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INCREASED RESISTANCE OF THERMALLY MODIFIED NORWAY SPRUCE TIMBER (TMT) AGAINST BROWN ROT DECAY BY *OLIGOPORUS PLACENTA* – STUDY ON THE MODE OF PROTECTIVE ACTION

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ABSTRACT

The reasons for the increased decay resistance of thermally modified timber (TMT) are not yet fully understood and were therefore exemplarily examined for thermally modified spruce against *Oligoporus placenta*. Spruce specimens heat treated at 200 and 220°C were successively extracted and additionally treated with 2%-KOH solution to modify the moisture sorption properties. The extracts were surveyed regarding their inhibitory effect on fungal growth, while the extracted specimens were tested against *O. placenta* to determine the decay resistance. Furthermore, maximum swelling and equilibrium moisture content (EMC) were determined. The decrease in swelling and EMC as well as the increased decay resistance of TMT were not affected by extraction and further on, none of the extracts revealed an inhibitory effect. In contrast, the alkali treatment provoked enhanced moisture sorption of TMT specimens and lead to increased fungal decay at the same time. Thus it is assumed, that the reduced moisture sorption of TMT is mainly responsible for the increased decay resistance.

KEY WORDS: Norway spruce, extraction, fungal decay, heat treatment, KOH treatment, moisture sorption, swelling properties

INTRODUCTION

The ability of thermal modification processes to improve the resistance against fungal decay of wooden products was intensively investigated and promoted in recent years (Brischke and Rapp 2004, Syrjänen and Kangas 2000, Boonstra and Tjeerdsma 2006, Del Menezzi

and Tomaselli 2006). On account of the increased durability of thermally modified timber (TMT) compared to untreated material, its application above ground in European Hazard Class 3 (EHC 3, EN 335-1, 1992) is recommended and established (Jämsä and Viitaniemi 1998, Wienhaus 1999). This improvement in biological durability is a result of the impact of heat that modifies the cell wall structure (Kollmann and Fengel 1965, Leithoff and Peek 1998) and therefore it is not exclusively related to a specific industrial heat treatment process (Welzbacher and Rapp 2007).

However, possible reasons for the increased biological durability of TMT are discussed controversially, and can be outlined in four conceivable hypotheses:

1) Formation of extractives during the thermal decomposition that act as biocides (*i.a.* Kamdem et al. 2000, Mazela et al. 2003),
2) Reduction of water sorption, which limits the growth and enzymatic activity of wood decay fungi (*i.a.* Vernois 2001, Boonstra and Tjeerdsma 2006) coming along with a reduced percentage of micro voids in the cell wall (Junghans et al. 2005) that restricts the accessibility of wood degrading agents (Schwarze and Spycher 2005),
3) Rearrangement of the carbohydrate- and lignin-structure that leads to a non-recognition by enzymes released by wood-destroying fungi (*i.a.* Hakkou et al. 2005, Paul et al. 2006),
4) Degradation and removal of the nutritive source (mainly hemicelluloses) for fungal decay (*i.a* Militz 2002, Weiland and Guyonnet 2003).

The hypotheses 3) and 4) have been addressed frequently in the recent past and are regarded as potential approaches to explain the changes in biological durability (Weiland and Guyonnet 2003, Boonstra and Tjeerdsma 2006). The present study contributes to the discussion about the reasons for the increased durability of TMT by examining potential approaches with particular consideration of hypothesis 1) and 2).

For this purpose, the inhibitory effect of extracts from TMT on fungal growth as well as the resistance against fungal decay of extracted heat treated spruce specimens was examined. Furthermore, the water sorption properties of heat treated and extracted specimens were changed by alkali treatment and investigated regarding their contribution to improved fungal resistance.

MATERIAL AND METHODS

Preparation of wood specimens

Axially matched specimens of 5x20x20 mm (long. x rad. x tang.) were cut from 16 different stakes of Norway spruce (*Picea abies* Karst.) and used in all tests. 192 spruce sapwood specimens were submitted to two different heat treatments, in groups of 6 tightly wrapped in aluminum foil to minimize oxidation processes. After oven drying at 103°C, the heat treatments were carried out in a drying oven at 200°C and 220°C for 3 h. The decrease in mass caused by gasification of wood substance during the thermal modification process was used as a measure of the heat treatment intensity. It was determined by weighing the oven-dried specimens before and after the heat treatment to the nearest 0.001 g.

