

Aging of wood under long-term storage in a salt environment

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Received: 23 July 2015 / Published online: 11 May 2016

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Abstract Aging of archeological wood in a salt environment was assessed in Hallstatt, Upper Austria, where Bronze Age wooden findings of the archeological salt mining site were compared with recent wood samples from the same site. Three species (*Picea abies*, *Abies alba* and *Fagus sylvatica*) were investigated. Recent samples covered all main geological units within the salt valley. FTIR spectroscopy was used to determine differences in wood chemistry. Principal component analysis was used to display the segregation of the data set according to the different factors and to give a measure of the strength of the effects. Results revealed that deacetylation processes took place at the wood material. The aging process affects wood chemistry significantly stronger than differences in earlywood and latewood. Together these two factors accounted for 80 % of data variability. As a third factor, the tree species segregated the data set. Geological differences in the sampling sites were not mirrored in the FTIR spectra.

Introduction

Organic matter usually undergoes a rapid transformation process in our environment. Depending on the recalcitrance of the molecules, mineralization and humification take place within days to few years after exposure. Several conditions can inhibit these processes and lead to subfossil materials. Dry (deserts), cold (ice, permafrost) and wet (groundwater, lake sediments, bogs and moors) conditions are favorable to keep good preservation, but also salt or some metal oxides can prevent

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aging processes (Jacomet and Kreuz 1999). Archeological wood samples found in marine sediments are, for example, the wrecks of the Swedish “Wasa” (Rowell and Barbour 1990)—sank in 1628 and salvaged in the harbor of Stockholm 1961—the English “Mary Rose”—sank in 1545 and salvaged 1982—or the two Russian frigates St. Mikael and St. Nikolai—sank in 1747 and 1790 and salvaged in the Gulf of Finland (Reunanen et al. 1989, 1990).

The salt mine in Hallstatt, Upper Austria, housed prehistoric mining activities during the Bronze Age (1500–1100 BC) and the Early Iron Age (850–350 BC). Due to a geological disaster, both mines were filled with material. Huge amounts of soil, stones and whole trees liquidated the mining activities. Since that time, all organic material was covered by salt-rich clay. Since the nineteenth century, the prehistoric mines have been studied and therefore excavated. The high amount of organic findings gives reason for the high value of the archeological site of Hallstatt. Very rare insights into prehistoric life are possible due to textiles, wood handicrafts, leather, excrements and other findings, which usually are rotted and decomposed in other sites (Kern et al. 2009; Reschreiter 2013). In the salt environment, microbial activity can be seen as negligible (Sorokin et al. 2013). The good preservation of the findings regarding shape and even colors suggests that no aging process at all has taken place. Among the organic findings in Hallstatt, wood plays a special role due to the high number of wood pieces and the good status of preservation (Grabner et al. 2010). The latter allows dating by dendrochronology. Up to now, no study assessed the chemistry of these wood findings.

Fourier transform infrared (FTIR) spectroscopy is a proven method to assess wood and its main compounds lignin, cellulose and hemicellulose (Costa e Silva et al. 1999; Meder et al. 1999; Pandey 1999), but also other wood compounds. Attenuated total reflection (ATR) is a special technique which allows to measure surfaces directly and point by point. It combines two advantages. Influences due to sample preparation with milling are avoided (Schwanninger et al. 2004), and earlywood can be measured separately if it is large enough (Mohebbi 2008; Müller et al. 2009).

It was the target of this study to reveal the influence of a salt environment over a period of 3500 years on wood chemistry. Better knowledge of wood preservation or degradation under special environmental conditions can complement archeological findings and support data interpretation. In order to clearly detect changes due to aging, other effects such as differences in earlywood and latewood and the tree species (Emandi et al. 2011) or heterogeneity of the geological underground and differences in the orographic origin (Rana et al. 2008) were taken into account.

Materials and methods

Recent and prehistoric wood samples were taken in Hallstatt, Upper Austria. Altogether, 120 wood cores were measured. The heterogeneity of the geological situation in the salt valley of Hallstatt was covered by wood samples of trees grown on all important geological zones which are limestone, depleted Haselgebirge and a mixture of both (Mandl et al. 2012). Haselgebirge is the predominant breccia rock of

the salt deposit in Hallstatt, containing mainly chlorides, clay, gypsum and carbonates. Depleted Haselgebirge is the cover material at the top, where rainwater leached mainly chlorides and gypsum. Furthermore, trees from different orographic origin were sampled (650 and about 1000 m asl.).

