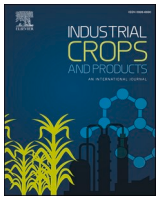
Industrial Crops & Products 223 (2025) 120284

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Mild thermal treatment assists fungal preprocessing of softwood sawdust for production of fermentable sugar

Feng Chen [a,b,1], Carlos Martín [c,d,2], Leif J. Jönsson [c,3], Madhavi Latha Gandla [c,4], Sarah J. Klausen [d,5], Luis A. Romero Soto [d,6], Shaojun Xiong [a,*,7]

[a] Swedish University of Agricultural Sciences, Department of Forest Biomaterials and Technology, Umeå, SE 901 83, Sweden
[b] Anhui Agricultural University, College of Horticulture, Hefei 230036, China
[c] Umeå University, Department of Chemistry, Umeå, SE 901 87, Sweden
[d] Inland Norway University of Applied Sciences, Department of Biotechnology, Hamar N-2317, Norway

ARTICLE INFO

Keywords:
Shiitake mushroom
Fungal pretreatment
Lignocellulose
Terpenes
Enzymatic saccharification
Biorefinery

ABSTRACT

Preheating with hot air at 85 – 125 °C was evaluated for its effectiveness in removing terpenes and terpenoids in softwood sawdust, thereby enhancing fungal preprocessing and subsequent saccharification of softwood-based mushroom substrates. Sawdust from pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) H. Karst.) was pre-heated prior to shiitake (*Lentinula edodes* (Berk.) Pegler) cultivation. Preheating removed up to 96 % of terpenes in pine- based substrates and up to 50 % in spruce-based substrates. Additionally, preheating decreased total terpenoids content in spruce by up to 78 %. For the pine-based substrate, the mild heating generally led to faster colonisation and improved mushroom yield, with the fastest mycelia colonisation and highest yield observed for 105 °C treatment. This temperature was associated with the lowest content of total terpenes and absence of major monoterpenes. The content of terpenes and terpenoids continued to decrease during cultivation, alongside fungal degradation of lignocellulose. As a result of more extensive lignin degradation, the enzymatic digestibility of cellulose was higher for spruce-based spent mushroom substrate than for pine-based one (up to 89 % vs. 49 % conversion). Enzymatic digestibility showed a negative correlation with the α-pinene content, and a positive correlation with increasing preheating temperatures.

1. Introduction

Studies have shown that the cultivated edible white-rot fungi can degrade selectively lignin and hemicellulose contained in the growing substrate (Lin et al., 2015; Wan and Li, 2012). After a harvest of mushroom fruit bodies, the resulting spent mushroom substrate (SMS) is enriched in cellulose, leading to an enhanced enzymatic digestibility compared with the initial mushroom substrate (IMS) (Chen et al., 2022a). The fermentable sugar may then be processed into bioethanol fuel. This allows for an effective production of fermentable sugar from cellulose in SMS without any further pretreatment. In previous studies, the enzymatic digestibility of cellulose in a birch-based substrate increased by nearly 4 times to 85 % (w/w), after a cultivation of shiitake mushroom (*Lentinula edodes*) (Chen et al., 2022b; Xiong et al., 2019). At the same time, a good yield of mushroom fruit bodies rich in non-meat protein was achieved. Exploration of SMS as a source of fermentable sugars would facilitate an integrated production of edible fungi and biofuels, which will benefit economically the mushroom industry and contribute to its sustainable development. Mushroom industry generates massive amounts of SMS that is today extensively considered as a waste

* Corresponding author.
E-mail address: Shaojun.Xiong@slu.se (S. Xiong).
[1] ORCID: 0000-0003-3369-7273
[2] ORCID: 0000-0002-4258-0512
[3] ORCID: 0000-0003-3866-0111
[4] ORCID: 0000-0003-2798-6298
[5] ORCID: 0009-0002-6466-5530
[6] ORCID: 0000-0001-7409-1943
[7] ORCID: 0000-0001-5700-9493

https://doi.org/10.1016/j.indcrop.2024.120284
Received 19 August 2024; Received in revised form 26 November 2024; Accepted 7 December 2024
Available online 10 December 2024

(Wei et al., 2020). The global production of shiitake alone generates annually up to 12 million tons dry mass, suggesting a large potential resource for biofuel production while maintaining an environmental friendliness.

Hardwood (e.g., oak, birch, beech, alder, etc.) is a common substrate for commercial shiitake mushroom production, either in the form of sawdust or wood chips, and is supplemented by crop-based by-products such as wheat bran serving as nitrogen additive (Royse et al., 2017). Softwoods, such as pine and spruce, are rarely used for production of e.g. shiitake mushroom fruit bodies. This is in contrast to the fact that most forest and wood resources in northern regions consist of coniferous tree species such as spruce and pine. For example, spruce and pine constitute about 80 % of the wood in Sweden (Fridman, 2023). Thus, extending the mushroom cultivation substrate range from hardwood to softwood is important. However, biomass from softwood is difficult to colonize for most white-rot fungi (Croan, 2004). The reasons are poorly understood, but higher contents of lignin and extractives, such as terpenes, in softwood can decrease its susceptibility to fungal growth (Couturier et al., 2015).

Terpenes and terpenoids are plant secondary metabolites and a large group of extractives that are present in most tree species (Brennan et al., 2021). They are mainly found in the resin of the heartwood and sapwood of conifer trees (softwood species), but a small fraction is contained in the sapwood of hardwood species (Back, 2002). Terpenes are hydrocarbons derived from isoprene (C_5H_8). Monoterpenes, such as α- and β-pinene, 3-carene, limonene, and myrcene, contain two isoprene units and are the simplest terpenes (Thomsett et al., 2016). Terpenoids are also composed of isoprene units, but they contain additional functional groups (Manninen at al., 2002). The composition terpenes varies both between and within species (Kopaczyk et al., 2020).

Terpenes and terpenoids, together with other extractives, have a protective function against plant pathogens and herbivores (Kopaczyk et al., 2020). They have antimicrobial properties and protect trees against attacks from microbes, including fungi (Valette et al., 2017) and bacteria (Muilu-Mäkelä et al., 2022). The inhibitory effects of terpenes (Croan, 2004) and terpenoids (Marsui et al., 2001) on fungal growth have previously been reported.

There are reports on using extractives-degrading fungi, like *Ophilostoma spp.*, to improve the susceptibility of conifer biomass to edible white-rot fungi (Croan, 2004). However, the effect of physiochemical pre-removal of some of the extractives, such as terpenes, on fungal growth has rarely been investigated. Since terpenes are volatile, e.g., monoterpenes comprising about 80 % of the volatile organic compounds (VOC) in softwood (Muilu-Mäkelä et al., 2022), devolatilization could be a strategy for reducing the content of terpenes and thereby potentially improve the susceptibility of softwood to the growth of white-rot fungi. Monoterpenes and many terpenoids have relatively low boiling points. Some of them have boiling points of around 150 °C, but they start to evaporate at lower temperatures (Wagner et al., 2020). Thus, it is not surprising that heating results in a decrease of the content of volatile extractives (Brito et al., 2008; Hyttinen et al., 2010; Poletto et al., 2012). Recently, studies showed that hot air (75 – 100 °C) pasteurization of substrate for growing mushrooms resulted in not only considerable energy savings, but also in effective mycelial growth and production of shiitake fruit bodies, compared with conventional autoclaving methods used globally (Wei et al., 2020). Thus, we hypothesise that heating softwood biomass with hot air, prior to mushroom cultivation, may reduce the content of terpenes and improve its susceptibility to colonisation by edible white-rot fungi.

During mushroom cultivation on a wood substrate, lignocellulose components are degraded by the growing white-rot fungus. A question to be addressed by this study is how removing terpenes by the preheating will impact the fungal degradation of lignin, hemicelluloses, and cellulose, and the subsequent enzymatic saccharification of SMS after mushroom cultivation. Answering this question will contribute to clarifying the importance of the removal of extractives' for achieving both a satisfactory mushroom growth and an efficient fungal preprocessing to decrease the recalcitrance of softwood for subsequent bioconversion. Thus, it is important to evaluate the composition and properties of SMS to assess its potential to be used as feedstock for biorefinery processes leading to ethanol and other bio-based products.

Using shiitake as a model mushroom and investigating the chemical changes in the preheated substrates, this study aims at evaluating how a preheating treatment affects the content of terpenes and terpenoids in softwood, and how that is reflected in mushroom cultivation, substrate degradation, and enzymatic saccharification of residual cellulose in the spent mushroom substrate.