Pine sapwood (*Pinus sylvestris* L.) specimens of 5x20x20 mm (long. x rad. x tang.) were used in addition as controls and virulence samples in biological laboratory tests.

Extraction of wood specimens

Heat treated und untreated spruce specimens were extracted successively with petroleum ether, acetone/water (9:1), and methanol/water (4:1). In each case, one wood specimen and 20 ml extracting agent were filled in a test tube (Greiner, 30 mm diameter/ 115 mm height) and placed in an ultrasonic bath (Bandelin Sonorex Super RK102H) for the first extraction phase of 45 minutes at 20°C. Afterwards the test tubes were put in an overhead shaker with a rotary frequency of 80 min^{-1} for 15 hours at 20°C. The different extractive solutions were separated and stored deep frozen at –20°C for further utilization. The mass loss by extraction of each specimen was calculated as a percentage of the initial dry mass by oven drying the specimens at 103°C and weighing to the nearest 0.001 g.

Furthermore, unextracted and successively extracted specimens were alkali-treated with a 2% solution of caustic potash (KOH) at 100°C for 4 hours and afterwards kept immersed in the particular KOH solution for 18 hours at 20°C. The pH-value of KOH treated specimens either remained non-neutralized or it was neutralized with a solution of 8% oxalic acid ($C_2H_2O_4$) or by elution with deionised water. In the same way, the KOH solution used for alkali treatment was either kept non-neutralized or neutralized with a solution of 8-percent oxalic acid for further utilization in fungal growth tests.

Swelling properties and equilibrium moisture content

Maximum swelling of the specimens was determined after water vacuum-pressure impregnation (vacuum: 2 kPa, 30 min; pressure: 800 kPa, 30 min) and subsequent water storage at 60°C for 24 h to determine the change in dimensional stability caused by the heat treatment as well as by the extraction. Furthermore, maximum swelling of extracted and unextracted specimens was also determined after treatment with KOH solution at 100°C for 4 hours and subsequent soaking in the KOH solution for 18 hours at 20°C. In both cases, the swelling was determined by measuring the oven dry and wet dimension of the specimens according to EN 52 184 (1979).

The equilibrium moisture content (EMC) of n = 26 specimens for each combination of heat treatment and extraction/ KOH-treatment was determined at 20°C/75%RH. Therefore the specimens were placed in desiccators at 20°C over saturated salt solution (NaCl, RH of 75%) until constant mass was reached (48 days). To assure an equal climate inside the desiccators and to prevent growth of mould, each of the desiccators was equipped with an axial fan, placed between the salt solution and the specimens, securing a constant air stream of 1.3 m/s. EMC at 20°C and 75%RH was calculated according to DIN 52 183 (1977).

Fungal growth tests

The extracts of untreated and heat treated wood specimens were tested in terms of their inhibitory effect on fungal growth. Therefore the extracts of eight wood specimens from each successive extraction were filled in a volumetric flask of 750 ml and topped up with 160 ml of the particular extractive solvent used. The solved extracts were then applied to filter paper strips of 40 x10 mm and 0.046 g, which were cut from filter paper circles of 125 mm diameter, Type 595, Schleicher & Schüll, Dassel, Germany. Based on the initial extractive content of wood samples, expressed as mass loss by extraction, a basic (c=1), a twofold (c=2) and a threefold (c=3) concentration of extracts was applied on filter paper strips to examine the inhibitory effect of the extracts in dependency of their concentration.

The filter paper strips were then placed on malt agar in the centre of the Petri dishes (90 mm

diameter) at 22°C and 70%RH. Cultures of *Oligoporus placenta* [*Oligoporus placenta* var. Monticula = (Fr.) Gilbertson et Ryv. FPRL 280 BAM, 8/1997] were inoculated at the periphery of the Petri dishes in a distance of 40 mm to the broad faces of the filter paper strips. Untreated paper strips were used as controls. Furthermore, the pure extraction solvents (petroleum ether, acetone, methanol, KOH, oxalic acid, deionised water) were also tested on filter paper strips in a basic (c=1) and a twofold (c=2) concentration.

