WOOD RESEARCH
62 (2): 2017
181-196

DETERMINATION OF THE PHENOLIC EXTRACTIVE CONTENT IN SWEET CHESTNUT (*CASTANEA SATIVA* MILL.) WOOD

Simon Eichhorn
Georg-August-University Göttingen Faculty of Forest Sciences and Forest Ecology, Section Wood Biology and Wood Products
Göttingen, Germany

Sebastian Erfurt
Georg-August-University Göttingen Faculty of Forest Sciences and Forest Ecology, Section Wood Biology and Wood Products
Göttingen, Germany

Tamás Hofmann
University of Sopron, Institute of Chemistry
Sopron, Hungary

Stefan Seegmüller
Rhenish-Palatine Research, Institute of Forest Ecology and Forestry (Fawf)
Trippstadt, Germany

Róbert Németh
University of Sopron, Institute of Wood Sciences
Sopron, Hungary

František Hapla
Georg-August-University Göttingen, Faculty of Forest Sciences and Forest Ecology, Section Wood Biology and Wood Products
Göttingen, Germany

(Received May 2016)

ABSTRACT

The reason for the excellent natural durability of Sweet chestnut wood can be primarily explained with the extractives incrusted in the wood cell wall. These compounds basically involve tannins, which protect the heartwood against wood decaying microorganisms. Research

carried out on the wood of ring-porous broadleaved species revealed that there is a significant radial variation in the concentration of phenolic extractives. The present research focused on the radial distribution of total phenol and ellagitannin content in the heartwood of Sweet chestnut stems, originating from different forest stands. It was also investigated if there was a significant correlation between water supply of the treesand the distribution of phenolic compounds. Total phenol and ellagitannin contents generally increased from juvenile wood towards the sapwood/heartwood boundary, lowest values were determined in sapwood tissues. Respecting water supply it was established that the heartwood of trees growing in a dryer foreststand, contained significantly higher phenolic extractives compared to trees in well water-supplied, fresh forest sites. However, ellagitannin contents didn't significantly differ between sites. The utilization of the wood of naturally durable European species, including Sweet chestnut, will gain in importance in the future basing on the growing common ecological awareness of the population.

KEYWORDS: Sweet chestnut, sapwood and heartwood, phenolic extractive sand ellagitannins, UV-VIS spectrometry, Folin-Ciocâlteu assay.

INTRODUCTION

Background

As a result of the extensive research carried out in the field of wood modification new products are developed from low and medium durable European wood species which serve as the substitutes for naturally durable wood of tropical species. At the same time domestic broadleaved species with outstanding durability parameters sink almost into oblivion. The heartwood of Sweet chestnut (*Castanea sativa* Mill.), Pedunculate- and Sessile oak (*Quercus robur* L., *Quercus petraea* Liebl.) as well as of Black locust (*Robinia pseudacacia* L.) could be regarded as alternatives to tropical wood basing on their high natural durability (Militz et al. 2003).

Looking from the background of climate change, it is especially the Sweet chestnut which shows a high potential to become a promising industrial wood species on the long run (Wambsganß et al. 2013). The excellent properties of its wood make Sweet chestnut valuable for an economic utilization (Militz et al. 2003, Wambsganß et al. 2013). According to the standard DIN EN 350 - 2 (1994) the heartwood of Sweet chestnut is classified into durability class 2, indicating that it can be used for several years of outdoor application without any additional chemical wood protection (Militz et al. 2003, Wambsganß et al. 2013).

The natural durability of the heartwood against wood decaying microorganisms is explained with the presence of incrusted extractives (secondary metabolites) (Scalbert 1992, Viriot et al. 1994, Faix 2007). These extractives do not only imply durability but also have a decisive impact on the timber use for cooperage, providing body and color to aged wine and spirits. This holds mostly true for Italy, but is also a good future prospect for high value chestnut use in Middle and Eastern Europe (Sachsse 1984).

In oak trees wood extractives vary between tree species and regions (Vivas 1997). But there is also radial variation of these extractives in oak trunks (Tillmanns 1957, Mosedale and Savill 1996), which is possibly dependent on wood species (Pedunculate or Sessile oak) as well as on other individual factors. The age of the heartwood, the rate of heartwood formation, as well as ecological factors could also influence the distribution of these extractives across the trunk, resulting in differences in the durability parameters of the heartwood (Tillmanns 1957). For example, oak and black locust heartwood phenolics increase along the radius from pith towards

the sapwood/heartwood boundary, reflecting heartwood formation and aging (Scheffer et al. 1944, Peng et al. 1991, Viriot et al. 1994). In oak there is a remarkable site aridity impact on heartwood phenolics reflecting drought constraints as drivers especially for tannin formation as an antioxidative means of the trees (Böhm et al. 2013).

Phenolic extractives

Phenolic extractives can be classified into several different groups according to their chemical structure: lignans, stableness, flavonoids, tannins. These compounds exhibit a characteristic biocide effect (Faix 2007). Their presence decides on the natural durability of the heartwood against wood decaying fungi and bacteria. Primarily it is the stilbenes and tannins which exhibit a fungicide effect, whereas stilbenes also have an anti-termite effect (Scalbert 1991, Viriot et al. 1994, Higuchi 1996, Hon and Shiraishi 2001). The fungicide effect of lignans is quite low. Some of the flavonoids determine the color of the wood, nevertheless their contribution to natural wood durability is also significant (e.g. in the case of Douglas fir heartwood) (Kennedy 1956).