2. Materials and methods

2.1. Materials and experimental settings

Wood sawdust from three tree species, namely white birch (*Betula pubescens Ehrh.)*, Scots pine (*Pinus sylvestris* L.) and Norway spruce (*Picea abies* (L.) H. Karst.) was used as major ingredients in preparation of mushroom substrates. The birch wood was harvested from a natural forest in Vännäs, Sweden and ground into sawdust-size particles. Sawdust of pine and spruce was collected from Sävar sawmill nearby Umeå, Sweden. The collected fresh sawdusts were immediately packed in airtight plastic bags and then kept in a freezer at −18 °C before further processing, to avoid any potential losses or contamination of volatile components. All sawdusts are from debarked stemwood consisting of both heartwood and sapwood fractions.

Sawdust from pine and spruce was subjected to preheating treatment following the experimental setup shown in Fig. 1. The preheating was performed using a hot-air dryer where three batches of either pine or spruce chips were heated at 85 °C, 105 °C and 125 °C, respectively, for 16 h. The three temperatures were selected so that the highest value would be slightly below the boiling point of monoterpenes and to benefit from existing knowledge and logistics from previous studies on hot-air pasteurization at 85 – 100 °C (Wei et al., 2020; Xiong et al., 2019). The choice of 16 h heating was referenced the way to determine the dry mass /moisture content of wet sawdust at 105 °C according to ISO standard (ISO 18134–3). The 16 h heating was also one of the durations previously tested for hot air pasteurisation. It was hypothesised that the hot air pasteurisation might remove inhibitory terpenes from the

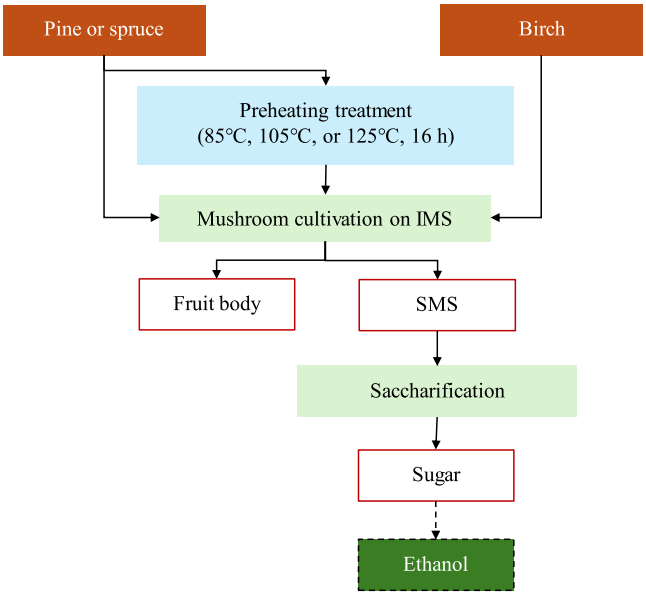


Fig. 1. Schematic representation of the experimental setup. Ethanol production (represented with dotted loop and lines) is not included in this study. IMS – initial mushroom substrate; SMS – spent mushroom substrate.

softwood by evaporation.

All wood sawdust materials, both with and without preheating, were further ground to a homogeneous particle size < 2.8 mm before being used as major substrate ingredients for shiitake cultivation. Birch sawdust, without preheating and adopted as a model substrate species in previous studies (Chen et al., 2022a,b), was used as a reference. The initial mushroom substrates (IMS), based on either spruce, pine or birch, were prepared by mixing the sawdust with wheat bran and barley grain at ratios of 80:10:10 (% of dry weight). The pH was adjusted to approximately 6.3–6.4 by adding $CaCO_3$ at 1 % of the dry mass (DM) of the initial substrate formulation. Water was added to obtain a moisture content of approximately 65 % (wet based, w/w). After a thorough mixing, the moisturised substrates were put in transparent polypropylene containers ("micro-boxes", 125 × 65 × 80 mm), resulting in 200 g wet mass in each container. The containers were sealed with a lid equipped with microporous filters for gas exchange and biofiltration. Each treatment included four replicates (micro-boxes). The C/N ratios of the final mixture were slightly different, 90.5, 93.0 and 94.7 for birch-, pine- and spruce-based substrates, respectively. The content of total nitrogen (TN) was determined, together with content of total carbon (TC), using an elemental analyzer-isotope ratio mass spectrometer (DeltaV, Thermo Fisher Scientific, Germany). Substrate pasteurisation was performed in an oven at 85 °C for 4 h to deactivate competing microbes. After that, the substrate containers were left overnight in the oven to cool down to room temperature before inoculation.

2.2. Inoculation and shiitake mushroom cultivation

Each substrate container was inoculated with 5 g of shiitake spawn M3790 (2.5 % of wet mass) under a laminar flow bench. After that, the containers were incubated under controlled conditions at around 21–22 °C in a dark room. When all six sides of the cultivation box were fully covered with mycelia, the colonisation period was considered complete. This "observation standard" of full colonisation was based on previous pilot study by observing intersected substrate block over time. When the fruit bodies emerged, the lid of the container was removed, the temperature was lowered to 18 °C, the humidity was increased to 90 % and the light (< 500 lx) was turned on in the climate cabinet until the harvest was completed.

The mushroom fruit bodies were harvested manually and freeze-dried and then weighed. The yield (g/kg) was defined as the weight of fresh fruit bodies per kg dry initial substrate, where the fresh weight was normalised to 90 % moisture content. Upon the harvest, the SMS from each entire substrate container was collected immediately, dried at 45 °C, milled to ≤ 0.5 mm and stored in airtight plastic bags before further analysis.

2.3. Analysis of substrate composition

The determination of extractives was performed by successive extractions with water and ethanol according to the standard NREL protocol (Sluiter et al., 2005). After the extraction, the extractive-free materials were air-dried until the dry matter content was above 90 %. Determination of structural components was performed using analytical acid hydrolysis (Sluiter et al., 2008). Klason lignin was determined gravimetrically after drying the filtration residue at 105 °C. Acid-soluble lignin was determined by measuring the absorbance of the hydrolysates at 240 nm using a spectrophotometer (Shimadzu, Kyoto, Japan). Glucose and xylose in the hydrolysates were analysed with HPLC (High-Performance Liquid Chromatography), using an Aminex HPX-87H column and an RI detector. Elution was performed with isocratic flow of a 5 mM aqueous solution of sulphuric acid. The flow rate was 0.6 mL/min and the column temperature was set to 55 °C.

2.4. Determination of terpenes and terpenoids

The contents of terpenes and total terpenoids in the IMS and the SMS were determined by Celignis Analytical (Limerick, Ireland; https://www.celignis.com). The terpenes were extracted with dichloromethane (DCM) after a brief sonication. It was followed by identification of monoterpenes in the extracts by gas chromatography (GC) coupled to flame ionization detection (FID), and total terpenoids as linalool equivalents.

2.5. Enzymatic saccharification of substrates

An analytical enzymatic saccharification approach was used for evaluating the susceptibility of the initial substrates and SMSs to saccharification by cellulases. An assay adapted to SMS saccharification and previously described by Xiong et al. (2019) was used. The biomass was suspended in 50 mM sodium citrate buffer (pH 5.2) at a 5 % (w/w) consistency. After that, the commercial enzyme preparation Cellic CTec2 (produced by Novozymes and commercialized from Sigma-Aldrich Chemie GmbH, Steinheim, Germany), a blend of cellulases, β-glucosidases and hemicellulases, was added at a loading of 100 CMCase units/g biomass, based on previously used dosage (Chen et al., 2022b). After adding the enzyme blend, the flasks containing the reaction mixture were incubated for 72 h in a Thermo Scientific compact digital waving rotator (Thermo Fischer Scientific, Waltham, MA, USA) placed in a Termaks B4115 incubator set at 45 °C and 100 rpm. At the end of the saccharification, the hydrolysate was separated from the residual solids by centrifugation, and a sample was diluted, filtered, and subjected to HPLC (Shimadzu, Kyoto, Japan) analysis for quantification of glucose. The enzymatic digestibility was calculated as the fraction of hydrolysed glucan in present.

2.6. Data analysis

All data presented in this study, except for mushroom yield and/or those stated otherwise, are based on dry weight including the percentage (% w/w). The statistical differences in relevant data between different treatments were assessed by one-way ANOVA followed by Post Hoc Multiple Comparisons (Duncan). A quadratic regression analysis was conducted followed by ANOVA *F*-test to assess the significance of the potential correlation between α-pinene and enzymatic digestibility. The SPSS statistical analysis software (IBM SPSS version 26.0) was adopted for the analyses.