Only earlywood was measured from *Picea abies* and *Abies alba*. Both prehistoric and recent samples of *Fagus sylvatica* had such narrow tree rings that it was not possible to measure earlywood-like tissue separately. Therefore, 18 samples of prehistoric and recent samples of *Picea abies* with narrow tree rings were chosen and additionally measured at the whole ring to quantify this effect.

Sixty-eight cores from living *Picea abies*, 32 from living *Abies alba* and 20 from living *Fagus sylvatica* as well as 39 cores from archeological findings were analyzed (Table 1). Recent core samples were taken by random sampling of living trees on the respective geological underground. Cores with 5.5 mm diameter were taken using usual forestry increment borers, dried at room temperature, and polished on one side. Prehistoric samples were taken from inside the archeological mining site with the help of a machine-driven hollow corer. All findings were introduced into the mine either by Bronze Age men as mine timber or during a mass movement together with debris. In both cases, one can be quite sure that wood came locally from the high valley of the salt mine as the transport of huge logs would have been very complicated. Drilling cores with 7 mm diameter were taken, dried at room temperature, and polished on one side. Storage conditions in the mine can be described as follows: The wood was embedded in mass movement material. This material consists of partly depleted Haselgebirge with water contents between 10 and 15 % DM. The wood was stored under wet conditions with a saturated salt solution in its pores. The salt consists of 95 % NaCl. The solution features a pH slightly above 7. Other inorganic compounds play only a subordinate role.

Fourier transform infrared (FTIR) spectroscopy was applied to characterize wood samples. Eight to twelve measurements were taken along the core samples which were fixed between the sample holder and the optical crystal of a Bruker® Helios FTIR micro sampler (Tensor 27). This device allows spot measurements with a spatial resolution down to 250 µm. The replicates were necessary to account for the variability due to the different age of the trees. FTIR spectra were recorded in the ATR (attenuated total reflection) mode in the mid infrared area (4000–400 cm⁻¹).

Table 1 Sample numbers of different tree species and regions; all samples of FS are measured on narrow tree rings, PA and AA only on earlywood; numbers of PA behind the slash are additional samples measured with narrow tree rings

Origin	FS (<i>Fagus sylvatica</i>)	PA (<i>Picea abies</i>)	AA (<i>Abies alba</i>)
Prehistoric samples	9	10/10	10
DMW (depleted Haselgebirge)	4	10/8	11
BLW (mixture with limestone)	4	10	
HWS (limestone)	3	10	11
HLA (mixture with limestone, 350 m lower)		10	

Thirty-two scans were collected at a spectral resolution of 4 cm^{-1} . The replicates of the spectra were vector normalized and averaged.

Principal component analysis (PCA) was performed with The Unscrambler[®] X 10.1 software to display the influencing factors.

Results and discussion

All samples

Spectral regions from 3485 to 2430 cm^{-1} and from 1838 to 400 cm^{-1} were included in the analysis as these regions potentially contribute to any separation. Two main effects separated the samples into four sets: age and whether only earlywood or a mixture with latewood was measured. The spectral region from 1118 to 895 cm^{-1} had a main impact on the separation. This spectral region contains prominent C–O bands of cellulose (Fengel and Ludwig 1991). The intensity of the cellulose band is influenced by the aging process (Fig. 2), but additionally by the tree species. In order to improve the assignment of the PCs to the different effects, the region was therefore excluded from PCA. Figure 1 displays the resulting PCA. PC 1 explains 57 % of data variability and separates the data set into two groups with a clear distance in between which indicates a strong distinguishing effect. These two groups represent the prehistoric samples on the one side and all recent samples on the other side with no exception (Fig. 1a). Therefore, the loadings plot of PC 1 explains differences in the spectra reasoned by age of the sample groups. The loadings plot displays four strong spectral regions influencing PC 1: the OH-stretching region from 3000 to 3300 cm^{-1} and two comparatively narrow maxima at 1730 and 1235 cm^{-1} (Fig. 1e). In Fig. 1b, the same scores plot is shown, but the data set is marked according to the type of measurement. The second PC can be assigned to differences in measurement (only earlywood vs. narrow tree rings with a mixture of earlywood and latewood). The PC explains 23 % of data variability. To display this effect more clearly, the prehistoric samples were removed in a second step (Fig. 3). Figure 1c again shows the same scores plot, but in this case arranged according to the tree species. *Abies alba* (AA) and *Picea abies* (PA) samples are well mixed. The separation of *Fagus sylvatica* (FS) samples can be reasoned at least partly by measuring only narrow rings at these samples. Further details are discussed in combination with Fig. 4. Finally, in Fig. 1d, data are arranged according to the sampling site (corresponding to different geological underground). For this factor, no obvious segregation was found.