The heartwood of Sweet chestnut is exceptionally rich in tannins (Endres et al. 1962, Bouffier 2005, Sanz et al. 2010). The tannin content of Sweet chestnut heartwood is 8-10 % on average (Peng et al. 1991, Lohmann et al. 2003, Wagenführ 2007). According to Trendelenburg (1955) the content of hydrolysable tannins can reach values as high as 16 % in the heartwood.

Tannins can be classified as hydrolysable and condensed tannins (Freudenberg 1920, Endres et al. 1962, Scalbert et al. 1989, Peng et al. 1991). Hydrolysable tannins are either gallotannins or ellagitannins. The heartwood of Sweet chestnut contains primarily hydrolysable tannins, predominantly ellagitannins (Endres et al., 1962, Viriot et al. 1993, Sanz et al. 2010). The most important ellagitannin compounds in Sweet chestnut heartwood are Castalagin and *Vescalagin* (Mayer et al. 1967), which are the anomers of *di-hexahydroxydiphenoyl-glucose*. These two compounds comprise 80 % of the hydrolysable tannin content of the heartwood of Sweet chestnut (Mayer et al., 1967, Mayer et al. 1971). Apart from this, *Castalin, Vescalin* and other minor compounds are also important tannin components of the heartwood. They are the respective tannin hydrolysis products of the two prominent ellagitannins, which do not contain ellagic acid moieties (Mayer et al. 1959, Mayer et al. 1967).

Aims of the research

The radial distribution of extractives determined in Oak and Black locust heartwood presupposes that similar tendencies could be observedinSweet chestnut heartwood. Nevertheless, detailed results on the heartwood of Sweet chestnut can hardly be found in the literature. The present research focuses on the determination of the radial variation of the total phenol and ellagitannin content ranging from juvenile heartwood to the adult sapwood tissues, by means of the Folin-Ciocâlteu assay.

There are to our knowledge no studies into site effects on tannin contents in Sweet chestnut. Therefore the study highlights possible site effects on phenolic extractives with special emphasis on ellagitannins. This will not only help to identify possibly extraordinary durable trees, but also individually appropriate trunks for wine aging by addressing site characteristics.

MATERIAL AND METHODS

The present research was carried out in the framework of a cooperation involving the Georg-August-Universität Göttingen (D), University of West Hungary Sopron (H) and the Federal Institute of Forest Ecology and Forestry, Rhineland Palatinate (D).

Investigated forest stands and assignment of trees

Wood for the present research originated from trees, growing in selected Sweet chestnut forest stands in the Haardt region of the Palatine Northern Vosges Mountains (Rhineland-Palatinate, Germany). Altogether 5 stands were assigned for sample collection and 5 trees (stands P_1-P3) or 8 trees (stands P_4-P_5) were selected randomly at each location, involving a total number of 31 trees (Tab. 2). All trees were dominant or predominant as determined by Kraft tree classes (Kramer 1988). Wood discs were cut from each stem at a height of 1.3 m as orientating experiments didn't show any significant gradient in total phenolics or ellagitannins along the trunk (Tab. 1).

Tab. 1: Total phenolics and ellagitannins along the trunk of Sweet chestnut trees.

Trunk position	Total phenolics[$] (µmol pe[+] / g dw[#])	Ellagitannins (µmol eae[•]/ g dw)
Butt	448.21 (143.87)[a]	324.05 (59.62)[A]
Crown base	447.71 (140.17)[a]	320.54 (102.04)[A]

[$],values in parentheses are standard deviations, [+], pe, phenyl moiety equivalents; [#], dw, dry weight, [•], eae, ellagic acid equivalents

The orientating investigation was done with 3 individual trees, every tree being characterized by 3 independent samples from the butt and from the crown base end of the trunk. Samples were normally distributed and had homogene variances. Therefore they were compared by ANOVA. The uniform indices show that there were no significant ($p \leq 0.05$) differences between trunk sections.

The samples originating from stands P_1-P_3 were used for the determination of the radial distributions of the total phenol content without any further investigation of site condition impact. The samples from stands P_4 and P_5 were used for the determination of the radial distributions of the total phenol and ellagitannin content with special respect to site conditions. All sites were of compatible geological origin and had oligo- to mesotropheous mostly sandy soils, but differed remarkably in water supply (Tab. 2).

Tab. 2: Description of Sweet chestnut plots (forest stands).

	Plot P_1*	Plot P_2*	Plot P_3*	Plot P_4*	Plot P_5*
Number of sample trees (N)	5	5	5	8	8
Stand age*	63	39	24	2 - 63	7 - 138
Bedrock	Lower bunter to Upper Permian	Lower bunter to Upper Permian	Lower bunter to Upper Permian	Lower Permian	Bunter to Lower Permian
Soil texture	Clay- sand	Sand	Clay- sand	Clay- sand	Sand/ poorsand
Soil type	Brown (forest) soils	Brown (forest) soils	Brown (forest) soils	Brown (forest) soils	Brown (forest) soils
Precipitation (mm a^{-1})	675 – 750	750 – 850	750 – 850	675 – 750	675 - 750
Soil depth	deep soils	middle soils	deep soils	middle to deep soils	middle to deep soils
Water balance level	humid	moderately humid	humid	humid	moderately humid to very dry

* Plots P_1, P_2 and P_3 and P_4 are pure stands. Plot P_5 is a heartwood mixed stand.