3. Results and discussion

3.1. Content of terpenes and terpenoids in initial mushroom substrates (IMS)

A total of six monoterpenes were detected (Fig. 2). The content of total terpenes in untreated pine IMS (22.3 ± 1.3 μg/g biomass DM) was five-fold higher than the content in the untreated spruce and the birch IMS (4.0 ± 0.2 μg/g for both) (Table 1). The most abundant terpene detected in pine was α-pinene, the content of which (10.3 ± 0.5 μg/g) represented 46 % of the total terpene content in untreated IMS. The second most abundant terpene in pine was 3-carene (4.4 ± 0.4 μg/g), while the contents of β-pinene (2.4 ± 0.1 μg/g) and sabinene (above 2.3 ± 0.1 μg/g) were also important. In spruce and birch, 3-carene was the main terpene detected. These results are in good agreement with the reported terpene composition of these three wood species (Kopaczyk et al., 2020). The results also agree with the reports by Hyttinen et al. (2010) regarding the major terpenes emitted from heated Scots pine.

The content of monoterpenes in birch was higher than expected, considering that birch extractives are typically richer in higher terpenes, such as betulin and betulinic acid, than in monoterpenes (Smolander et al., 2006). However, it should be kept in mind, that different

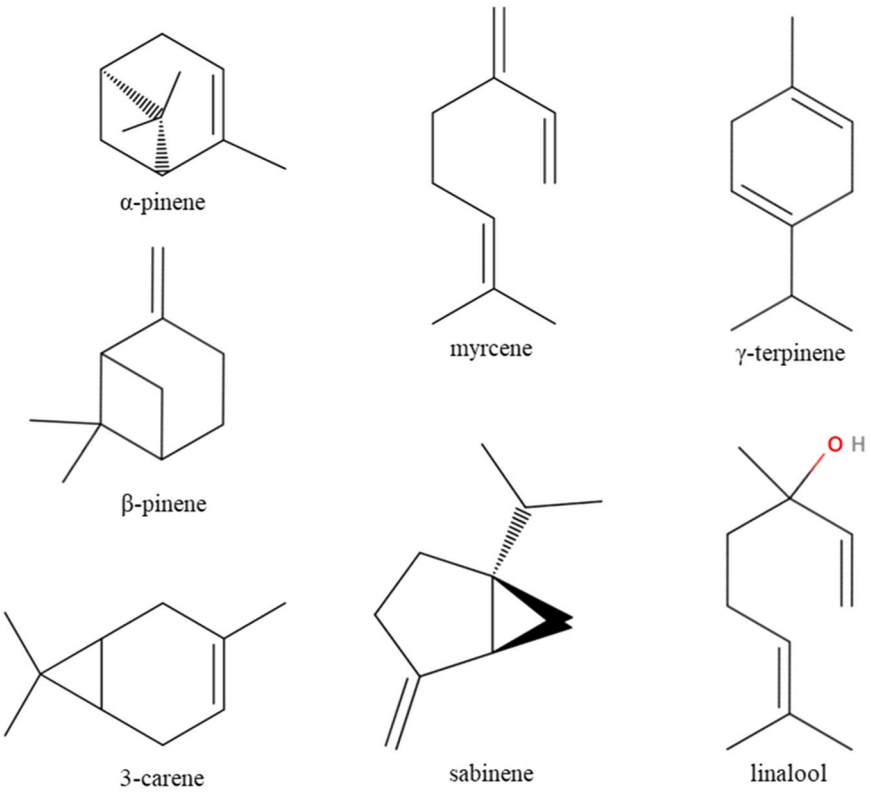


Fig. 2. Molecular structures of the terpenes and terpenoids detected in the initial mushroom substrates (IMS) and spent mushroom substrates (SMS).

Table 1
Contents of terpenes, terpenoids and the main lignocellulosic constituents in the untreated and preheated initial mushroom substrates (IMS). Standard deviations are shown in parentheses. Each determination was achieved by analysing a pooled sample from four replicated substrate blocks.

Components	Pine				Spruce				Birch
	Untreated	85 °C	105 °C	125 °C	Untreated	85 °C	105 °C	125 °C	Untreated
Terpenes, μg/g DM									
α-Pinene	10.3 (0.5)	3.9 (0.4)	ND	0.6 (0.1)	0.5 (<0.1)	0.6 (0.1)	ND	ND	ND
β-Pinene	2.4 (0.1)	2.4 (<0.1)	ND	0.5 (<0.1)	0.5 (<0.1)	ND	ND	ND	1.2 (<0.1)
3-Carene	4.4 (0.4)	3.4 (<0.1)	ND	1.0 (<0.1)	3.0 (0.2)	2.2 (0.2)	2.1 (0.2)	2.0 (0.1)	2.6 (0.1)
Myrcene	1.4 (0.1)	0.6 (<0.1)	0.9 (0.1)	0.6 (0.1)	ND	ND	ND	ND	ND
Sabinene	2.3 (<0.1)	1.7 (0.1)	ND	ND	ND	ND	ND	ND	ND
γ-Terpinene	0.6 (0.1)	3.2 (0.3)	ND	ND	ND	ND	ND	ND	0.2 (<0.1)
Total terpenes	22.3 (1.3)	15.2 (0.4)	0.9 (0.1)	2.7 (0.1)	4.0 (0.2)	2.8 (0.3)	2.1 (0.2)	2.0 (0.1)	4.0 (0.2)
Terpenoids									
Linalool, μg/g DM	2.1 (0.1)	4.3 (0.4)	2.4 (0.3)	1.1 (0.1)	0.1 (<0.1)	ND	ND	0.3 (<0.1)	0.2 (<0.1)
Total terpenoids, mg/g DM	2.2 (0.1)	2.1 (<0.1)	3.8 (0.2)	2.3 (0.1)	2.6 (0.2)	1.8 (0.1)	1.1 (0.1)	0.5 (<0.1)	2.2 (0.1)
Lignocellulosic constituents, % DM									
Glucan	38.1 (0.6)	35.9 (1.5)	33.7 (1.0)	34.3 (0.4)	36.1 (1.2)	36.2 (0,9)	34.6 (0.1)	36.4 (0.6)	29.1 (0.8)
Lignin[a]	26.7(0.3)	26.6 (0.3)	26.0 (0.2)	26.6 (0.5)	27.6 (0.1)	29.8 (0.0)	28.1 (0.0)	28.6 (0.2)	24.6 (0.4)
Mannan	9.0 (0,1)	8.6 (0,1)	8.0 (0.2)	7.7 (0.2)	8.2 (0.2)	8.4 (0.2)	7.5 (0.5)	8.3 (0.1)	1.3 (0.0)
Xylan	5.5 (0.1)	5.2 (0.2)	4.9 (0.2)	5.0 (0.2)	5.4 (0.1)	5.1 (0.3)	4.8 (0.2)	5.3 (0.2)	13.0 (0.5)
Arabinan	1.6 (0.0)	1.4 (0.0)	1.3 (0.0)	1.4 (0.0)	1.2 (0.0)	1.1 (0.1)	1.1 (0.0)	1.2 (0.1)	0.6 (0.0)
Galactan	1.3 (0.0)	1.3 (0.0)	1.2 (0.0)	1.2 (0.0)	1.3 (0.0)	1.3 (0.0)	1.1 (0.1)	1.3 (0.0)	1.2 (0.0)
Extractives	10.6 (0.6)	10.8 (0.4)	12.0 (0.7)	12.4 (0.6)	9.6 (0.5)	9.0 (0.3)	10.6 (0.1)	10.4 (0.4)	10.9 (0.3)

[a] Sum of acid-insoluble (Klason) lignin and acid-soluble lignin.

monoterpenes have been reported in birch exposed to the presence of conifers (Smolander et al., 2006), grown at high latitutudes (Haapanala et al., 2009), or grown in the vicinity of herbivore-damaged trees (Kopaczyk et al., 2020). In this study, the birch was harvested from a mixed forest of birch and conifers (pine and spruce) in Vännäs, northern Sweden. This may explain why a high content of terpenes was found in the birch substrate. The birch material was handled and stored generally in the same way as for softwoods, despite in different facilities, with caution not to expose potential contamination sources during the experiments. The possibility of cross contamination was small, although not absolutely excluded.

As expected, preheating decreased the total terpene content (Table 1) in both pine and spruce substrates. The decrease was larger for preheating at 105 °C and 125 °C than at 85 °C, and it was more remarkable for pine than for spruce. The largest decrease was observed for pine preheated at 105 °C, where the total terpenes content was reduced by 96 %, followed by 88 % at 125 °C. After preheating at 105 °C, almost all the identified terpenes, except myrcene, were completely removed from pine, while at 125 °C, some compounds, such as 3-carene and α- and β-pinene, still remained in the preheated

substrate. For spruce, around 50 % of the initial content of total terpenes was removed after preheating at either 105 or 125 °C, whereas 30 % was removed at 85 °C.

