Proceedings IRG Annual Meeting (ISSN 2000-8953)

IRG/WP 17-20629

THE INTERNATIONAL RESEARCH GROUP ON WOOD PROTECTION

Section 2 **Test methodology and assessment**

Multivariate modeling of mould growth in relation to extractives in dried Scots pine sapwood

Olov Karlsson, Olena Myronycheva, Margot Sehlstedt-Persson, Micael Öhman, Dick Sandberg

Luleå University of Technology
Wood Science and Engineering
Forskargatan 1
SE-931 87, Skellefteå, Sweden

Poster paper prepared for the IRG48 Scientific Conference on Wood Protection
Ghent, Belgium
4-8 June 2017

IRG SECRETARIAT
Box 5609
SE-114 86 Stockholm
Sweden
www.irg-wp.com

Multivariate Modeling of Mould Growth in Relation to Extractives in Dried Scots Pine Sapwood

Olov Karlsson, Olena Myronycheva, Margot Sehlstedt-Persson, Micael Öhman, Dick Sandberg

Div. of Wood Sci. Eng., Luleå University of Technology, olov.karlsson@ltu.se, olena.myronycheva@ltu.se, margot.sehlstedt-persson@ltu.se, micael.ohman@ltu.se, dick.sandberg@ltu.se

ABSTRACT

Influence of extractives on mould growth on Scots pine sapwood dried in air or in kiln was studied. Boards were sprayed with water mixtures of spores of the fungal species *Penicillium* sp., *Aspergillus* sp., *Mucor* sp., *Paecilomyces* sp., *Trichoderma* sp., treated at a temperature of 22°C at 90% RH, classified into a percentage of covered area. Acetone and water extracts were isolated and analyzed for sugars, nitrogen, ash, resin/fatty acids, glycerol, and phenols. A multivariate Orthogonal Partial Least Squares (OPLS) regression model was developed to study relations between the extent of mould coverage of boards and chemical content. The model describes 51% variability in X and 69% in Y with prediction power of 55%. The results indicated that total acetone soluble extractives and sugars like glucose contributed to increased mould growth whereas fatty acids prevent mould growth.

Keywords: multivariate analysis, sapwood, fungi

1. INTRODUCTION

To be able to move towards a sustainable economy, further development in use of wood is of great importance. However, some challenges need to be addressed which could hinder a successful usage such as maintenance costs in multi-storey buildings, entrapment of moisture in passive houses, climate changes, use of less harmful preservative and surface treatments, etc. Outdoors, a fungal growth regarding moulds is mostly related to aesthetic issues, and increased maintenance cost whereas indoor released toxins could influence people's health. Mould growth on wooden boards can be linked to many factors such as moisture content and its variation due to climate conditions, temperature, sawing pattern, wood surface roughness (Johansson *et al.* 2012, Viitanen 1994). Often mould growth on sawn timber is observed near the edges which are close to where the living tree grow, rather than in middle parts of the flat side. Higher content of nutrients like sugars has been found in kiln-dried than in air-dried boards (Ahmed *et al.* 2013; Sehlstedt-Persson *et al.* 2011, Terziev & Boutelje 2007). This phenomenon is due to the migration of capillary water during the first parts of industrial drying, and that soluble compounds follow and accumulates on the wood surface. Hindrance of such flow during drying could explain the development of sticker marks sometimes found in moulded side-boards. The presence of other nutrients on wooden boards such as nitrogen containing or fatty extractives has been declared (Theander *et al.* 1993). The wood surface also contains other types of extractives, and some of them are antioxidants and could influence or even hinder mould growth. The purpose was to study the influence of the chemical composition of extractives on colonization of selected mould fungi on Scots pine sapwood.