When the untreated control-strips were completely covered by the mycelium, the filter paper strips were evaluated with respect to the inhibitory effect of extracts on fungal growth. Therefore the particular area of filter paper strips, which was actually covered by mycelium *(covered area$_{actual}$)* as well as the amount of mycelium covered space of filter paper controls *(covered area$_{potential}$)* were determined and used to calculate the relative inhibitory effect *(inhibition$_{relative}$)* of the extracts (Equation 1).

Equation 1: Calculation of the relative inhibition of the extracts

$$inhibition_{relative} = (1 - \frac{\text{covered area}_{\text{actual}}}{\text{covered area}_{\text{potential}}}) * 100\ [\%]$$

Four replicates per concentration (c=1, c=2, and c=3) for each extractive agent were tested.

Fungal decay tests

In addition to the tests of the inhibitory effect of extracts on fungal growth, basidiomycete tests with *Oligoporus placenta* according to a modified EN 113 (1996) were performed to determine the resistance of differently heat treated and extracted specimens against wood destroying fungi. *O. placenta* was used in this study, because it is regarded as being the most critical fungus for TMT in laboratory tests (*c.f.* Welzbacher and Rapp 2007). 12 replicates were tested for each combination of heat treatment (103°C/16h, 200°C/3h, 220°C/3h), extraction (unextracted, successively extracted) and KOH treatment (non KOH-treated or KOH treated). The wood specimens were incubated in Petri dishes of 90 mm diameter. Each Petri dish contained three specimens of the same treatment combination of heat treatment and extraction, and one control specimen of pine sapwood. Four additional specimens of each combination of heat treatment and extraction were used as control specimens in order to calculate correction factors.

Deviant to the specifications given in EN 113 (1996), the specimens were incubated for a shortened period of 8 weeks at 22°C and 70% RH. The initial dry mass and the final dry mass after incubation were determined by oven drying the specimens at 103°C and weighing to the nearest 0.001 g. The mass loss of each specimen was calculated as a percentage of the initial dry mass.

RESULTS AND DISCUSSION

Decrease in mass by heat treatment and mass loss by extraction

The decrease in mass of spruce specimens caused by the heat treatment at 220°C for 3 h was 6.7% (+/- 0.32%), whereas the decrease in mass of specimens caused by the heat treatment at 200°C for 3 h was 2.2% (+/- 0.16%). The successive extraction of the different heat treated und untreated spruce wood specimen caused mass loss in the range from 1.5 – 2.4%, as shown in Tab. 1. With increasing heat treatment intensity, the total mass loss by extraction also increased.

Tab. 1: Oven dry density, total mass loss by successive extraction as well as individual mass loss depending on the single extractive solvents of untreated and heat treated spruce specimens. Standard deviation in brackets

Heat-treatment	Oven dry density [g/cm³]		Mass loss [%] of specimens by extraction			
	before extraction	after extraction	total (a + b + c)	a) petroleum ether	b) acetone/ water	c) methanol/ water
103°C	0.49 (0.03)	0.50 (0.03)	1.47 (0.53)	0.11 (0.15)	0.62 (0.52)	0.74 (0.20)
200°C	0.49 (0.03)	0.50 (0.03)	2.20 (0.28)	0.10 (0.17)	1.43 (0.28)	0.66 (0.22)
220°C	0.48 (0.02)	0.48 (0.03)	2.40 (0.21)	-0.04 (0.23)	2.03 (0.25)	0.41 (0.09)

Maximum swelling and EMC

Differences in maximum tangential swelling between unextracted and successively extracted specimens were not found, independent from the heat treatment and soaking medium (liquid water or KOH solution) applied. On this account, swelling values of unextracted (n=26) and extracted (n=26) specimens of the same heat treatment combination were combined, as shown in Fig. 1.