The content of total terpenoids was slightly higher for untreated spruce (2.6 ± 0.2 mg/g) than for untreated pine and birch (2.2 ± 0.1 mg/g) (Table 1). Preheating decreased total terpenoids for both softwood IMS. For spruce, the decrease was proportional to the temperature, i.e., 27 % of the initial content was removed at 85 °C, 58 % at 105 °C, and 81 % at 125 °C, whereas no clear trend was observed for pine.

3.2. Content of main lignocellulosic constituents in IMS

Glucan and lignin were the main constituents in all IMS samples (Table 1). The softwood-based untreated IMS contained more glucan (36.1 ± 1.2 for spriuce and 38.1 ± 0.6 % for pine) and total lignin (27.6 ± 0.1 for spruce and 26.7 ± 0.3 % for pine) than the birch-based one, which contained 29.1 ± 0.8 % glucan and 24.6 ± 0.4 % lignin. The softwood hemicelluloses were dominated by mannan followed by xylan and some contribution of arabinan and galactan. In birch, xylan was the dominant hemicellulosic polysaccharide (13.0 ± 0.5 %), but small fractions of mannan, galactan and arabinan were also detected. This is in accordance with the typical composition of hemicelluloses, which are dominated by galactoglucomannan in softwood and acetylated glucuronoxylan in hardwood with some content of arabinoglucuronoxylan and glucomannan in softwood and hardwood, respectively (Fengel and Wegener, 1989). Pine IMS had a higher content of total carbohydrates than the birch and spruce IMS. The ratio of total carbohydrates (glucan + mannan + xylan + arabinan + galactan) to lignin (C/L) in IMS was 2.1 for untreated pine, 2.0 for birch, and 1.9 for untreated spruce.

Preheating did not affect the lignin content in IMS (Table 1). However, some changes were observed for the content of carbohydrates and extractives. For pine-based IMS, an evident decrease of the content of glucan, mannan, and xylan occurred, while arabinan and galactan were less affected. For spruce-based IMS, the decrease was less remarkable. Concomitantly, there was a trend of increase in the content of total extractives from 10.6 ± 0.6–12.0 ± 0.7–12.4 ± 0.6 % in pine, and 9.6 ± 0.5–10.4 ± 0.4–10.6 ± 0.1 % in spruce. An explanation for these changes could be a partial thermal degradation or decomposition of some carbohydrate components, as it has previously been reported for thermal treatment of different wood species (Brito et al., 2008; Poletto et al., 2012). One can assume that the heating led to splitting of some glycosidic bonds in hemicelluloses, which made them prone to extraction leading to an apparently higher content of extractives. The decrease of the content of hemicelluloses resulted in a decreased carbohydrate-to-lignin ratio in the preheated substrates. It is interesting that the most notable decrease of the content of carbohydrates was observed at 105 °C.

3.3. Shiitake growth and fruit body production

Significant differences in mycelial growth were observed for the mushroom substrates (Fig. 3a). Generally, a quicker full colonisation by shiitake mycelium was achieved for pine substrates. Full colonisation of the pine-based substrate took 19.3 ± 0.5–22.8 ± 1.3 days, compared with 27.0 ± 0.1–32.5 ± 1.7 days for spruce-based substrate. The colonisation time for the untreated birch substrate used as reference was 24.3 ± 4.0 days, which is comparable to untreated pine, but significantly ($p < 0.05$) lower than that for untreated spruce-based substrate (32.5 ± 1.7 days). For the softwood-based substrates, the shortest time for full mycelial colonisation occurred in the pine substrate subjected to 105 °C preheating, whereas the longest time was observed for untreated spruce substrates. The preheating decreased the colonisation time significantly for the pine substrate treated at 105 °C and for spruce

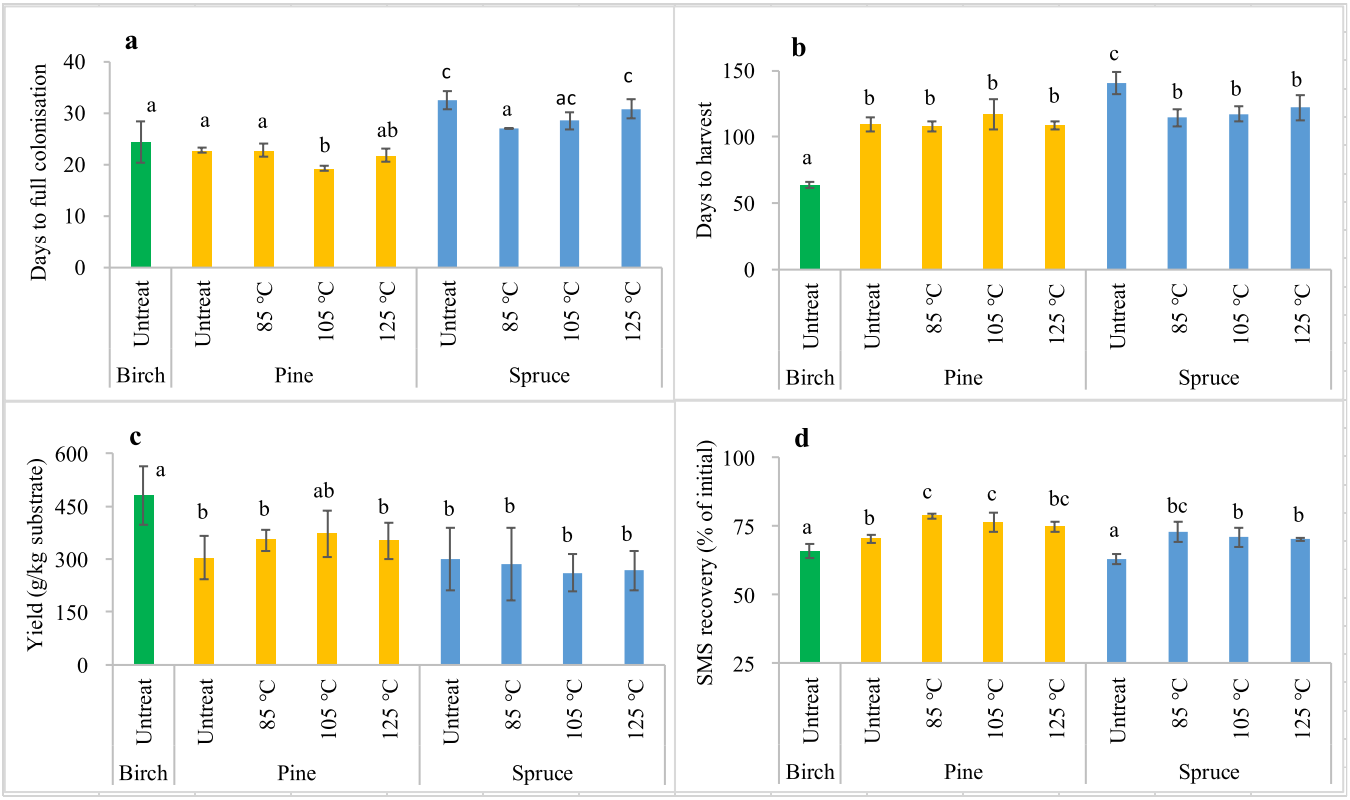


Fig. 3. Parameters of mushroom cultivation. a, time to full mycelial colonization of the substrate; b, time to harvest; c, fruit-body yield (g of fresh mushroom (90 % moisture) per kg dry initial mushroom substrate (IMS); and d, recovery of spent mushroom substrate (SMS). Each bar refers to mean value from at least four replicates. The error bars represent standard deviation. Different letters indicate significant differences at $p < 0.05$ between the treatments within each sub-figure.

substrates treated at 85–105 °C (Fig. 3a). Those preheating temperatures also led to the highest reduction of the content of terpenes' in the IMS (Table 1). This suggests that the speed of mycelial colonisation of preheated softwood substrates correlates well with the removal of terpenes during the treatment.

The fruit-bodies cultivated on the birch substrate were harvested after around 64.0 ± 2.3 days (Fig. 2b) of cultivation, which was consistent with that resulting from the same substrate composition in the previous study (66–68 days; Chen et al., 2022a). On the other hand, it took significantly longer cultivation time ($p < 0.05$) to get fruit bodies from all softwood-based substrates (108.8 ± 3.3 – 140.5 ± 8.2 days). The preheating treatment shortened the cultivation time required for harvesting fruit-bodies from spruce-based substrates, whereas it did not significantly affect that from pine-based substrates. Notably, all four pine-based substrates resulted in comparable lengths of cultivation time despite differences in preheating temperatures.