Figure 2 visualizes the effect on the OH-stretching region. Average spectra are shown of all prehistoric and all recent samples. The peak maximum shifts from 3350 (recent samples) to 3306 cm^{-1} (prehistoric samples), but the whole peak shifts from 2930 to 3358 cm^{-1} . The region from 3200 to 3700 cm^{-1} is assigned to OH vibrations (Fengel 1993). The shift of the peak could be explained by the higher amount of OH bonds remaining after a deacetylation process (Stefke et al. 2008). The region from 1709 to 1738 cm^{-1} (including 1730 cm^{-1}) can be assigned to the C=O stretch in unconjugated ketones, carbonyl stretching vibration of the acetyl group of

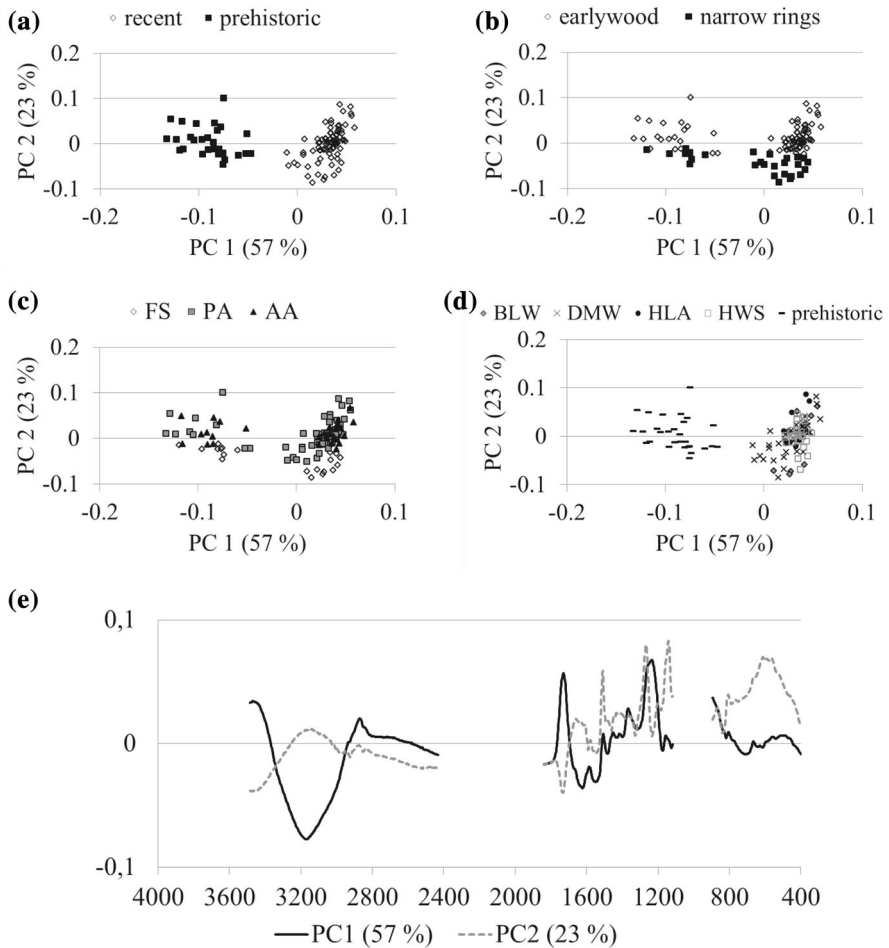


Fig. 1 PCA of all 120 samples; scores plot with the samples marked up according to (a) age, (b) measurement type, (c) tree species, and (d) sampling site, (e) displays the loadings plot of the first two PCs

hemicellulose (Terpáková et al. 2012) and in ester groups frequently of carbohydrate origin (Faix 1991). It is assumed that hemicelluloses degraded by breaking acetyl groups in xylan (Esteves et al. 2013). The region from 1225 to 1235 cm^{-1} originates from the OH vibration (deformation in plane), also from COOH (Fengel and Ludwig 1991). The decrease of this band can also be assigned to deacetylation processes (Mohebbi 2008; Stefke et al. 2008). Pizzo et al. (2015) also found a decrease of the bands at 1730 and 1230 cm^{-1} when they assessed waterlogged wood samples by ATR-FTIR spectroscopy. Stich (2006) compared recent and waterlogged wood of *Pinus sylvestris*. The waterlogged samples were found in the sediment of a lake in Finland. Based on spectroscopic and chemical results, he concluded that after 4000–5000 years of storage in situ hydrolysis had taken place. Similar results were found for prehistoric *Sequoia* and *Sequoiadendron* wood under comparable storage conditions (Uçar et al. 2005).