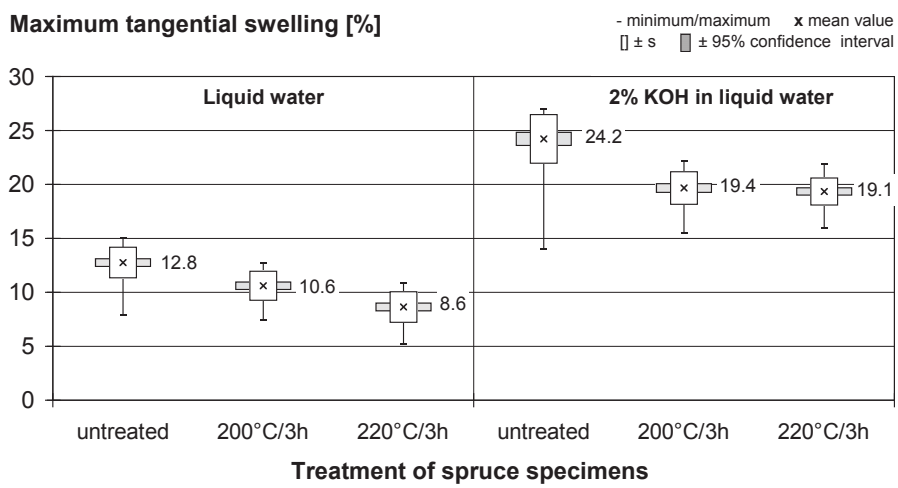

Fig. 1: Maximum tangential swelling of heat treated spruce specimens after 24h soaking in liquid water or in a solution of 2% KOH in liquid water (n=52).

The maximum tangential swelling was decreased significantly with increasing heat treatment intensity in swelling tests with liquid water. This was not unexpected since the correlation between improved dimensional stability with increasing treatment intensity is commonly accepted (*i.a.* Stamm et al. 1946). However, the KOH-treatment resulted in total reversion of dimensional stabilization caused by the heat treatment: An average tangential swelling of approximately 19% was

found for the heat treated spruce specimens and 24.2% for spruce controls. This increase in swelling of thermally modified specimens coincides with results from Kamdem et al. (2002) and Seborg et al. (1953). There, heat treated spruce was swollen in 18% sodium hydroxide solution (NaOH) for 24 h. As a consequence of the increased swelling of the heat treated specimens, Kamdem et al. (2002) concluded, that ether cross linking bonds, potentially responsible for dimensional stabilization of TMT, were not established during heat treatment, because alkali solutions are not able to break ether linkages (Seborg et al. 1953). It is conceivable, that the reduced swelling of TMT compared to controls results from cross-linking of the lignin-network, as described by Burmester (1975) and Boonstra and Tjeerdsma (2006), which is partly broken up by the alkali-treatment leading to the reconstitution of swelling behaviour. Consequently, the rearrangement of lignin structure is rather concurrently causative for the reduced sorption properties leading to increased fungal resistance than increasing the decay resistance by constricted enzymatic-recognition.

Analogous to the swelling tests, no differences in EMC were found between extracted (n=26) and unextracted (n=26) specimens of the same heat-treatment, and therefore these groups of specimens were also combined (n=52) to examine differences in EMC related to different heat treatment intensities and due to the alkali treatment. A reduced EMC with increasing heat treatment intensity was found for spruce specimens without alkali treatment (Fig. 2), which is in line with previous results from Popper et al. (2005) and can be explained by less moisture accessible hydroxyl groups of heat treated specimens compared to controls (Boonstra and Tjeerdsma 2006).