The yield of one flush of fresh fruit-bodies (containing 90 % water) is shown in Fig. 3c. The cultivation on the birch reference substrate achieved the highest yield, on average of 481 g fresh mushrooms (with 90 % water) per kg dry initial substrate (corresponding to 48.1 % biological efficiency). The yield harvested from the birch substrate was significantly ($p < 0.05$) higher than those most achieved from pine (302–371 g/kg) and spruce (261–300 g/kg) substrates. The shiitake yield from birch substrate in this study is within the range (437–651 g/kg) reported in previous studies with hardwood substrates (Chen et al., 2022a; Lin et al., 2015; Xiong et al., 2019). The preheating treatment slightly improved the yield of fruit bodies from the cultivation on pine-based substrates, but it did not exert any improving effect on the yields from spruce-based substrates. The yields of fruit-bodies from preheated pine substrates ranged from 350.7 ± 51.3–371.4 ± 66.1 g/kg, while it was 302.6 ± 61.3 g/kg for untreated pine substrate. In contrast, the corresponding yields in preheated spruce substrates were 260.7 ± 52.7–285.7 ± 103.3 g/kg, which was in general lower than the untreated substrate (300.0 ± 88.8 g/kg), although the differences were not statistically significant. It is noteworthy that the highest yield from pine substrates was associated with preheating at 105 °C where the lowest content of total terpenes was observed, and where no presence of α-pinene, sabinene, β-pinene, 3-carene, and γ-terpinene was detected. This result is in line with previous reports on antifungal activity of terpenes (Li et al., 2015). The antifungal activity of α-pinene and other terpenes and terpenoids has previously been associated with their ability to disrupt the cell membrane, due to their lipophilic nature and low molecular weight, causing cell death or inhibiting reproduction (Nazzaro et al., 2017). Other extractive compounds, such as resin acids, stilbenes, lignans, and di- and triterpenes, might also have contributed to the inhibition of shiitake growth since they are known to have antifungal activity (Ristinmaa et al., 2023). However, a thorough assessment of the inhibitory effect of each group of compounds is beyond the aim of the current study.

The recovery of SMS, quantified as the mass fraction of recovered material out of the initial substrate, displayed statistically significant differences ($p < 0.05$) between the different cultivation substrates (Fig. 3d). The lowest SMS recovery (64.8 ± 2.6 %) was observed for birch reference, which is in the same range as the values achieved in previous studies using birch as substrate (64.4–66.6 %; Chen et al., 2022a,b). The SMS recoveries of preheated softwood-based substrate were in the range 70.2 ± 0.6 – 78.7 ± 1.0 %, which is significantly higher ($p < 0.05$), than for birch-based substrate. The SMS recovery was comparable for cultivations on both softwood substrates, despite being slightly higher for pine than for spruce. Although the cultivation on the substrate treated at 85 °C resulted in a slightly higher SMS recovery, the effect of preheating temperature on the SMS recovery was not significant. The SMS recovery (Fig. 3d) was generally lower for the substrates, where the mushroom yield was higher (Fig. 3c), which is can be explained by a more extensive utilization of the lignocellulose constituents for substrates with better fungal growth and higher mushroom production.

3.4. Compositional change in the substrate after shiitake cultivation

3.4.1. Terpenes and terpenoids

In addition to the decrease of the content of terpenes and terpenoids observed during the preheating treatments (Table 1), the characterisation of SMS revealed that a further reduction in all untreated and most pre-heated substrates from the initial content occurred during fungal cultivation (Table 2).

The calculation of the relative mass change provides a good indication of the decreases for the different substrates. For preheated pine-based substrates, there was a general trend that the mass reductions of total terpenes were more remarkable for substrates with less removal during preheating. For example, the mass of total terpenes was reduced by ca. 64 % for 85 °C (Table 2), where the lowest removal by preheating was observed (Table 1), and by ca. 42 % for 125 °C, where a moderate removal during preheating was observed. For spruce, the trend was different, and removals between 92 % and 100 % were observed for all three preheated substrates. From these results, it might be inferred that the terpenes that were not removed from spruce during the preheating treatment are more volatile than those not removed from pine during preheating. Therefore, the terpenes remaining in the spruce-based substrates have a higher degree of removal during fungal cultivation than those remaining in the pine-based substrates. For non-treated substrates, the mass reduction of total terpenes was around 90 % for birch, 67 % for pine, and 57 % for spruce.

The mass reduction of total terpenoids in untreated softwood-based substrates was 51–55 %, while it was 32 % for birch (Table 2). For pine, the mass removal of terpenoids during cultivation was lower for the treated substrates than for the untreated one, and the removal decreased with the increase of the preheating temperature. Also, for spruce, the removal of terpenoids was much less for substrates treated at 85 – 105 °C than for untreated substrate (6.7–9.7 % vs. 51.5 %), whereas an increase of mass was detected for the substrate treated at 125°C. There was no clear trend correlating the heating temperature with the mass change.

The decrease of the contents of terpenes and terpenoids in the substrates during cultivation might not be fully explained by this study by evaporation. The evaporation would be one reason, but it is difficult to understand why substances that did not evaporate during preheating at 85 – 125 °C would have evaporated under cultivation conditions. Another explanation could be fungal degradation. Degradation of terpenes by white-rot fungi during bio-pretreatment of pine by *Ceriporiopsis subvermispora* has been reported (Vasco-Correa et al., 2019). However, no previous studies on degradation of terpenes by *L. edodes* have been found in the literature, and a specifically dedicated study would be required for a full clarification. Another issue to be clarified is why the content of some terpenes and terpenoids increased after the cultivation (Table 2 vs. Table 1). A deeper study of the particularities of the dynamics of fungal metabolism of terpenes would be required to elucidate this. Although the metabolism of terpenes and terpenoids in fungi has been investigated (González-Hernández et al., 2023), information relevant for *L. edodes* is scarce. Differences in the content of terpenes and terpenoids between the investigated wood species are clear: more individual terpenes were detected in pine than in birch and spruce substrates (Tables 1 and 2). The interaction between fungal metabolic activities and the wood species used in the substrate formulation needs further investigation.

3.4.2. Major changes in the lignocellulosic constituents of SMS

Fig. 4 illustrates major compositional changes of lignocellulose in the substrates after the mushroom cultivation. The changes were mostly resulting from lignocellulose degradation by shiitake. The changes resulted from a mass balance of the whole process, where the mass fraction of each constituent remaining in the SMS and its composition

Table 2
Contents of terpenes and terpenoids in spent mushroom substrate (SMS) and relative mass change compared with the initial mushroom substrate (IMS). The standard deviation are shown in parentheses. Each determination was achieved by analysing a pooled sample from four replicated substrate blocks.

	Pine				Spruce				Birch
	Untreat	85 °C	105 °C	125 °C	Untreat	85 °C	105 °C	125 °C	Untreat
Terpenes, μg/g DM									
α-Pinene	5.0 (0.3)	3.4 (0.3)	NA[a]	0.9 (<0.1)	0.7 (<0.1)	0.3 (<0.1)	ND	ND	ND
β-Pinene	1.8 (0.2)	1.6 (<0.1)	NA	1.2 (0.1)	1.1 (<0.1)	ND	ND	ND	ND
3-Carene	1.1 (0.1)	0.8 (0.1)	NA	ND	0.5 (<0.1)	ND	ND	0.2 (<0.1)	0.6 (<0.1)
Myrcene	0.5 (<0.1)	ND	NA	ND	ND	ND	ND	ND	ND
Sabinene	1.7 (0.1)	1.2 (<0.1)	NA	ND	0.4 (<0.1)	ND	ND	ND	ND
γ-Terpinene	ND	ND	NA	ND	ND	ND	ND	ND	ND
Total terpenes	10.0 (0.7)	7.0 (0.4)	NA	2.1 (0.1)	2.7 (<0.1)	0.3 (<0.1)	ND	0.2 (<0.1)	0.6 (<0.1)
Terpenoids									
Linalool, *μg/g DM*	ND	ND	ND	ND	0.2 (<0.1)	ND	ND	ND	ND
Total terpenoids, mg/g DM	1.4 (0.1)	1.5 (<0.1)	3.5 (0.2)	2.3 (0.1)	2.0 (<0.1)	2.3 (0.1)	1.4 (0.1)	1.1 (0.1)	2.3 (0.1)
Mass change[b], %									
Total terpenes	−66.8	−63.8	NA	−41.9	−57.4	−92.2	−100.0	−93.0	−90.3
Total terpenoids	−55.2	−43.8	−29.7	−25.3	−51.5	−6.7	−9.7	54.5	−32.2

[a] NA, data not available; ND, not detectable.
[b] Relative mass change (%) = (mass in SMS – mass in IMS)* 100/mass in IMS.

