



## Scientific Report, COST STSM

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### ***Introduction***

The over-all aim of this STSM was to learn the methodology of microanalysis of wood developed at the host institution. A scheme for chemical multi-analysis of wood tissues on micro-samples (<10 mg) has been brought into practice. The use of micro-samples can be essential in the non-invasive assessment of wood cultural heritages where a chemical evaluation is crucial to make an appropriate conservation or treatment of wooden artefacts. We focused on the chemical degradation of water-logged conserved wood and especially the alteration of hemicelluloses and formation of free organic acids which are evident signs of active degradation processes. The wood consists of approximately one third lignin and the understanding of its chemistry may also be essential to evaluate the degradation mechanisms in the wood. Lignin was analysed in order to evaluate eventual differences between affected and non affected wood. We used these methods developed and on fresh oak wood, wood material from the Swedish war-ship *Vasa* and other two other ship. A possible combination of the techniques used at the host and home institutions may lead to future collaboration and a fruitful contribution of science into the field of conservation.

## **Materials**

Two types of samples were analysed: surface samples and inner samples. From former analyses within the “Save the *Vasa*”-project we know that the surface samples have higher sulfur content and display some influence of former bacterial degradation. The inner samples had no signs of microbial degradation. However, in samples with high iron content and low sulfur content, degradation of cellulose and hemicelluloses has occurred [1, 2]. Reference samples from recent oak wood and a reference samples from the Swedish ship *Elefanten*, which was recovered in 1930-ties and kept in museum environment, were also analysed.

## **Methods used**

### ***Acid methanolysis***

By acid methanolysis the composition and total amounts of non-cellulosic carbohydrates is determined (i.e. hemicelluloses, pectins and low molecular oligomers). The acid methanolysis was performed with 2 ml of 2 M anhydrous HCl/MeOH at 100°C, with subsequent neutralization, derivatization and gas chromatographic analysis according to Sundberg *et al.* [3].

The aim of analysis of waterlogged wood by this method was mainly the determination of xylose and glucose. The content of xylose is of interest since the xylan is partly depolymerized in degraded wood of the *Vasa*. Increased levels of glucose may indicate severe depolymerization of cellulose or a change its crystalline properties.

The results obtained are presented in Table 1. The results are expressed in amounts per gram dry wood by adjusting for the content of polyethylene glycol and sulfates in the samples.

## Trends and correlations

All sugars except GlcA displayed lower levels compared to recent wood.

### Xylan

- Surface samples with high content of sulfur accompanied by overlaying salt precipitation showed decreased contents of carbohydrates compared to the unaffected samples, see Fig 1. The plausible explanation is former microbial activity degrading polysaccharides in the surface region. If the salt affected samples are excluded, the average xylan content is somewhat lower (18 %) compared to in recent oak (heartwood, 25 %) [4].
- Inner samples with high iron content, which have documented chemical degradation (depolymerisation) of cellulose and/or hemicellulose, did not show a significant decrease of the xylan content.
- The average ratio of GlucA:Xyl within the analysed samples was 0.10 compared to 0.12 in recent wood. However, some trends were found (Figure 2). The ratio is negatively correlated to the sulfate (and sulfur) content. Two different trends of the ratio as a function of pH can be seen within the surface and inner samples. (Figure 3).

### Glucose

- The average content of glucose is at the same levels as found in recent oak.
- The glucose content shows a positive correlation with the depth (Figure 4), a negative correlation with the content of PEG and a negative correlation with the content of sulfur. High contents of both PEG and sulfur are found in the surface regions in the Vasa wood. That means that PEG and sulfur correlated to higher activity of former microbiology during the time in the sea and conservation. Such activity does not necessary need to be linked to a particular wood degradation process. A biological “background” activity may very well consume the glucose, which is susceptible as a general energy source. Another explanation could be diffusion of glucose from the wood leading to lower levels nearer the surface. This is, however, not seen for the other sugars with exception of galactose.

### Other sugars

- A positive correlation between rhamnose and galactose were found, Figure 5.
- A positive correlation between glucose and galactose were found, Figure 6.

### ***Thermo-chemolysis (low-temperature pyrolysis)***

Pyrolysis is a technique for the analysis of lignin structure and decay in wood and other sample matrices. With regard to the analysis of lignin, TMAH (tetramethyl ammonium hydroxide) thermo-chemolysis is an analytical procedure that combines the bas catalyzed decomposition of specific structural bonds in lignin with simultaneous derivatization of phenols, alcohols and acids with a methyl group. This technique minimizes the risk of decarboxylation in the lignin and gives derivatized functional groups possible to analyze by gas chromatography.