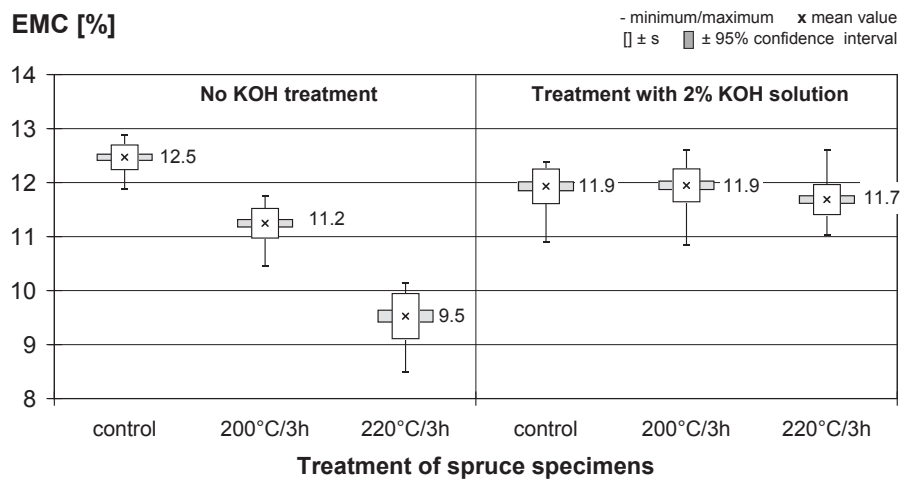

Fig. 2: Equilibrium moisture content (EMC) of heat treated spruce specimens untreated or treated with a 2% KOH solution after 48 d exposure in 20°C/75%RH (n=52).

However, an alkali treatment with 2% KOH solution compensated the reduced hygroscopicity of the heat treated specimens completely: average EMC values in a range from 11.7 to 11.9% were found for all spruce specimens after the KOH-treatment, independent from the prior heat treatment or extraction (Fig. 2). This leads to the assumption, that the reconstitution of the hydrophilic behaviour of heat treated wood after alkali treatment is not exclusively related to already existing moisture accessible hydroxyl groups of hemicelluloses, which are commonly

considered as being mainly responsible for the sorption properties of wood (*i.a.* Kollmann and Fengel 1965, Burmester 1975), since hemicelluloses are dominantly degraded by the heat-treatment (Wienhaus 1999). Furthermore, remaining accessible hemicelluloses of the heat treated specimens were probably removed by the alkali treatment or prior extraction (Fengel 1966). On this account, the reconstituted hydrophilic character of the heat- and alkali-treated specimens is more likely attributed to the creation of new voids within the cell wall by the KOH-treatment, which promote the accessibility to new laid open hydrogen-bonds or hydroxyl groups.

Inhibitory effect of extracts

None of the extracts from successive extraction (petroleum ether, acetone, methanol) of the differently heat treated spruce specimens revealed an inhibitory effect on the growth of *O. placenta,* independent from the extract concentration applied to the filter paper strips. Furthermore, the single extracts were tested in combinations with each other to consider possible synergistic effects, but also no inhibitory effect was found. Comparable results were reported by Buro (1954), where untreated controls impregnated with the acetone-chloroform extracts from heat treated softwood timber did not show any inhibitory effect on fungal growth. Consequently, these results disapprove the hypothesis regarding the formation of toxic products during the heat treatment that contribute to the improved fungal resistance of TMT. Only in the case of non pH-neutralized extracts from the KOH treatment, an inhibition of fungal growth was found (Tab. 2).

Tab. 2: Average value of relative inhibition of extracts from heat-treated spruce specimens on the growth of Oligoporus placenta; n=4 specimens tested per concentration.

		Concentration	
Extractive solvent	Heat treatment	c=1	c=2
KOH, extract non-neutralized	103°C/16h	0.4%	83.9%
	200°C/3h	0.2%	33.3%
	220°C/3h	0.2%	71.8%
KOH, extract neutralized with oxalic acid	103°C/16h	0.0%	0.0%
	200°C/3h	0.0%	0.0%
	220°C/3h	0.0%	0.0%
Pure KOH (2%)	-	3.8%	-
Pure oxalic acid (8%)	-	0.0%	-

However, if the pH-value of the extracts from the KOH treatment (pH 13.8) was neutralized with oxalic acid (pH 6.3 after neutralization), the inhibition of fungal growth was also neutralized. This shows, that the inhibition caused by the extracts from the KOH treatment was a consequence of the resulting pH value, since microbial activity in general is prevented outside pH 2 and 12 (Schmidt 2006), whereas fungal activity in particular is already limited above pH 8.5 (Wallhäußer and Schmidt 1967). The optimum pH range for wood destroying basidiomycetes is between 5 and 6, but among various wood fungi (Sharp 1975, Schmidt and Liese 1978) *O. placenta* is able to change the pH value of the substrate by pH regulation through the excretion of organic acids, particularly oxalic acid (Schmidt 2006). Consequently, the activity and growth of *O. placenta* was not inhibited by the addition of oxalic acid.