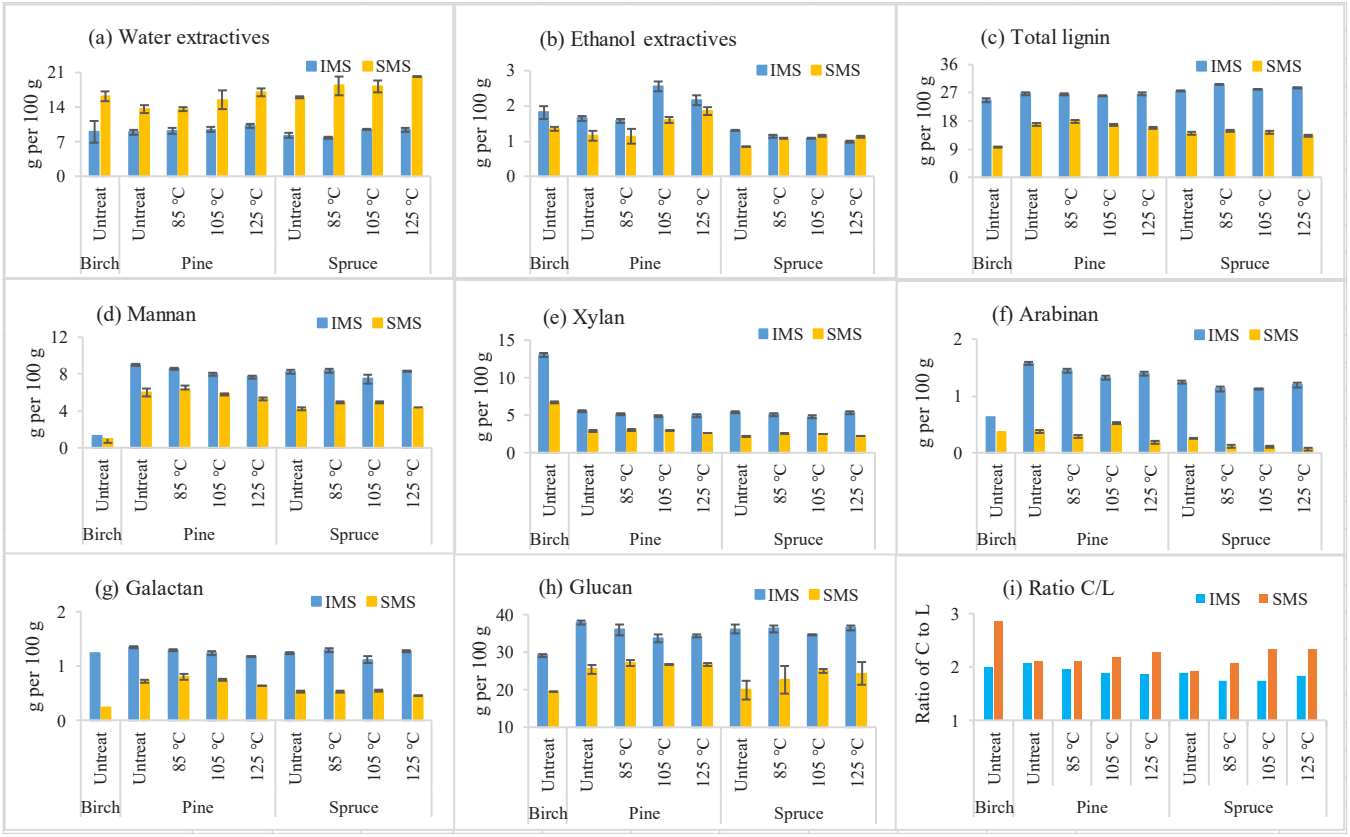


Fig. 4. Mass fractions (oven-dry based; mean±SD) of lignocellulosic components (a-h) remaining in spent mushroom substrates (SMS) and initial mushroom substrate (IMS). Each bar shows the mean from the analysis of a pooled sample of four replicates. The carbohydrate-to-lignin ratio (C/L) is also shown (i).

are compared with that in the IMS. As expected, the mass changes differed between the three different substrates and between different preheating treatments.

A change of the pattern of extractives was observed for all the materials after the cultivation. The SMS contained more water extractives than the IMS (Fig. 4a), whereas the IMS contained more ethanol extractives than the SMS (Fig. 4b). This difference is probably due to the presence of more polar low-molecule mass substance in SMS, coming either from the mycelium or from by-products formed during fungal degradation of wood polymers (Klausen et al., 2023). Being less polar than water, ethanol is better solvent for non-polar substances, which might have been more abundant in the IMS. Among the untreated substrates, spruce-based SMS had the highest increment of water extractives fraction (91.2 % of initial mass), followed by the birch reference (77.8 %) and pine-based SMS (51.6 %). This pattern matches well with that the longest cultivation period was observed for untreated spruce (Fig. 3b) and the highest mushroom yield was achieved with birch substrates (Fig. 3c). The mass fraction of water extractives was higher in the SMS of the preheated softwood than in SMS of untreated softwood. That is in rather good agreement with the pattern of lignin mass reduction and the carbohydrate-to-lignin ratio in SMSs, which are discussed in the following paragraphs. Although the decrease of the mass of

ethanol extractives was slightly higher for pine than for spruce, the differences in quantity were rather small for all the IMS and the SMS. The SMS from preheated substrates contained slightly more ethanol extractives than those from untreated ones, but the values generally remained below the levels observed for the IMS. For pine-based substrates, some temperature-correlated increase of the mass fraction of ethanol extractives was detected in the SMS subjected to preheating treatments, but that trend was not observed for spruce-based substrates.

The cultivation led to a decrease of the lignin mass fraction (Fig. 4c). The largest decrease was observed for the birch reference. Relatively lower decreases of the lignin mass fraction occurred for softwood, especially for the untreated substrates. Notably, relative mass reduction of lignin was larger in the SMS of treated softwood, except for the pine-based substrate treated at 85 °C, than in those of untreated softwood. The mass decrease for lignin is due to its degradation during cultivation of shiitake, which is a white-rot lignin-degrading fungus.

All the analyzed hemicellulosic polysaccharides in mushroom substrate exhibited a clear decrease in mass fraction after cultivation. The mass fraction of mannan, a component of galactoglucomannan of softwood hemicelluloses, decreased more for spruce (34–50 %) than for pine (26–34 %) (Fig. 4d). Even if there was some increase of mannan degradation proportionally to the preheating temperature, the decrease of mass fraction of mannan in both softwood materials was larger for untreated substrates than for the average of the treated ones (34 % vs. 27 % for pine, and 50 % vs. 41 % for spruce). The decrease of mass fraction of xylan was also more remarkable for spruce than for pine, with an average reduction of 53 % for spruce and 44 % for pine (Fig. 4e). The mass fraction of arabinan (Fig. 4f) and galactan (Fig. 4g) also decreased after cultivation. The mass fraction decrease was larger for arabinan (61–86 % for pine, 80–94 % for spruce) than for galactan (38–46 % for pine, and 51–64 % for spruce). A stronger decrease of arabinan and galactan was observed for spruce than for pine, which follows the same trend detected for mannan and xylan. For spruce, the decrease of arabinan was larger for treated substrates, but for pine no trend was evident. For galactan, no clear differences depending on the treatment conditions were detected for any of the softwood species. The larger decrease of the arabinan mass fraction is in accordance with results in previous literature showing that arabinose side-chain moieties are cleaved relatively easy compared with other hemicellulosic carbohydrates (Mäki-Arvela et al., 2011). That has been proven before for hydrothermal pretreatment under 150 °C (Ilanidis et al., 2021), and we are showing now that it is valid also for bio-pretreatment, which occurs under much milder conditions.

The glucan fraction also decreased during fungal cultivation (Fig. 4h). In general, the decrease was larger for spruce- (28–45 %) than for pine-based (21–33 %) substrates, and it was attenuated in SMS from preheated spruce (average 33 %) and pine SMS (average 22 %), compared with those untreated SMS (45 % and 33 % for spruce and pine, respectively). This trend was exactly the same as that observed for mannan (Fig. 4d). In pine- and spruce-based substrates, glucan is related not only to cellulose but also to hemicelluloses, considering that galactoglucomannans are the main constituents of softwood hemicellulose (Fengel and Wegener, 1989). Although the enzyme systems of white-rot fungi are able to degrade both cellulose and glucomannan (Liu et al., 2018), they may preferentially degrade first hemicelluloses and lignin and then cellulose (Qi et al., 2022). Consequently, considering the reported kinetics and the experimental trend, one can infer that the observed decrease of glucan content was probably mainly due to the degradation of glucomannan rather than cellulose. Another important conclusion is that preheating contributes to a better preservation of cellulose, which is an important feature if the enzymatic saccharification of the SMS is considered.