Different types of samples were analyzed for comparison; recent oak wood (*Quercus Robur*), degraded (2B) and un-degraded (8B) *Vasa* wood, and a reference samples from the Swedish ship *Elefanten*, which was recovered in 1930-ties and kept in museum environment.

Wood samples, approx. 2 mg were freeze-dried and (meal, 40 mesh) then derivatized with THAM prior to thermolysis. Samples were analyzed with and without pretreatment before drying and derivatization. The pretreatment included of extraction by water or DTPA (iron chelator) in order to evaluate any effects of PEG, salt and iron compounds on the thermo-chemolysis process.

### Results

Interferences of PEG were evident in such way that results from samples with different PEG concentrations could not be evaluated properly. The influence of iron was also distinct in the iron rich sample 2B.

When comparing thermo-chemolysis results from samples pretreated with DTPA, some general results can be derived. The degraded sample showed major changes in the lignin structure as indicated by the change of intensity of a number of monomers (Table 2) compared to recent oak and un-degraded samples. The un-degraded samples (Recent oak, Elefanten and 8B) displayed a similar fragmentation profile without any major differences.

Evaluation of the details in these results is not finished as complementary analysis of degraded wood may be needed. The use of  $^{13}\text{C}$ -TMAH thermolysis should be taken in to consideration in order to distinguish between methoxyl groups originally from the monomer and methoxyl groups from TMAH derivatization.

### ***Low-molecular organic acids (oxalic acid)***

The degraded wood of Vasa typically exhibits a low pH. The accompanying low pH may be a result of degradation as well as being a promoter of such processes, indicated by the presence of various acids. For example, oxidation and hydrolysis of iron-sulfur compounds produce protons, oxidation of wood polymers increases the number of carboxylic acid groups [5] enabling hydrolysis, and free organic acids might form by degradation of both mono- and polysaccharides [6] [7]. The content of formic and acetic acid has been analysed by other methods. Oxalic acid has a certain importance as a degradation indicator and was analysed by gas chromatography after silylation derivatization in this STSM.

Four samples: recent oak wood (*Quercus Robur*), degraded (2B) and un-degraded (8B) Vasa wood, and *Elefanten* (see above). Approx 10 mg of wood material was extracted in 2 ml of water for 2 h, repeated three times. The last extraction lasted for 12 h. The supernatants were dried by evaporation (Rotavapor) by adding pure acetone. An internal standard of succinic acid was added before evaporation. After drying, the material was silylated by adding pyridine (100 ul), hexamethylsilazane (200ul) and trimethylchlorosilane (100 ul) as a catalyst. Aliquots were analyzed by gas chromatography. Identification of the analytes were

#### Preliminary results

Only very low concentrations of oxalic acid were found in the un-degraded samples whereas the degraded sample (2B) had a content of approx 8 mg/g.

### ***Over all conclusions of the mission***

This COST mission has been a very valuable opportunity to learn new methods and analyze wood samples from the Vasa with these alternative methods in order to determine links to degradation patterns in the wood. Changes in the lignin monomer composition as indicated

by thermo-chemolysis have not been used so far in the analysis of the *Vasa* wood. The sugar composition of hemicelluloses and pectin as well as the content of oxalic acid of gave valuable supplementary information of the degradation status of the wood. A general decrease of xylan but no evident cellulose degradation indicated by the glucose content was observed. The oxalic acid was increased in the degraded samples. This acid may play an important part in the degradation chemistry. It is a product of degradation but may enhance Fenton type of reaction leading to hydroxyl radical formation with subsequent depolymerisation of the wood polymers.

The use of micro-samples was especially interesting since it makes possible to use the methods as non-invasive tool on wood cultural heritages which normally is prohibited from sampling.

Table 1 Content of sugars by acid methanolysis

	Ara	Rha	Xyl	Man	Gal	Glc	4-O-MeGlcA	GlcA	GalA
CB16A	2	3	46	2	5	6	5	4	13
CB16B	3	4	140	5	9	8	15	4	17
CB2A	3	3	81	2	7	7	8	4	10
CB2B	3	8	146	3	24	25	17	5	17
CB6A	3	4	152	4	7	13	14	5	16
CB6B	3	4	157	3	9	14	15	5	17
65518y	9	4	26	2	11	8	2	3	24
65518i	6	4	159	12	12	18	16	2	15
65519y	6	4	139	3	20	9	16	3	14
65519i	3	4	129	3	10	13	13	2	14
HS10-100	4	5	138	3	18	28	16	5	18

Table 2. Relative signal intensity of lignin monomer between degraded and un-degraded samples by THAM-chemo-thermolysis