2. EXPERIMENTAL METHODS

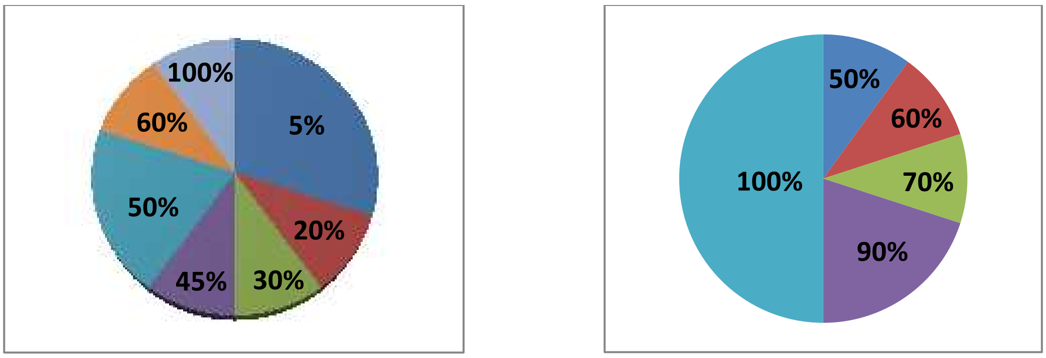
Sapwood surface of ten kiln or air dried Scots pine sideboards (220L x 100W x 25T mm) were studied. Air drying was performed indoor whereas boards from Scots pine were conventionally kiln dried for 44 hours to a moisture content of 16%. Mould growth of the surfaces was tested according to SP Method 4927:2013. A mixture of five mould fungi previously isolated from dried boards (Sehlstedt-Persson *et al.* 2011) was sprayed on the board surfaces. The spore suspension contained the following fungal species: *Penicillium* sp., *Aspergillus* sp., *Mucor* sp., *Paecilomyces* sp., *Trichoderma* sp. The samples were treated at a temperature of 22ºC at 90% RH in a chamber with a filter for the exchange of air. Visual inspection of the studied samples classified into a percentage of covered area used as evaluation (Fig. 1). The acetone solubles were isolated by extraction of milled wood samples with acetone using Soxhlet apparatus followed by drying in air. Ash content was determined gravimetrically. Sugars in water extracts from milled wood were analyzed using HPLC (Karlsson *et al.* 2012). Resin acids, fatty acids, and glycerol were analyzed after trimethylsilylation (BSTFA/TMSCl 2:1) at 70ºC for 20 minutes of dried acetone solubles with GS/MS (SUPELCO SLB-5 MS) using 1-methylnaphtalene as an internal standard. Folin-Ciocalteau (FC) was used to study the presence of phenols in acetone solubles. The amount of nitrogen was determined by the Kjeldahl method. Statistical analyses were performed using software SPSS 21 and SIMCA 14.

Figure 1: Surface view of air and kiln dried Scots pine sapwood boards after mold test

3. RESULTS AND DISCUSSION

Mould growth and extractives in sapwood surface layer

Moulds covering wood boards dried under industrial kiln conditions and in the air could be seen in Fig. 1. Totally ten boards of each drying type were analyzed and on average 86% of the surface of kiln dried samples was covered with mould compared with air dried in which 37% of the surface was covered. The variability of mould covered area was relatively large within the air dried as well as within the kiln dried boards (Fig. 2).



a) Air dried b) Kiln dried

Figure 2: Variability of the area coverage of mould (%) on sapwood of pine boards dried in (a) air or in (b) an industrial kiln

It could be seen that mould growth in about 1/3 of the air dried boards was very low (5%), but still, boards with high mould growth was observed (Fig. 2). Within kiln dried boards half of the analyzed samples were totally covered with mould (100%). Mould fungi are a primary colonizer usually and interact with the outermost surface of the wood as could be seen in Fig. 3, where hyphae are mostly situated on the wood surface.

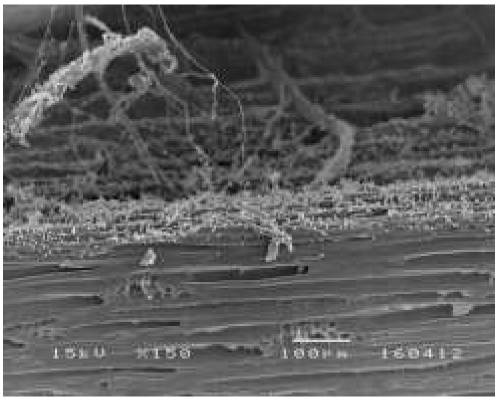


Figure 3: Scanning electron micrograph of mould growth on a kiln-dried wood surface

However, as can be seen on the boards, the growth is uneven and very much related to the spatial distribution on the wood surfaces with a sometimes quite irregular pattern (Fig. 2). The irregular pattern of mould growth has been proposed to be related to the presence and distribution of nutrient and sugars on the wood surface (Sehlstedt-Persson *et al.* 2011, Terziev & Boutelje 2007). This study also focuses on whether the extent of mould growth could be related to the presence of also other extractives in the surface area. In Table 1, content of various extractives isolated from the surface area of air- and kiln-dried boards is presented. It could be seen that higher content of the sugars: glucose, fructose, and sucrose, was found in kiln dried boards than in the ones dried in air. Nitrogen content includes compounds like proteins and could work as a nutrient for mould (Theander *et al.* 1993). They were also higher in kiln dried boards than in air dried ones (Table 1). Variability of data among these samples was large which makes it difficult to draw definite conclusions regarding the role of these nutrients in promoting mould growth and analysis by using multivariate tools was done (see below).

