraded most of the lignin present in *P. sylvestris* wood-based substrate, in which case the lignin fraction was dropped to 6.6 % of the dry weight. Based on the results of this study, the wood degradation by *G. lucidum* significantly reduced the fraction of lignin in *P. sylvestris*. These results agree with Kuhar et al. (2018), who reported a reduction in the lignin fraction of *Pinus radiata* and *Populus nigra* after the cultivation of *G. lucidum*. The cultivation of *G. lucidum* could lead to novel biotechnological pre-treatment of wood side-streams of *P. sylvestris*, such as in the bleaching process or biomechanical pulping due to the successful delignification of the lignocellulose (Blanchette 1991; Kang et al. 2007). Further research analyzing the changes in lignin structure and other cell wall components (i.e. Loyd et al. 2018) in both *P. tremula* and *P. sylvestris* is needed.

The proportion of inorganic compounds in both wood species increased after the degradation by *G. lucidum* as an outcome of the reduced organic fractions (Figure 1E). Therefore, for the same volume of material, there is a greater weight of inorganic compounds in the degraded wood samples. The ash content in sound wood is often within 0.6–2.7 % (Sannigrahi et al. 2010), which aligns with the findings of this study.

# 4 Conclusions

Changes in the wood components were observed for *P. tremula* and *P. sylvestris* wood-based substrates after the cultivation of *G. lucidum*. Carbohydrates and lignin of *P. tremula* were consumed in even proportions during the cultivation of *G. lucidum*, allowing the production of fruiting bodies and fungal polysaccharides. The wood degradation by *G. lucidum* significantly reduced the fraction of lignin in *P. sylvestris* substrate. On the contrary, the carbohydrate proportion in *P. sylvestris* increased after degradation. Further research is needed to understand the changes in the lignin structures and in other cell wall components of both poplar and Scots pine wood.

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