All plots were grown in coppice management

This allows for stand comparison, especially as forest stand P_4 was characterized as well water supplied (humid), whereas stand P_5 was poorlysupplied (dry).

Preparation of wood samples

The assignment of the samples from the wood discs was done differently for stands P_1-P_3 and P_4-P_5. Sections with dimensions of 5 x 5 cm (height, width) including the pith were cut out of the sample discs taken from forest stands P_1-P_3. Out of the prepared sections the samples (0.2 g amounts of wood grist) were taken from the latewood of defined growth rings by the use of a pillar drill (2 mm in diameter; Fig. 1).

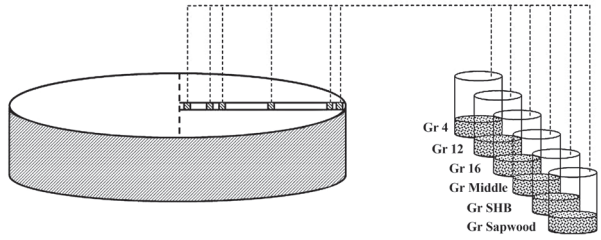

Fig. 1: Sampling of the trees of the stands P_1, P_2 and P_3.

Tab. 3 gives a classification of the samples taken from stands P_1-P_3. Fig. 2 depicts the distribution of growth rings/samples along the prepared sections originating from the same stands.

Tab. 3: Number of trees and samples for each plot (forest stand). n=75 samples (stands P_1-P_3); n=48 samples/ 16 mixed samples (stands P_4-P_5).

	Plot P_1	Plot P_2	Plot P_3	Plot P_4	Plot P_5
Trees per stand	5	5	5	8	8
Samples per tree	6	5	4	3	3
Mixed samples for one tree*	-	-	-	1	1
Samples per stand	30	25	20	24	24
Mixed samples per samples per stand*	-	-	-	8	8

* From each disc three equivalently-sized sections were cut, including an angle of 120°. The outer heartwood parts of these 3 sections were combined into one mixed sample.

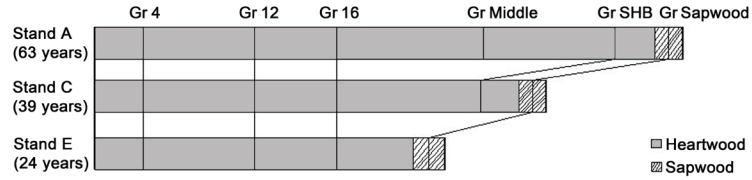

Fig. 2: Radial distribution of the samples in different stands P_1, P_2 and P_3 (Gr 4, Gr 12, Gr 16 = growth ring 4, 12 and 16 counted from the pith; Gr "Middle" corresponds to the growth ring halfway between 16 and „SHB"; Gr „SHB" = fifth growth ring of the heartwood counted from the visible sapwood/ heartwood boundary).

The discs, deriving from the P_4-P_5 forest stand trees, were sectioned into three equivalently-sized sections including an angle of 120°. According to Mosedaleet al. (1996) samples were taken from the sapwood, the 10 youngest heartwood year rings (outer heartwood) and from the pith (inner heartwood) of these sections, representing the decisive areas across the stem discs. In order to prevent preparation artefacts samples were ground under liquid nitrogen at 2,700 Hz for 2 min in a ball and tube mill to a fine powder (Sartorius Sedim Biotech GmbH, Göttingen, Germany). Due to the results of orientating experiments samples from the three 120° sections within a disc were combined (Böhm et al. 2013). Tab. 3 gives a classification of the samples taken from stands P_4-P_5.

Analysis of phenolic extractives

Determination of the total amount of polyphenolic compounds in the wood samples was carried out using the Folin-Ciocâlteu assay (FCR).Determinations of ellagitannins were done by Reichelt-Schwab-reaction. The required sample preparations were done in two different ways for the samples deriving from different forest stands (grouping P_1, P_2, P_3, P_4 and P_5 respectively)

Extraction of samples from forest stands P_1, P_2, P_3

For the extraction of wood samples 0.1 g amounts of wood grist and 10 ml extraction solvent (methanol: water 80:20 v/v) were transferred into an Erlenmayer flask and mixed by the use of magnetic stirrer (IKA RO 15 P, IKA Labortechnik GmbH, Staufen, Germany) for 24 hours under dimmed light conditions. Extracts were centrifuged (5 min, 18000 $1 \cdot min^{-1}$) and the supernatant was separated and used for further analysis.

Extraction of samples from forest stands P_4 and P_5

50 mg ± 5 mg of the fine ground wood powder were extracted in 1 ml ethanol: water (50:50 v/v) by shaking them parallel to the longitudinal axis of the PP extraction tubes for 4 hours on a KS200basic-laboratory shaker (IKA Labortechnik GmbH, Staufen, Germany) at 500 Hz. Extracts were centrifuged (15 min, 15000 $1 \cdot min^{-1}$, at 4°C) and the supernatant was collected and used for further analysis. According to orientating experiments this resulted in a minimum 95 % extraction rate (Böhm et al. 2013).