Mass loss by fungal decay

In addition to the tests on inhibitory effects of extracts, fungal decay tests with the differently treated spruce specimens were performed against *O. placenta*, together with pine sapwood virulence and control specimens, which showed sufficient mass loss (virulence: 30.9% +/- 3.90%, control: 29.5% +/- 4.72%) in accordance with EN 113 (1996).

As can be seen from Tab. 3, the mass loss of heat treated specimens caused by *O. placenta* decreased significantly with increasing heat treatment intensity (spruce 103°C: 23.9%; 200°C/3 h: 22.4%; 220°C/3 h: 6.3%), which coincides with results found by other authors (i.a. Vitaniemii 1997, Welzbacher et al. 2007). Additionally, Paul et al. (2006) and Mazela et al. (2004) also mentioned limited improvement of resistance to fungal decay for heat treatment temperatures below 200°C, which is in line with findings from Jämsä and Viitaniemi (1998) and Syrjänen and Kangas (2000) who recommended a minimum heat treatment temperature of 220°C for a sufficient increase of the resistance to fungal decay.

However, similar to results from Buro (1954), Kamdem et al. (2002) and Hakkou et al. (2005), no influence of the successive extraction on mass loss of the differently heat treated spruce specimens was found: The average mass loss of extracted heat treated specimens (200°C/3 h: 21.5%; 220°C/3 h: 6.3%) was equal to the average mass loss of axially matched unextracted heat treated specimens. In addition, the mass loss of extracted non-heat treated specimens (25.8%) was only slightly increased compared to the mass loss of unextracted parallels (23.9%).

Tab. 3: Oven dry density and mass loss by Oligoporus placenta of the different heat treated and extracted spruce specimens. Extracted = successively extracted, KOH = treated with a 2% solution of KOH, eluted = pH-neutralization by elution, oxalic acid = pH-neutralization with a solution of 8% oxalic acid. Standard deviation in brackets.

Material	Density [g/cm³]	Mass loss by *O. placenta* [%]
Spruce 103°C/16h		
unextracted	0.49 (0.03)	23.9 (4.20)
extracted	0.50 (0.03)	25.8 (6.57)
extracted, KOH, eluted	0.51 (0.03)	25.6 (12.41)
KOH	0.48 (0.02)	2.6 (0.57)
KOH, eluted	0.51 (0.03)	28.1 (9.88)
KOH, oxalic acid	0.52 (0.02)	23.8 (11.06)
Spruce heat-treated at 200°C for 3 h		
unextracted	0.49 (0.03)	22.4 (5.22)
extracted	0.50 (0.03)	21.5 (4.89)
extracted, KOH, eluted	0.50 (0.02)	33.2 (8.45)
KOH	0.48 (0.01)	3.0 (0.48)
KOH, eluted	0.50 (0.03)	28.7 (8.87)
KOH, oxalic acid	0.50 (0.02)	23.1 (4.20)
Spruce heat-treated at 220°C for 3 h		
unextracted	0.48 (0.02)	6.3 (2.72)
extracted	0.48 (0.03)	6.3 (2.42)
extracted, KOH, eluted	0.50 (0.02)	26.5 (12.02)
KOH	0.47 (0.02)	3.3 (0.33)
KOH, eluted	0.50 (0.03)	28.7 (8.87)
KOH, oxalic acid	0.51 (0.03)	20.2 (2.81)
Pine sapwood control	0.60 (0.04)	29.5 (4.72)

The treatment with KOH proved to have the strongest impact on the mass loss caused by *O. placenta*. Provided that the pH value of the KOH treated specimens was neutralized before inoculation, either by elution with deionised water (resulting pH 6.6) or with oxalic acid (resulting pH 6.3), the originally achieved reduction in mass loss due to thermal modification was reversed completely. All pH-neutralized KOH-treated specimens, independent from the prior heat treatment or extraction applied, showed mass loss by fungal decay similar to the mass loss of untreated spruce in a range from 20.2% (220°C/3 h: KOH, oxalic acid) up to 33.2% (200°C/3 h: extracted, KOH, eluted). Only in the case of specimens that remained non-neutralized after KOH treatment (pH 13.8), the mass loss by fungal decay was decreased significantly to 2.99%, which results from the high pH-value that prevents microbial activity (Schmidt 2006).