In summary, the mass changes (i.e. mass differences between IMS and SMS) for most components after cultivation in pine substrates were generally smaller than those observed for spruce-based ones (Fig. 4a, c-h), with the only exception being the ethanol extractives (Fig. 4b). The observed mass change pattern indicates that lignocellulose degradation by shiitake was generally stronger in spruce- than in pine-based substrates.

The combination of heat treatment and fungal activity resulted in an increase of the carbohydrate-to-lignin ratio (C/L) ratio (Fig. 4i) and glucan-to-lignin ratio (G/L; data not shown) in the SMS compared with the IMS. This might have resulted in improved susceptibility to enzymatic saccharification of the glucan contained in the SMS, even if the increment was not as high as that observed for the hardwood SMS. Notably, a larger increase in C/L (and G/L) was observed for spruce SMS than for pine SMS.

3.5. Enzymatic saccharification

Analytical enzymatic saccharification (Gandla et al., 2018) was applied to both SMS and IMS to investigate how the preheating treatments and the mushroom cultivation affected cellulose susceptibility to saccharifying enzymes. The saccharification of all the SMS resulted in higher enzymatic digestibility of glucan, i.e., cellulose, than that of the IMS (Fig. 5). This indicates that mushroom cultivation enhanced cellulose accessibility for the enzymes and thus the yield of sugars produced from the substrate. The increase of the enzymatic digestibility of glucan (Fig. 5) was detected for both untreated and preheated substrates.

Among the SMS of untreated materials, birch reference substrate resulted in the highest enzymatic digestibility (82 %), which was around four times higher than the value achieved for birch IMS (Fig. 5). Although this value is slightly lower than those reported in previous studies, e.g., 86.7 % (Xiong et al., 2019) and 92.8 % (Chen et al., 2022a), the trend is the same and the ability of shiitake cultivation to make cellulose susceptible to enzymatic saccharification is confirmed. The saccharification of the SMS of the two untreated softwood species resulted in a higher increase of the enzymatic digestibility for spruce-based substrate than for pine-based substrate. A nearly six-fold increase of the enzymatic digestibility, from 10.6 % in the IMS to 58.1 % in the SMS, was observed for spruce. For pine, the increase was, however, more moderate, from 24.6 % to 36.4 %. It is remarkable that the pine SMS resulted in a lower saccharification than the spruce SMS, despite glucan mass recovery was higher in the former than in the latter (Fig. 4h). The poorer enzymatic digestibility of the pine-based SMS might be related to the still higher content of total terpenes (10 μg/g) compared with that in spruce SMS (2.7 μg/g; Table 2).

The enzymatic saccharification of SMSs from substrates subjected to preheating treatments resulted in higher enzymatic digestibility values than those observed for SMSs from untreated substrates. The enzymatic digestibility increased proportionally with the heating temperatures (Fig. 5). Preheating of the substrate exerted a larger positive effect on the enzymatic digestibility for the spruce- (up to 89.2 %) than for the pine-based substrates (up to 48.9 %). This is consistent with the fact that spruce SMSs exhibited a larger degradation of lignin and hemicellulose (Fig. 4c-g) and contained less terpenes and terpenoids than pine SMS (Table 2). It is noteworthy that all the pine SMSs and the SMS of spruce treated at 85 ºC containing α-pinene (Table 2) displayed lower enzymatic digestibility than the SMS of spruce treated at 105 and 125 ºC, which did not contain α-pinene. This hints a plausible inverse correlation between the α-pinene content in the SMSs and their enzymatic digestibility. It has been shown that some terpenes inhibit β-glucosidase (Adamczyk et al., 2015) and that pine wood extractives inhibit hydrolase enzymes (Belt et al., 2018), but to our best knowledge this is the first report suggesting that α-pinene might inhibit the whole enzymatic saccharification process.

4. Conclusions

Preheating using hot air partially removed terpenes and terpenoids from softwood, but the efficiency varied, as more terpenes were removed from pine- while more terpenoids removed from spruce-based

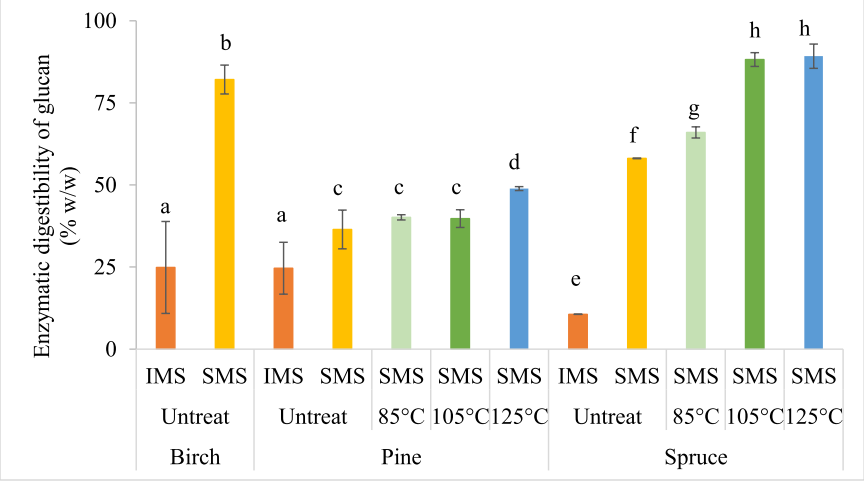


Fig. 5. Enzymatic digestibility of spent mushroom substrate (SMS) of pine and spruce submitted to different preheating treatments. Untreated initial mushroom substrate (IMS) and corresponding SMS are included. Birch IMS and SMS are included as references. Bars show mean values of three replicates with standard deviation. Data are dry-mass based and show significant differences between the treatments with different letters ($p < 0.05$).

substrate. Preheating of pine resulted in 16–22 % higher mushroom yield than for unpreheated one, but lower than for untreated birch. Terpenes and terpenoids decreased further in SMS after fungal cultivation, in parallel with degradation of main lignocellulosic components. A larger lignin degradation (49–53 % of IMS) and better enzymatic saccharification (66–89 % of glucan) was observed for spruce-based than for pine-based substrates (33–41 % and 40–49 %, respectively). The enzymatic saccharification correlated negatively with the α-pinene contents in SMS, but exhibited a positive correlation with the preheating temperature.

CRediT authorship contribution statement

Luis Soto: Investigation. **Sarah Klausen:** Investigation. **Shaojun Xiong:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Carlos Martín:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Feng Chen:** Writing – original draft, Methodology, Investigation, Data curation. **Madhavi Gandla:** Investigation. **Leif Jönsson:** Writing – review & editing, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Swedish State Department of Innovation through the BioInnovation program (VINNOVA 2016-05104, 2017-02705), Swedish Research Council (2022-02404, 2022-02760), Bio4Energy, and NordForsk (grant number 132066). Authors are very grateful to Johan Fredriksson at Sävar Sawmill, Norra Skogen, for providing the raw materials and assistances.

Data Availability

Data will be made available on request.