Increased relative signal intensity ( $\sim >4\times$ )	Decreased relative signal intensity ( $\sim <0.25\times$ )	Approx the same signal intensity
veratrol	1-veratrylglycerol trimethyl ether	1,4-dimethoxybenzene
3-methoxybenzaldehyde	1-(3,4,5-trimethoxyphenyl)-1,2,3-trimethoxypropane	4-vinylveratrol
dimethoxytoluene	1-(3,4,5-trimethoxyphenyl)-dimethoxybutane	4-methoxyveratrol
1,2,3-trimethoxybenzene		3,4,5-trimethoxytoluene
4-(1-propenyl) veratrol		veratraldehyde
3,4,5-trimethoxy propenylbenzene		veratryl-methyl-ketone
tetramethoxy biphenyl		veratric acid (methyl ester)
hexamethoxystilbene + acid 26:0		3,4,5-trimethoxybenzaldehyde
lirioresinol, dimethyl ether		2-veratryleten-1-ol methyl ether
1-veratryleten-1-ol methyl ether		3-veratrylpropan-1-ol methyl ether
veratrylacetone		1-veratrylprop-1-en-1-ol methyl ether
3,4,5-trimethoxyacetophenone		3,4,5-trimethoxybenzoic acid (methyl ester)
pentamethoxystilbene		(methoxypropenyl)-veratrol
		1-(3,4,5-trimethoxyphenyl)-2-methoxyethene
		1-(3,4,5-trimethoxyphenyl)-1-methoxy-prop-1-ene

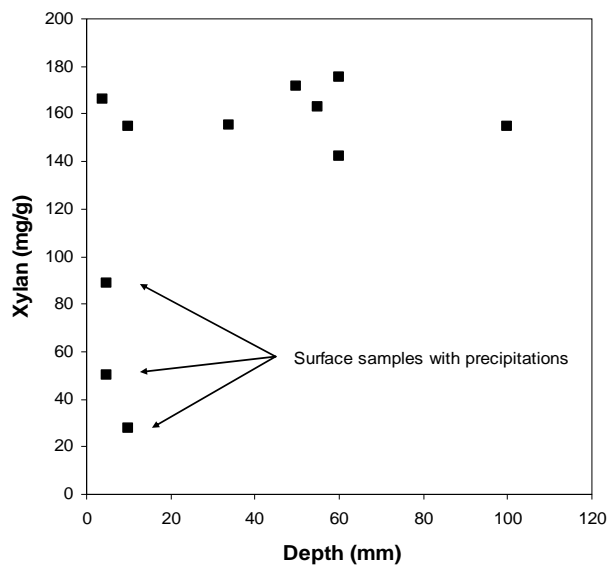


Figure 1. Xylan content as a function of depth.

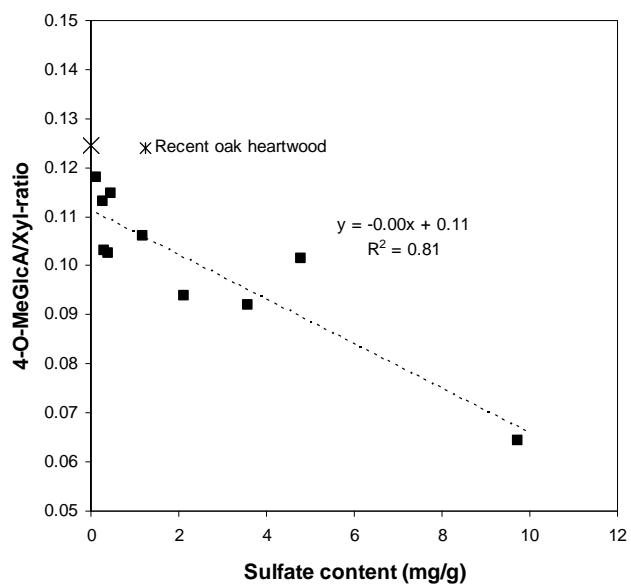


Figure 2. 4-O-MeGlcA/Xyl-ratio as a function of sulfate.