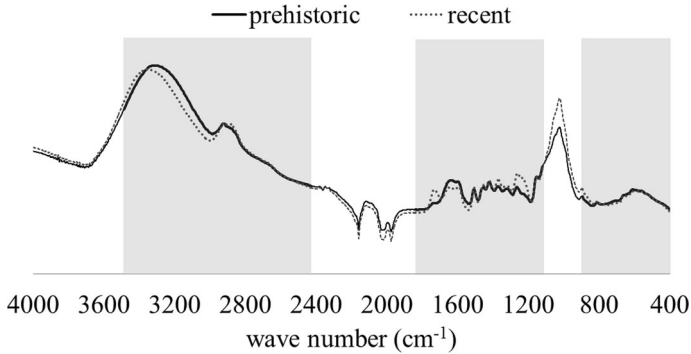


Fig. 2 Averaged FTIR spectra of the prehistoric and recent samples, gray boxes indicate the regions included in the PCA

Only recent samples

For a better assignment of the factor “type of measurement,” prehistoric samples were excluded from the data matrix and PCA was recalculated. This changed the orientation of the data set. Results are presented in Fig. 3. The first PC explains 58 % of data variability and separates the measurements of narrow tree rings and those of only earlywood. The loadings plot (Fig. 3c) displays three maxima at 1510,

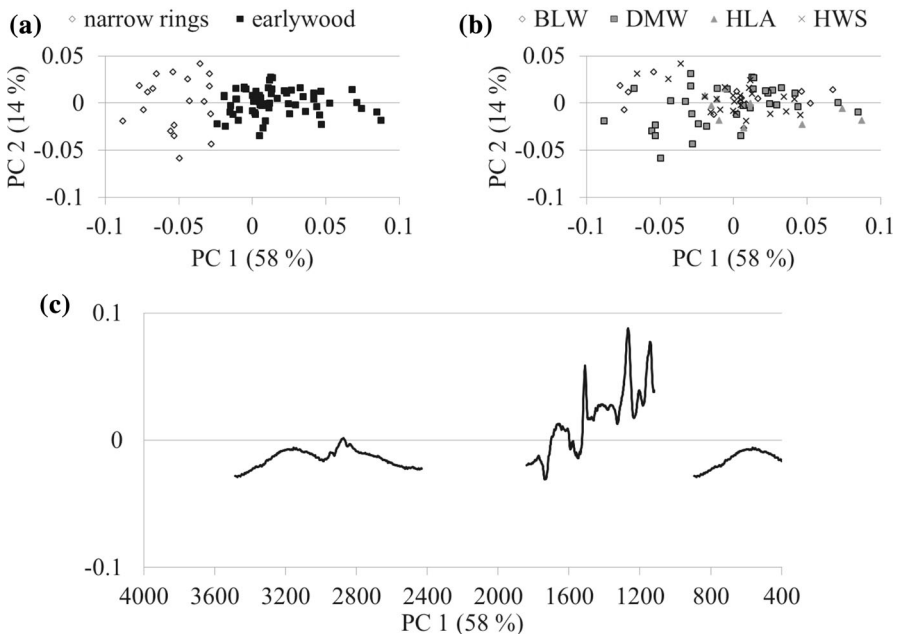


Fig. 3 PCA of recent 81 samples; scores plot with the samples marked up according to (a) measurement type and (b) sampling site, (c) displays the loadings plot of the first principal component

1267 and 1140 cm^{-1} , which are all linked to lignin (Faix 1991). The loadings of PC 1 in this PCA correspond well to the loadings of PC 2 in Fig. 1e. This can be expected, as the factor “type of measurement” is relevant in both cases. The factor “sampling site” cannot be found in the data structure even in this PCA (Fig. 3b).