Table 1: Extractives content in the surface of dried boards of sapwood of Scots pine

Analysed compounds	**Air dried [%]**	**Kiln dried [%]**
Sucrose	0.086	0.416
Glucose	0.160	0.805
Fructose	0.132	0.603
Nitrogen	0.149	0.267
Aceton solubles	3.594	5.707
Palmitic acid	0.045	0.024
Oleic acid	0.360	0.073
Linoleic acid	0.068	0.020
Stearic acid	0.029	0.033
Pimaric Acid	0.158	0.194
Isopimaric acid	0.105	0.149
Dehydroabietic acid	0.437	0.455
Abietic acid	0.081	0.085
Phenols	0.044	0.081
Glycerol	0.043	0.082
Ash	0.329	0.906

Spread in data could be due to the formation of coloured Maillard and caramelization products (Theander *et al.* 1993), but also to the spatial distribution of nutrients as mentioned above and that compounds might migrate towards the wood surface to various extent during drying. The content of acetone soluble extractives was found to be significantly higher in the kiln- than in air-dried boards (Table 1). Fatty compounds are known to constitute the main part of isolated acetone-soluble extractives in the sapwood of Scots pine and could act as a nutrient and influence fungal growth. Fatty acids have been reported to have antifungal properties (Tumlinson & Engelberth 2008) and they were found in lesser amounts in the kiln- than in the air-dried boards (Table 1). The lower content in the kiln dried boards could be due to a faster degradation/evaporation at higher drying temperature during the kiln than under air-drying.

Resin acids were similar or somewhat higher in the kiln dried boards (Table 1). Phenols such as pinosylvins mostly found in the heartwood of Scots pine have antioxidant properties and may hinder fungal colonization. Small amounts of phenols were found in the sapwood of dried boards and data indicated that they might be higher in kiln-dried boards than in air-dried boards.