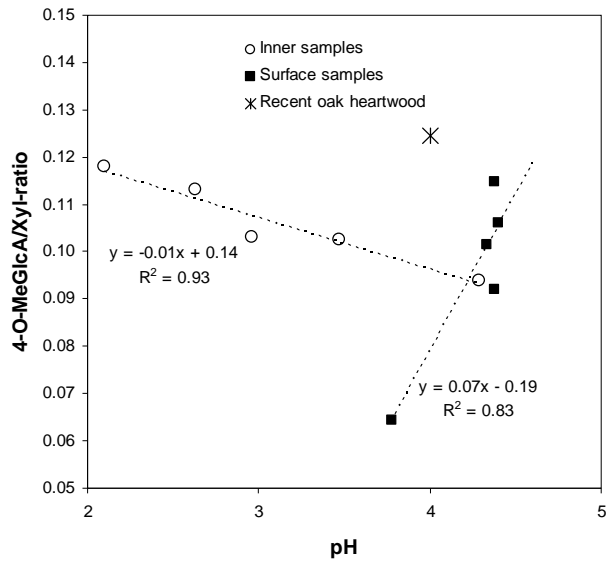


Figure 3. 4-O-MeGlcA/Xyl-ratio as a function of pH.

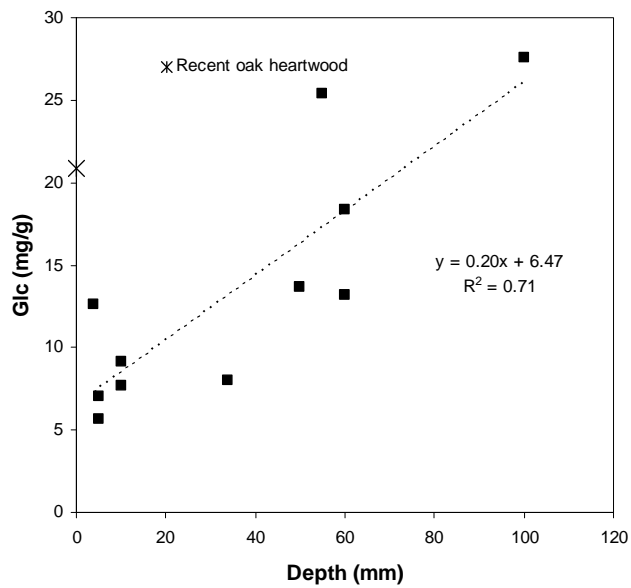


Figure 4. Glucose content as a function of depth.

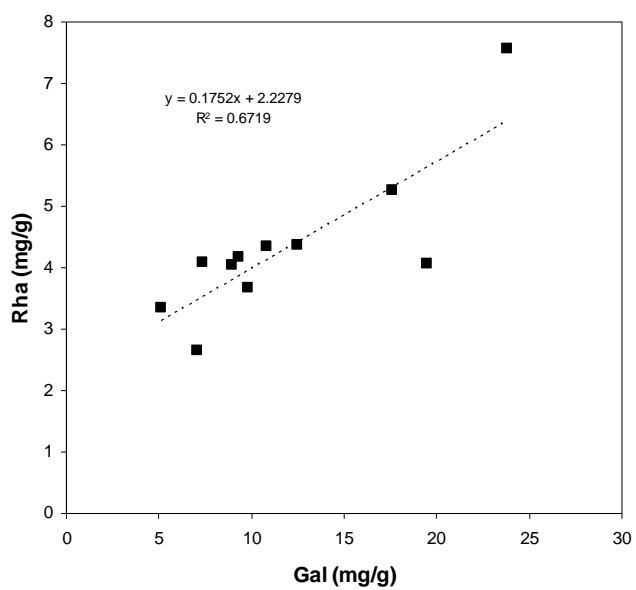


Figure 5. Correlation between rhamnose and galactose.

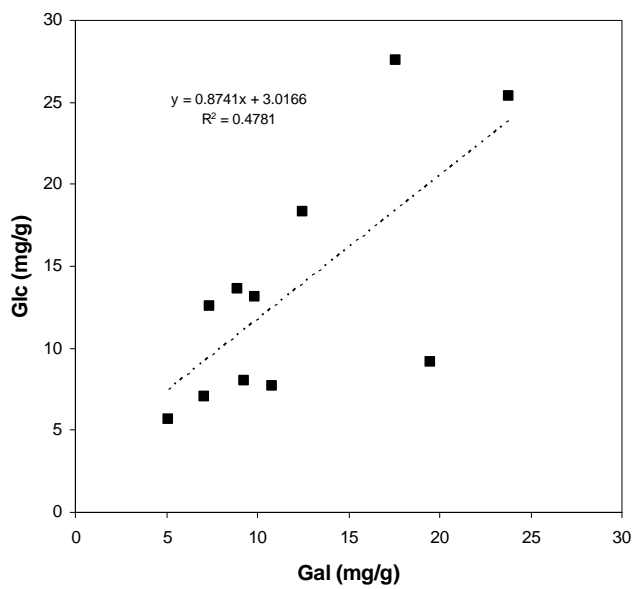


Figure 6. Correlation between glucose and galactose.

## *References*

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