Only narrow rings

Tree species result in different chemical wood composition. Differences in the ratio of lignin/cellulose/hemicelluloses, but also differences in extractives lead to different spectral patterns. To prove the effect of the factor “tree species,” the samples with only earlywood (*Picea abies* and *Abies alba*) and the samples with narrow rings (*Picea abies* and *Fagus sylvatica*) were analyzed separately. The softwood species *Picea abies* and *Abies alba* were not separated (PCA not shown), but *Picea abies* and *Fagus sylvatica* differed strongly as displayed in Fig. 4. PC 1 explains 47 % of data variability and mainly segregates the two species. No specimen overlaps the two groups. As displayed in the loadings plot, maxima at 1730 and 1230 cm^{-1} and minima at 1510 and 1270 cm^{-1} are mainly responsible for the segregation. Rana et al. (2008) investigated beech spectra and found (in the region of Fig. 4b) typical peaks at 1730, 1596, 1505, 1462, 1425, 1375, 1330 and 1235 cm^{-1} . Müller et al. (2009) investigated beech and fir samples and found in the softwood the corresponding peaks at 1738 (weaker than in beech), 1505, 1460, 1372, 1320 (as a shoulder from 1350 similar to Fig. 4b) and 1267 cm^{-1} . These

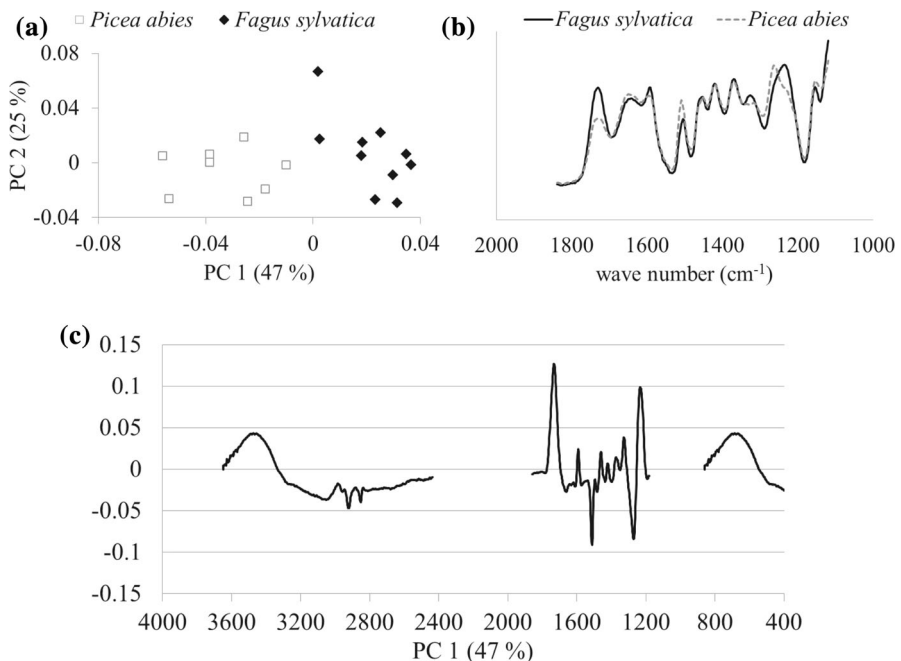


Fig. 4 PCA and IR spectra of recent 19 samples with narrow rings; (a) scores plot with the samples marked up according to the tree species; (b) averaged IR spectra in the wave number range from 1120 to 1838 cm^{-1} ; and (c) loadings plot of the first principal component

results correspond to the result given in Fig. 4c, especially the peaks at 1730, 1270 and 1230 cm^{-1} .

Conclusion

Aging of archeological wood by long-term storage in a salt environment leads to specific changes in wood chemistry. Deacetylation and degradation of hemicelluloses are the most probable processes responsible. In FTIR spectra, this leads on the one hand to a reduction of the bands in the region of 1730 and 1235 cm^{-1} , and on the other hand to a shift of the whole OH-region from 3000 to 3300 cm^{-1} . The deacetylation process may cause an increased susceptibility to microbial attack against the archeological wood findings. This conclusion can be deduced from the converse effect of acetylation. The effect of aging is the most important one influencing FTIR spectra being responsible for 57 % of data variability. Further 27 % accounted for the factor of earlywood and narrow tree rings, where earlywood and latewood were measured together. Only as the third important factor the tree species segregated the data set. Differences in the geological underground of the sampling sites were not found in the corresponding wood spectra.

Acknowledgments Open access funding provided by University of Natural Resources and Life Sciences Vienna (BOKU). The samples for this work were taken in the course of the FWF project ISOwood PP23647. We are grateful to the forest owner for the sampling permission.

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