Determination of the total phenol content

All total phenol determinations were made by means of the Folin-Ciocâlteu assay according to Singleton and Rossi (1965) either at 5 min reaction time at 50°C and subsequent recooling (P_1-P_3) or for 1 h at RT in the dark (P_4-P_5; Tab. 4).

Tab. 4: Overview of the dilution of the samples and the parameters of the total phenol assays used for the samples from different stands. Grey cells indicate the volumes applied in the Folin-Ciocâlteu assay.

		Samples of Plot P_1, P_2 and P_3		Samples of Plot P_4 and P_5	
		Heartwood samples	**Sapwood samples**	**Dilution I**	**Dilution II***
Volume of the extract		0.025 ml (5 %)	0.100 ml (20 %)	0.050 ml (5 %)	0.050 ml (5.88 %)
Dilution solution	**Methanol (80 %) and Water (20 %)**	0.475 ml (95 %)	0.400 ml (80 %)	-	-
	Water	-	-	0.950 ml (95 %)	0.800 ml (94.12 %)
Total diluted volume		0.500 ml (100 %)		1 ml (100 %)	0.850 ml (100 %)
		Samples of the stands P_1, P_2 and P_3		Samples of the stands P_4 and P_5	
Folin-Reagent [*Molybden-Tungsten-Ions*]		2.5 ml		0.05 ml	
Natrium carbonate [Na_2CO_3]		2.0 ml		0.1 ml	

*Extracted volume of Dilution II is the sum of the diluted extracts of dilution I

The assay results in a blue solution having an absorption maximum in the red and near infrared region (Möbius and Görtges 1974). The concentration of the total phenolic compounds was determined by the use of a spectrophotometer (Hitachi U-1500, Hitachi Ltd., Tokyo, Japan) at 760 nm (P_1-P_3) or at 750 nm (P_4-P_5; CADAS 100, Dr. Lange GmbH, Berlin, Germany). At least for P_4 and P_5 phenol contents were corrected with recovery rates (mean recovery 91 ± 10 %). Total phenol content was expressed in µmol equivalents of quercetin/ g dry weight or in µmol gallic acid equivalents (gae)/ g dry weight. Both measures are compatible as both reference compounds exhibit one hydrolysable phenol moiety accessible to FCR-staining. Therefore, all molarities in this paper are expressed as µmol phenyl equivalent (µmol pe). Dry matter was determined after 2 d of drying at 96°C to weight constancy. Measurements were run in triplicate for all samples.

Determination of the ellagitannin content

All ellagitannin determinations were made by Reichelt-Schwab-reaction according to Bate-Smith (1972). For this purpose, 921 µl of appropriately diluted extracts were acidified with 75 µl of 5 % acetic acid, oxygen was removed by bubbling through of pure nitrogen gas for 10 min. Afterwards the reaction was started by adding of 75 µl of 5 % (m/m) sodium nitrite in a pure nitrogen atmosphere. The reaction was completed after one h resulting in an orange dye. The extinction was measured at λ = 590 nm on a CADAS 100-spectrophotometer. Results were corrected with recovery rates (mean recovery 106 ± 8 %). Ellagitannin contents were expressed in µmol ellagic acid equivalents (eae)/ g dw. Dw was determined as described above. Measurements were run in triplicate for all samples.

Data evaluation

Results of the samples from stands P_1, P_2 and P_3 were evaluated byStatistica 11 (StatSoft Inc., Tulsa, USA) software and for P_4 and P_5 by SPSS® statistics 20.0 (IBM Corporation, New York, USA) was applied.

Evaluation of the samples from forest stands P_1, P_2 and P_3

Statistical evaluation involved the comparison of the total phenol contents between respective growth rings of the discs of a given forest stand. For the comparison of values, „One-way-ANOVA" analysis was run applying the Tukey HSD calculation method for a post-hoc test, identifying homogenous subgroups of the samples. In order to fulfill the requirements of the ANOVA analysis, values were checked for normal distribution (Kolmogorov-Smirnov test), then the variables were checked for the homogeneity of variances and the independence of variables (Barlett's Chi-square Test) (Hartung et al. 2002). Significant differences were investigated at a level of p=0.05.

Evaluation of the samples from forest stands P_4 and P_5

Statistical analysis was done to analyse if there were significant differences in the total phenol and ellagitannin contents across and along the trunk and between outer heartwood contents in a humid and a dry forest stand. Data were first checked for normal distribution by Kolmogorov-Smirnov tests for both forest stands, individually. Variances were checked for homogeneity, by applying Levene's test. If distributions didn´t differ significantly from the normal and variances were homogene, samples were analysed for significant differences by ANOVA, followed by post hoc Scheffé's test in case of more than two groups. If normal distribution could not be evidenced, the Kruskal Wallis tests were applied. Significant differences were stated atthe p≤ 0.05 level.