Since the differences in mass loss by fungal decay were only slight between extracted and unextracted specimens, as well as between the various KOH treated samples with subsequent pH-neutralization, two groups of specimens were formed to demonstrate the impact of KOH-treatment on the dependency of mass loss by *O. placenta* on decrease in mass by heat treatment: Group 1 = non KOH-treated specimens (no KOH), group 2 = KOH-treated and pH-neutralized specimens (KOH). As shown in Fig. 3, the relationship of increased heat treatment intensity and reduced mass loss by fungal decay was totally compensated by KOH-treatment.

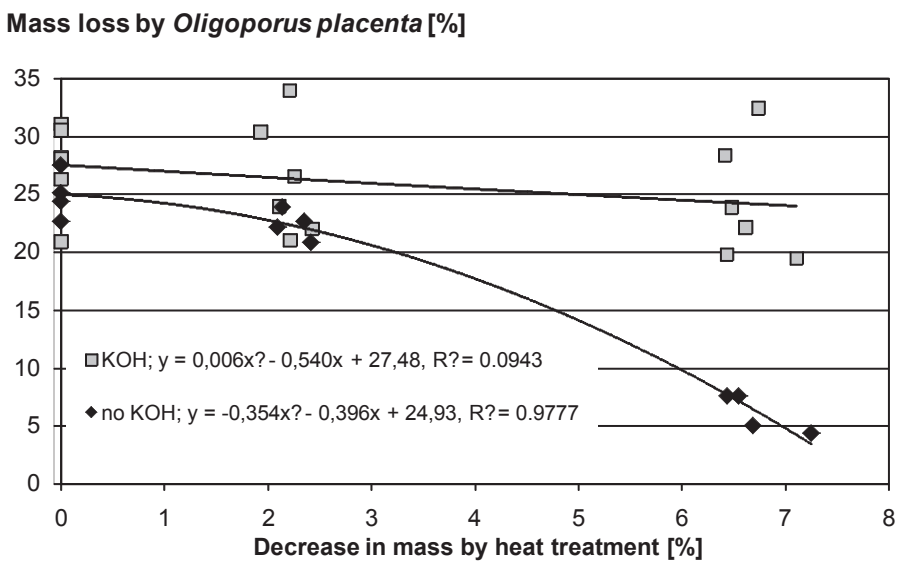

Fig. 3: Correlation between mass loss by Oligoporus placenta and decrease in mass of heat treated specimens (no KOH) as well as of heat treated specimens with KOH-treatment and subsequent pH-value neutralization. Each dot represents the mean value of 6 specimens.

These results indicate, that in contrast to findings from Buro (1954), Kamdem et al. (2002) and Hakkou et al. (2005), the reduced moisture adsorption contributes to the increase in durability of TMT, as it was also assumed by Tjeerdsma et al. (1998). However, the reduced moisture adsorption of TMT is closely associated with the modification of the microporous

structure (Popper et al. 2005) that leads to a decreased percentage of micro voids (Junghans et al. 2005) which might also result in retarded accessibility for fungal wood degrading agents at the same time (Schwarze et al. 2005). The interaction of these factors with other potential causes, such as the commonly considered non-recognition of nutritive source by enzymes responsible for fungal decay (Tjeerdsma et al. 1998, Paul et al. 2006), needs to be examined in further studies.

CONCLUSIONS

It was shown, that the reconstitution of the hydrophilic character of heat treated specimens by alkali treatment leads to increased mass loss by fungal decay at the same time. This indicates that the reduced moisture sorption coming along with the modification of microporous structure contributes to the increased fungal resistance of heat treated wood to a higher extent than it was commonly assumed. Furthermore, the extracts obtained by successive extraction of heat treated spruce specimens showed no inhibitory effect on the fungal growth of *O. placenta,* and in addition, the extraction itself had no influence of the mass loss rates obtained by fungal decay. Hence, the hypothesis regarding the potentially formed toxic byproducts during thermal modification, which may account for the improved fungal resistance of heat treated wood, is disapproved. However, more work is needed to examine the interaction of mechanisms (reduced moisture sorption, non-recognition of nutritive source by enzymes due to molecular modification and degradation of the nutritive source) behind the increased fungal resistance of heat treated wood.

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