References

Adamczyk, S., Adamczyk, B., Kitunen, V., Smolander, A., 2015. Monoterpenes and higher terpenes may inhibit enzyme activities in boreal forest soil. Soil Biol. Biochem. 87, 59–66.
Back, E.L., 2002. A pattern of parenchyma and canal resin chemistry in hardwoods and softwoods: 1. Review for softwoods. Nord. Pulp Pap. Res. J. 17, 153–158.
Belt, T., Mollerup, F., Hänninen, T., Rautkari, L., 2018. Inhibitory effects of Scots pine heartwood extractives on enzymatic holocellulose hydrolysis by wood decaying fungi. Int. Biodeter. Biodegr. 132, 150–156.
Brennan, M., Hentges, D., Cosgun, S., Dumarcay, S., Colin, F., Gérardin, C., Gérardin, P., 2021. Intraspecific variability of quantity and chemical composition of ethanolic knotwood extracts along the stems of three industrially important softwood species: *Abies alba*, *Picea abies* and *Pseudotsuga menziesii*. Holzforschung 75, 168–179.
Brito, J.O., Silva, F.G., Leao, M.M., Almeida, G., 2008. Chemical composition changes in eucalyptus and pinus woods submitted to heat treatment. Bioresour. Technol. 99, 8545–8548.
Chen, F., Martín, C., Finell, M., Xiong, S., 2022a. Enabling efficient bioconversion of birch biomass by Lentinula edodes: regulatory roles of nitrogen and bark additions on mushroom production and cellulose saccharification. Biomass Convers. Bior. 12, 1217–1227.
Chen, F., Xiong, S., Gandla, M.L., Stagge, S., Martín, C., 2022b. Spent mushroom substrates for ethanol production – Effect of chemical and structural factors on enzymatic saccharification and ethanolic fermentation of *Lentinula edodes*-pretreated hardwood. Bioresour. Technol. 347, 126381.
Couturier, M., Navarro, D., Chevret, D., 2015. Enhanced degradation of softwood versus hardwood by the white-rot fungus *Pycnoporus coccineus*. Biotechnol. Biofuels 8, 216.
Croan, S., 2004. Conversion of conifer wastes into edible and medicinal mushrooms. For. Prod. J. 54, 68–76.
Fengel, D., Wegener, G. Wood – Chemistry, Ultrastructure, Reactions. 2nd Edition, Walter de Gruyter, Berlin, 1989.
Fridman, J., 2023. Data om skog och mark. In: Fridman, J., Danell, K. (Eds.), Sveriges skogar under 100 år. Riksskogstaxeringen 1923-2023. Riksskogtaxeringen, pp. 55–105.
Gandla, M.L., Martín, C., Jönsson, L.J., 2018. Analytical enzymatic saccharification of lignocellulosic biomass for conversion to biofuels and bio-based chemicals. Energies 11, 2936.
González-Hernández, R.A., Valdez-Cruz, N.A., Macías-Rubalcava, M.L., Trujillo-Roldán, M.A., 2023. Overview of fungal terpene synthases and their regulation. World J. Microbiol. Biotechnol. 39, 194.
Haapanala, S., Ekberg, A., Hakola, H., Tarvainen, V., Rinne, J., Hellen, H., Arneth, A., 2009. Mountain birch - potentially large source of sesquiterpenes into high latitude atmosphere. Biogeosc 6, 2709–2718.
Hyttinen, M., Masalin-Weijo, M., Kalliokoski, P., Pasanen, P., 2010. Comparison of VOC emissions between air-dried and heat-treated Norway spruce (*Picea abies*), Scots pine (*Pinus sylvesteris*) and European aspen (*Populus tremula*) wood. Atmos. Environ. 44, 5028–5033.
Ilanidis, D., Stagge, S., Jönsson, L.J., Martín, C., 2021. Hydrothermal pretreatment of wheat straw: effects of temperature and acidity on by-product formation and inhibition of enzymatic hydrolysis and ethanolic fermentation. Agronomy 11, 487.
Klausen, S.J., Falck-Ytter, A.B., Strætkvern, K.O., Martin, C., 2023. Evaluation of the extraction of bioactive compounds and the saccharification of cellulose as a route for the valorization of spent mushroom substrate. Molecules 28, 5140.
Kopaczyk, J.P., Warguł, J., Jelonek, J., 2020. The variability of terpenes in conifers under developmental and environmental stimuli. Environ. Exp. Bot. 180, 104197.

Li, J., Tian, X.R., Gao, Y.Q., Shang, S., Feng, J., Zhang, X., 2015. A value-added use of volatile turpentine: antifungal activity and QSAR study of β-pinene derivatives against three agricultural fungi. RSC Adv. 5, 66947–66955.

Lin, Y.Q., Ge, X.M., Liu, Z., Li, Y.B., 2015. Integration of Shiitake cultivation and solid-state anaerobic digestion for utilization of woody biomass. Bioresour. Technol. 182, 128–135.

Liu, B., Krishnaswamyreddy, S., Muraleedharan, M.N., Olson, Å., Broberg, A., Ståhlberg, J., Sandgren, M., 2018. Side-by-side biochemical comparison of two lytic polysaccharide monooxygenases from the white-rot fungus *Heterobasidion irregulare* on their activity against crystalline cellulose and glucomannan. PLOS ONE 13, e0203430.

Mäki-Arvela, P., Salmi, T., Holmbom, B., Willför, S., Murzin, D.Y., 2011. Synthesis of sugars by hydrolysis of hemicelluloses—A review. Chem. Rev. 111, 5638–5666.

Manninen, A.M., Tarhanen, S., Vuorinen, M., Kainulaine, P., 2002. Comparing the variation of needle and wood terpenoids in Scots pine provenances. J. Chem. Ecol. 28, 211–228.

Marsui, T., Matsushita, Y.I., Sugamoto, K., Ogawa. K., 2001. Mycelial growth inhibition of shiitake (*Lentinula edodes*) by several terpenoids isolated from sugi (*Cryptomeria japonica*) wood. Mokuzai Gakkaishi 47, 58-62. JAPAN WOOD RES SOC. (in Japanese with English abstract).

Muilu-Mäkelä, R., Aapola, U., Tienaho, J., Uusitalo, H., Sarjala, T., 2022. Antibacterial and oxidative stress-protective effects of five monoterpenes from softwood. Molecules 27, 3891.

Nazzaro, F., Fratianni, F., Coppola, R., De Feo, V., 2017. Essential oils and antifungal activity. Pharmaceuticals 10, 86.

Poletto, M., Zattera, A.J., Forte, M.M., Santana, R.M., 2012. Thermal decomposition of wood: influence of wood components and cellulose crystallite size. Bioresour. Technol. 109, 148–153.

Qi, J., Jia, L., Liang, Y., Luo, B., Zhao, R., Zhang, C., Wen, J., Zhou, Y., Fan, M., Xia, Y., 2022. Fungi's selectivity in the biodegradation of *Dendrocalamus sinicus* decayed by white and brown rot fungi. Ind. Crops Prod. 188, 115726.

Ristinmaa, A.S., Tafur Rangel, A., Idström, A., Valenzuela, S., Kerkhoven, E.J., Pope, P.B., Hasani, M., Larsbrink, J., 2023. Resin acids play key roles in shaping microbial communities during degradation of spruce bark. Nat. Commun. 14, 8171.

Royse, D.J., Baars, J., Tan, Q., 2017. Current Overview of Mushroom Production in the World. In: Zied, D.C., Pardo-Giménez, A. (Eds.), Edible and medicinal mushrooms: technology and applications. Wiley, New York, pp. 5–13.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2008. Determination of structural carbohydrates and lignin in biomass. In: National Renewable Energy Laboratory Technical Report NREL/TP-510-42618.

Sluiter, A., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., 2005. Determination of extractives in biomass. In: National Renewable Energy Laboratory technical report NREL/TP-510-42619.

Smolander, A., Ketola, R.A., Kotiaho, T., Kanerva, S., Suominen, K., Kitunen, V., 2006. Volatile monoterpenes in soil atmosphere under birch and conifers: Effects on soil N transformations. Soil Biol. Biochem. 38, 3436–3442.

Thomsett, M.R., Storr, T.E., Monaghan, O.R., Stockman, R.A., Howdle, S.M., 2016. Progress in the synthesis of sustainable polymers from terpenes and terpenoids. Green. Mater. 4, 115–134.

Valette, N., Perrot, T., Sormani, R., Gelhaye, E., Morel-Rouhier, M., 2017. Antifungal activities of wood extractives. Fungal Biol. Rev. 31, 113–123.

Vasco-Correa, J., Luo, X.L., Li, Y., Shah, A., 2019. Comparative study of changes in composition and structure during sequential fungal pretreatment of non-sterile lignocellulosic feedstocks. Ind. Crops Prod. 133, 383–394.

Wagner, Z., Bendová, M., Rotrekl, J., 2020. Thermochemical properties of selected terpenes. J. Solut. Chem. 49, 1137–1153.

Wan, C.X., Li, Y.B., 2012. Fungal pretreatment of lignocellulosic biomass. Biotechnol. Adv. 30, 1447–1457.

Wei, M., Xiong, S., Chen, F., Geladi, P., Eilertsen, L., Myronycheva, O., Lestander, T.A., Thyrel, M., 2020. Energy smart hot-air pasteurisation as effective as energy intense autoclaving for fungal preprocessing of lignocellulose feedstock for bioethanol fuel production. Renew. Energy 155, 237–247.

Xiong, S.J., Martin, C., Eilertsen, L., Wei, M.G., Myronycheva, O., Larsson, S.H., Lestander, T.A., Atterhem, L., Jönsson, L.J., 2019. Energy-efficient substrate pasteurisation for combined production of shiitake mushroom (*Lentinula edodes*) and bioethanol. Bioresour. Technol. 274, 65–72.