RESULTS

Radial distribution of phenolic compounds

Respecting the tissues of the heartwood, juvenile wood (growth rings P_1_4, P_2_4 and P_3_4) contained the lowest average amounts of phenolic compounds (P_1_4: 163.3 µmol pe/ g dw; P_2_4: 139.9 µmol pe/ g dw; P_3_4: 170.0 µmol pe/ g dw). The phenol content of the growth rings P_1_12 (227.5 µmol pe/ g dw), P_2_12 (185.3 µmol pe/ g dw) and P_3_12 (231.6 µmol pe/ g dw) showed only small differences compared to P_1_16 (204.8 µmol pe/ g dw), P_2_16 (189.7 µmol pe/ g dw) and P_3_16 (226.6 µmol pe/ g dw). In the case of the stands P_1 and P_3 higher total phenol levels could be determined in the samples P_1_12 and P_3_12 compared to samples P_1_16 and P_3_16. Regarding forest stand P_2 the P_2_16 sample had only slightly higher concentration of phenols than sample P_2_12. The samples „Middle“, involving growth ring 36, was onlyanalysed in the case of the P_1 stand. This sample had a total phenol level of 249.2 µmol pe/ g dw. The sample „SHB“ (sapwood/ heartwood boundary) was analysed for stands P_1 and P_2, resulting values of P_1_SHB: 308.4 µmol pe/ g dw and P_2_SHB: 251.7 µmol pe/ g dw, which were the overall highest values inside the heartwood. Sapwood samples had significantly lower concentrations of polyphenols, compared to heartwood samples (P_1_Sapwood: 53.5 µmol pe/ g dw; P_2_Sapwood: 66.0 µmol pe/ g dw; P3_Sapwood: 36.8 µmol pe/ g dw).

In Fig. 3 the radial distribution of the phenolic extractives in the defined growth rings of all the discs and forest stands (P_1, P_2 and P_3) is shown. Juvenile wood (Total of growth ring_4) had an average of 157.7 µmol pe/ g dw. There is only a slight difference between the concentrations measured in growth ring 12 (214.8 µmol pe/ g dw) and growth ring 16 (207.0 µmol pe/ g dw). The total phenol content of the samples „Middle“ corresponds to the value of P_1_Middle (249.2 µmol pe/ g dw). Samples of the sapwood/ heartwood boundary showed an average concentration of 280.1 µmol pe/ g dw, whereas sapwood had an average of 42.1 µmol pe/ g dw.

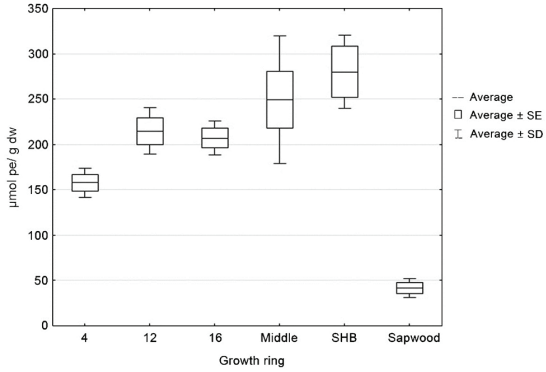

Fig. 3: Box plot of the radial distribution of the total phenol content in the growth rings involving all trees and stands (P_1, P_2 and P_3).

Number of samples: Growth ring 4 (n = 15)
Growth ring 12 (n = 15)
Growth ring 16 (n = 15)
Growth ring „Middle“ (n = 5)
Growth ring „SHB“ (n = 10)
Growth ring Sapwood (n = 15)

Generally, a steady increase of the total phenol concentration from the pith towards the outer heartwood tissues was observed. The difference in the concentration between juvenile wood and sapwood/ heartwood boundary was 78 % in average. The increase was significant at the $p \leq 0.05$ level (Tab.5).

Tab. 5: Total phenol contents of different growth rings for all stands (P_1, P_2 and P_3). Table indicates the results of the Post-Hoc test using Tukey HSD method for unequal N of the One way ANOVA analysis.

Growth ring	4	12	16	Middle	SHB	Sapwood
4						
12	0.02					
16	0.05	1.00				
Middle	0.03	0.85	0.70			
SHB	0.00	0.03	0.01	0.90		
Sapwood	0.00	0.00	0.00	0.00	0.00	

Significant differences at α=0.05 are indicated by grey colour.

There was also a significant drop of the concentration between sapwood/ heartwood boundary and sapwood, involving an almost 7-fold change (Tab. 5; Fig. 3). Basing on the results the average total phenol content of Sweet chestnut heartwood was calculated 221.6 µmol pe/g dw, while the average total phenol content of Sweet chestnut wood (involving all tissues of the heartwood and sapwood) was found 191.8 µmol pe/ g dw.

Statistical evaluation of defined growth rings of all the discs and forest stands (P_1, P_2 and P_3) revealed that sapwood has significantly lower content of phenolic extractives compared to the heartwood. The growth rings 4 and 12, 12 and „SHB" as well as growth rings 16 and „SHB" were identified as significantly different in terms of their total phenol content. Significant differences were also found between growth ring 4 and „Middle" as well as between growth ring 4 and „SHB". Comparing growth rings 12 and 16, 16 and „Middle" as well as „Middle" and „SHB" no significant differences were indicated, similarly as between growth rings 4 and 16, as well as between 12 and „Middle" (Tab. 5).

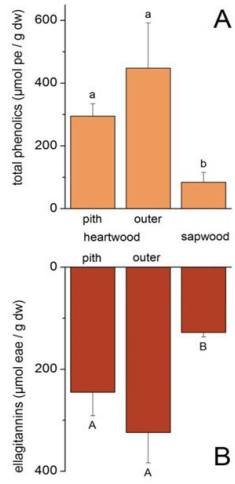

Fig. 4: Radial gradient of total phenolics (A) and ellagitannins (B) in Sweet chestnut from stand P_4.