Multivariate analysis

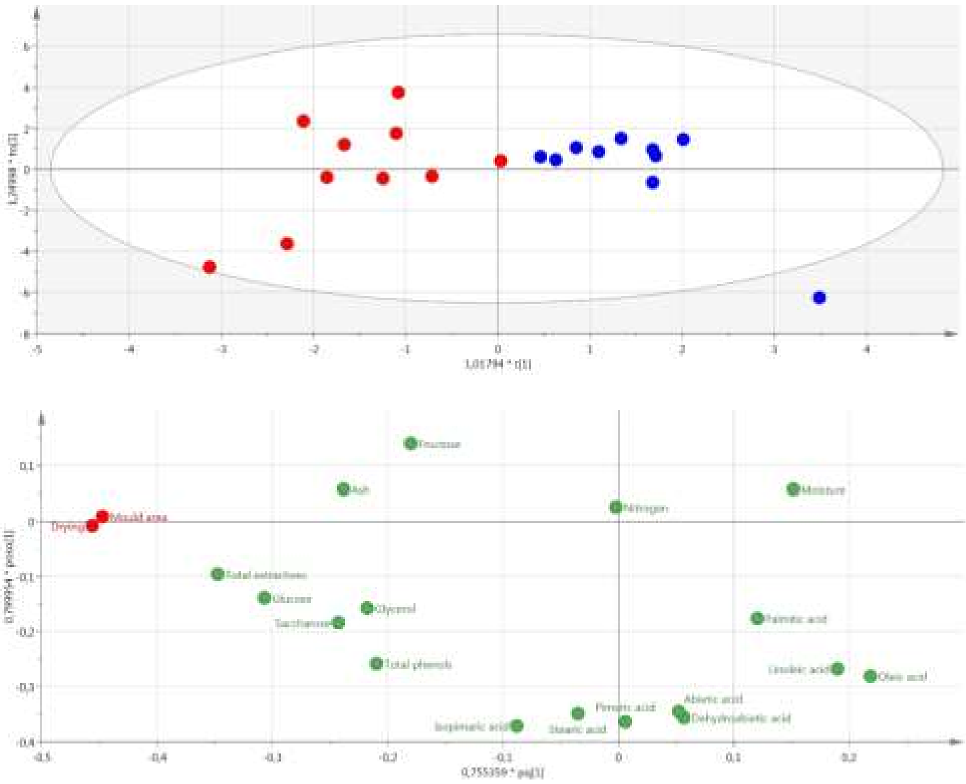


Figure 5: Score (upper) and loading (lower) plot of OPLS model from kiln-dried (red) and air-dried (blue) boards

In such a complex matrix of parameters, the multivariate analysis is a tool to study how each of the factors interacts with each other. An Orthogonal Partial Least Squares (OPLS) regression model based on the content of sugars, acetone-soluble compounds, moisture content, resin/fatty acids, phenols, ash, nitrogen, mould coverage was developed. This type of the model allowed us to separate systematic variation in measured chemical compounds and improve the predictive power of the Partial Least Squared regression. In our case, the *Drying type* and *Mould area* were selected as Y (responses), when *Glycerol, Palmitic, Oleic, Linoleic, Stearic, Pimaric, Isopimaric, Dehydroabietic, Abietic acids, Total Extractives, Total phenols, Nitrogen, Ash, Moisture content, Saccharose, Glucose, Fructose* were defined as X (variables). The developed OPLS model has two components, defining one predictive and one orthogonal component that covers 33% systematic variability in X variables. The model describes 51% variability in X and 69% in Y with prediction power of 55%. Fig. 5 shows the score and loading plot from the analysis. The analysis confirm that *Drying type* and *Mould area* strongly related to each other based on their presence in the loading plot (Fig. 5). It could also be seen that *Total extractives* content and other variables such as *Glucose* are situated quite close to *Mould area* in the loading plot whereas others like *Nitrogen* seemed not to contribute to the developed model. A negative influence on mould area is indicated for fatty acids such as for *Oleic acid* (Fig. 5).

4. CONCLUSIONS

To reach deeper in understanding on how mould grows on dried pine sapwood surfaces probably also other characterization parameters such as spatial distribution has to be included to obtain a more valid model. Furthermore, growth conditions of moulds depend on the species and the microclimate on the wood surface and its physical properties.

5. REFERENCES

Ahmed, S. A., Sehlstedt-Persson, M. & Morén, T. (2013). Development of a new rapid method for mould testing in a climate chamber: preliminary tests. Eur J Wood Wood Prod, 71(4), 451–461.

Johansson, P., Ekstrand-Tobin, A., Svensson, T. & Bok, G. (2012). Laboratory study to determine the critical moisture level for mould growth on building materials. Int Biodeter Biodegr, 73, 23–32.

Karlsson, O., Yang, Q., Sehlstedt-Persson, M. & Morén, T. (2012). Heat treatments of high temperature dried Norway spruce boards: Saccharides and furfurals in sapwood surfaces. BioResources 7(2), 2284–2299.

Sehlstedt-Persson, M., Karlsson, O., Wamming, T. & Morèn, T. (2011). Mold Growth on Sapwood Boards Exposed Outdoors: The Impact of Wood Drying. Forest Prod J, 61(2), 170–179.

Terziev, N. & Boutelje, J. (2007). Effect of Felling Time and Kiln-Drying on Color and Susceptibility of Wood to Mold and Fungal Stain During an Above-Ground Field Test. Wood Fiber Sci, 30(4), 360–367.

Theander, O., Bjurman, J., & Boutelje, J. B. (1993). Increase in the content of low-molecular carbohydrates at lumber surfaces during drying and correlations with nitrogen content, yellowing and mould growth. Wood Sci Technol, 27(5), 381–389.

Tumlinson, J.H., Engelberth, J., 2008. Fatty Acid-Derived Signals that Induce or Regulate Plant Defenses Against Herbivory, in: Schaller, A. (Ed.), Induced Plant Resistance to Herbivory. Springer Netherlands, Dordrecht, pp. 389–407.

Viitanen, H. (1994). Factors affecting the development of biodeterioration in wooden constructions. Mater Struc, 27(8), 483–493.