Means were calculated from 9 (pith and sapwood) or 24 (outer heartwood) samples. Samples were normally distributed and had homogene variances. Therefore, they were compared by ANOVA with post hoc Scheffé's test to identify homgene groups among samples. Different indices indicate significant ($p \leq 0.05$) differences between sample positions across the stem.

The same tendencies were found for stands P_4 and P_5, where there was a significant fourfold increase of total phenolics at the sapwood/ heartwood boundary (Fig. 4_A). This increase could be traced back to the ellagitannins. They were significantly threefold higher in the heartwood than in the sapwood (Fig. 4_B).Towards the pith, there was a non-significant tendency towards a decrease both in total phenolics and in ellagitannins (Fig. $4_{A, B}$).

Total phenolics and ellagitannins in the outer heartwood of trees from stands withdifferent water supply (P_4 and P_5)

The average total phenol content in the outer heartwood from trees grown under humid conditions in stand P_4 was 448.21 µmol pe/ g dw (± 11 %), while the same parameter was determined 612.55 µmol pe/ g dw (± 8 %) for the dry stand P_5. Difference between the two stands of differing aridity were significant, indicating that the outer heartwood of Sweet chestnut trees of the dry stand (P_5) have a 30 % higher total phenol content as compared to the humid stand (P_4; Fig. 5_A). However, the same didn't hold true for the ellagitannins in the outer heartwood, being with 324.05 µmol eae/ g dw (± 11 %) in the humid stand P_4 and 348.02 µmol eae/ g dw (±19 %) nearly the same irrespective of the water supply (Fig. 5_B).

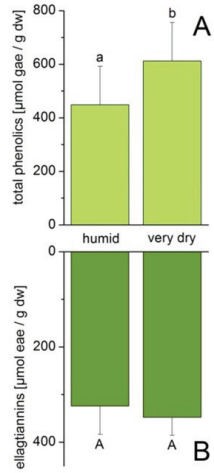

Fig. 5: Total phenolics (A) and ellagitannins (B) in Sweet chestnut from a humid and a dry site.

Means were calculated from samples of 8 individual trees per stand. Samples were normally distributed and had homogenevariances.

Therefore they were compared by ANOVA. Different indices indicate significant ($p \leq 0.05$) differences between total phenolics and ellagitannins.

DISCUSSION

According to the present findings there are an average 200 µmol pe/ g dw of total phenolics in Sweet chestnut wood. This agrees with former investigations, with ca. 240-300 µmol pe/ g dw being observed (Scalbert et al. 1989, Sanz et al. 2010). On the other hand, in the outer heartwood, values go up to 600 µmol pe/ g dw (Fig. 4_A). Considering the total phenol content of the heartwood of other common hardwood species, in Red oak, Wild cherry and Rowan berry there is an average value less than 360 µmol pe/ g dw (Scalbert et al. 1989, Sanz et al. 2010). White oak

species have ca. 240-390 µmol pe/ g dw in the outer heartwood (Scalbert et al. 1989), while e.g. Beech is very poor (5-20 µmol pe/ g dw) in phenolic extractives (Albert et al. 2003). Apparently, Sweet chestnut is among the tannin richest European tree species (Böhm et al. 2013). This is of special interest for cooperage flavors (vanillin, eugenol) that emanate from toasting (Vivas 1997). In agreement with this, wines aged in Sweet chestnut barriques were the richest in the respective flavors (Binder 2013).

The most important tannins in Sweet chestnut wood are the ellagitannins (Mayer et al. 1967). The present study showed that there are up to 350 µmol eae/ g dw in the outer heartwood of this tree species. Mostlyellagitannincontents in Sweet chestnut range between 60 and 250 µmol eae/ g dw, pointing out a good agreement with the present results (Scalbert et al. 1989, Sanz et al. 2010). Besides Sweet chestnut, ellagitannins are found in White oak and Black locust, whereas most Maple ssp., Red oaks and Wild cherry are virtually free of these compounds (Bate-Smith 1977, Scalbert et al. 1989). Ellagitannins are important for the aging of alcoholic beverages, providing them bogy and colour (Vivas 1997). This makes it clear, why Sweet chestnut ranges among the prominent cooperage wood species (Sacchse 1984). Furthermore, keeping in mind, that cooperage is at least in France and Rhineland-Palatinate (South West Germany) the major use of high value oak timber, Sweet chestnut appears to have the capacity for an extra substantial timber profit, as it is not yet at its full value (Phan-Hoang et al., 2007, Rérat 2008).

Results of the present study outlined that there is a continuous radial increase of the total phenol and ellagitannin content inside the heartwood, from pith towards the sapwood/ heartwood boundary. On the other hand, the sapwood contains significantly lower amounts of total polyphenols and ellagitannins as compared to the heartwood. This contradicts former findings, that there are similar ellagitannin contents in Sweet chestnut heartwood and sapwood. It rather underlines the current cooperage practise to reject sapwood from stave making (Böhm et al. 2013). In snowslide timbering the same holds true for the use of Sweet chestnut fascines, as ellagitannins were found the only fungicide compound in the wood (Hart and Hillis 1972).

Similar results concerning the within heartwood total phenolics and ellagitannindistribution in Sweet chestnut were published by Peng et al. (1991). Viriot et al. (1994) also confirm that the highest total ellagitannin concentrations could be measured in the sapwood/ heartwood transition zone inside the heartwood of Sweet chestnut and the concentration decreases towards the juvenile wood. In Black locust heartwood there is also an increasing concentration of extractives from pith towards the sapwood/ heartwood boundary (Scheffer et al. 1944). Peng et al. (1991) and Viriotet al. (1994) established the same tendency for ellagitannins in Sessile oak. Basing on the results of Hancock (1957) as well as of King and Clark-Lewis (1955) the radial increase of extractives (*Leucoanthocyanidin* and *Dihydroquecetin*) from pith towards the sapwood/ heartwood boundary was also evidenced for Douglas fir heartwood (Lelis 1995). Apparently, reactions of the heartwood formation are not confined to the sapwood/ heartwood boundary, but proceed with years, resulting in distinct features of the outer heartwood and the pith and/ or are going on differently in juvenile and adult wood (inner and outer heartwood). In the case of Oak species it has been hypothesized, that proceeding pithifying is due to tannin polymerization and incrusting into the cell walls. This results in decreasing contents of extractable total phenolics and ellagitannins (Mosedale et al. 1996). The juvenile heartwood hypothesis would lead to the finding, that young wood stores less extractable total phenolics (especially ellagitannins) than adult wood. However, this hypothesis needs to be more elucidated.

The present study showed that there were distinctly more total phenolics in the wood from a dry stand than in the wood of a humid stand (Fig. 5). Similar results were found for USA grown White oak samples: wood total phenol content depended on summer precipitation,

summer temperature and summer aridity (Miller et al. 1992). The same observation was made for Sessile oak stands in Rhineland-Palatinate (Böhm et al. 2013). Obviously, coloured-heartwood forming ring-porous broadleaved species (e.g. Chestnut, Oak species) store more extractable total phenolics under climatic constraints. A background for this could be that the pentagalloyl glucose forming galloyltranferase has a high temperature optimum of above 30°C (Schmidt et al. 1987). This is more probable in warm and dry stands. However, such a context remains to be elucidated.

However, there were no elevated ellagitannin amounts in the heartwood of trees from an arid site (Fig. 5 $_B$). The same observation was made for other volatile wood compounds in Sessile and Pedunculate oak (Prida et al. 2007). Obviously the phenol reaction to enhanced aridity does not necessarily imply a role for ellagitannin accumulation. It may be hypothesized instead, that the enhancement corresponds more to structural phenyl moiety bearing carbohydrates.

CONCLUSIONS

1. The aim of the present research was to investigate into the radial distribution of total phenol and ellagitannin contents from the pith (juvenile wood) towards the sapwood of Sweet chestnut.
2. It was also investigated if there was a correlation of heartwood phenolics and ellagitannins to environmental conditions (water supply).
3. Altogether 5 forest stands were assigned for the investigations in the Haardt region of the Palatine Northern Vosges (South West Germany), analyzing 5 and 8 sample trees in stands P_1, P_2, P_3 and P_4 and P_5 respectively (N=31).
4. Discs were taken from the trees at a height of 1.3 m. Samples were assigned in the discs either according to defined growth rings (P_1, P_2 and P_3) or to one defined growth ring zone ($P_4$and P_5). Samples were ground, extracted and the total phenol content and ellagitannins were determined, by Folin-Ciocâlteu assayand by Reichel-Schwab-reaction, respectively.
5. A characteristic radial distribution of the total phenol and ellagitannin content was observedin Sweet chestnut wood. Both compounds increase continuously in the heartwood from pith (juvenile wood) towards the sapwood/ heartwood boundary. Between the boundary and the sapwood there was a sharp decrease of the concentrations. Regarding the samples of the stands P_1, P_2 and P_3 (n=72) the average total phenol content of Sweet chestnut heartwood was determined (221.6 µmol pe/ g dw).
6. Significant differences were indicated between the total phenol content of the outer heartwood of the trees from a humid forest stand and a dry forest stand, with the dry stand samples having a 30 % higher total phenol content as compared to the samples of the humid stand. This, however, didn't hold true for ellagitannins, where there was no such gradient visible(P_4 and P_5).

ACKNOWLEDGMENT

The laboratory experiments of this investigation, regarding the plots P_1, P_2 and P_3, were carried out in cooperation with the Faculty of Wood Sciences and with the Institute of Chemistry at the University of West Hungary in Sopron. For their support and the scientific discourse, the authors thank explicitly. The FAWF thanks Mr. J. Edinger from the Rhenish-palatine forest service for his support for tree selection and Mr. M. Jochum for providing the samples in stands P_4 and P_5.

REFERENCES

1. Albert, L., Hofmann, T., Németh, Zs.I., Rétfalvi, T., Koloszár, J., Varga, Sz., Csepregi, I., 2003: Radial variation of total phenol content in beech (*Fagus sylvatica* L.) wood with and without red heartwood, Holz als Roh- und Werkstoff 61: 227-230.
2. Bate-Smith, E., 1972: Detection and determination of ellagitannins. Phytochemistry 11: 1421-1426.
3. Bate-Smith, E., 1977: Astringent tannins of Acer species. Phytochemistry 16: 1421-1426.
4. Binder, G., 2013: Ausbau von Spätburgunder in Holzfässern regionaler Herkunft. Vortrag, 66. Pfälzische Weinbautage: pp 59-62. Landwirtschaftskammer Rheinland-Pfalz und Dienstlesitungszentrum Ländlicher Raum Rheinpfalz. Neustadt an der Weinstraße.
5. Böhm, S., Große, S., Jochum, M., Letzel, T., Müller-Schick, C., Schmarr, H.G., Seegmüller, S., 2013: Fassholz aus Rheinland-Pfalz. Forschungsanstalt für Waldökologie und Forstwirtschaft Rheinland-Pfalz. Trippstadt, 94 pp.
6. Bouffier, V.A., 2005: Die Dannenfelser Esskastanien, Baumzeitung (1): 27-29.
7. DIN EN 350-2, 1994: Dauerhaftigkeit von Holz und Holzprodukten – Natürliche Dauerhaftigkeit von Vollholz. Teil 2: Leitfaden für die natürliche Dauerhaftigkeit und Tränkbarkeit von ausgewählten Holzarten von besonderer Bedeutung in Europa.
8. Endres, H., Howes, F.N., von Regel, C., 1962: Gerbstoffe/ Tanning Materials. In: Die Rohstoffe des Pflanzenreichs. Verlag von J. Cramer. Weinheim, 246 pp.
9. Faix, O., 2007: Chemie des Holzes. In: Taschenbuch der Holztechnik. Pp 47-74, Fachbuchverlag Leipzig im Carl Hanser Verlag. München.
10. Freudenberg, K., 1920: Die Chemie der natürlichen Gerbstoffe. Springer-Verlag. Berlin, 143 pp.
11. Hancock, W.V., 1957: The distribution of Dihydroquercetin and a Leucoanthocyanidin in a Douglas-fir tree, Forest Products Journal 7(10): 335-338.
12. Hart, J., Hillis, W., 1972: Inhibition of wood rotting fungi by ellagitannins in the heartwood of *Quercus alba*, Phytopathology 61: 620-626.
13. Hartung, J., Elpelt, B., Klösener K.H., 2002: Statistik – Lehr- und Handbuch der angewandten Statistik. Oldenbourg Verlag. München, Wien, 928 pp.
14. Higuchi, T., 1996: Biochemistry and molecular biology of wood. Springer-Verlag. Berlin, Heidelberg, New York, 307 pp.
15. Hon, D.N.S., Shiraishi, N., 2001: Wood and cellulosic chemistry. Dekker. New York, 914 pp.
16. Kramer, H., 1988: Waldwachstumslehre. Verlag Paul Parey. Hamburg und Berlin, 374 pp.
17. King, F.E., Clark-Lewis, J.W., 1955: The constitution and synthesis of Leucoanthocyanidins, Journal of the Chemical Society (10): 3384-3388.
18. Kennedy, R.W., 1956: Fungicidal toxicity of certain extraneous components of Douglas-Fir heartwood,Forest Products Journal 6(2): 80-84.
19. Lelis, R.C.C., 1995: Zur Bedeutung der Kerninhaltsstoffe obligatorisch verkernter Nadelbaumarten bei der Herstellung von feuchtebeständigen und biologisch resistenten Holzspanplatten, am Beispiel der Douglasie. Dissertation. University Göttingen, 237 pp.
20. Lohmann, U., 2003: Holzlexikon. DRW-Verlag. Leinfelden-Echterdingen, 1423 pp.
21. Mayer, W., Seitz, H., Jochims, J.C., Schauerte, K., Schilling, G., 1971: Struktur des Vescalagins, Liebigs Annalen der Chemie 751: 60-68.
22. Mayer, W., Gabler, W., Riester, A., Korger, H., 1967: Die Isolierung von Castalagin, Vescalagin, Castalin und Vescalin, Liebigs Annalen der Chemie 707: 177-181.

23. Mayer, W., Lauer, K., Gabler, W., Panther, U., Riester, A., 1959: Castalagin und Vescalagin, zwei neue, kristallisierte Ellagengerbstoffe aus dem Holz der Edelkastanie und Eiche, Die Naturwissenschaften 46(24): 669-670.
24. Militz, H., Busetto, D., Hapla, F., 2003: Investigation on natural durability and sorption properties of Italian Chestnut from coppice stands, Holz als Roh- und Werkstoff 61 (2): 133-141.
25. Miller, D., Howell, G., Michaelis, C., Dickmann, D., 1992: The content of phenolic acid and aldehyde flavor components of White oak as affected by site and species, American Journal of Enology and Viticulture 43: 333-338.
26. Möbius, C., Görtges, S., 1974: Phenolbestimmng für die Praxis, Die Wein-Wissenschaft 29: 241-251.
27. Mosedale, J., Savill, P., 1996: Variation of heartwood phenolics and oak lactones between the species and phenolical types of *Quercus petraea* and *Quercus robur*, Forestry 69: 47-55.
28. Mosedale, C., Charrier, B., Crouch, N., Janin, G., Savill, P., 1996: Variation in the composition and content of ellagitannins in the heartwood of European oaks (*Quercus robur* and *Quercus petraea*). A comparison of two French forests and variation with 2 heartwood age, Annales des Sciences Forestières 53: 1005-1018.
29. Peng, S., Scalbert, A., Monties, B., 1991: Insoluble ellagitannins in *Castanea sativa* and *Quercus petraea* woods, Phytochemistry 30(3): 775-778.
30. Phan-Hoang, D., Block, J., Eder, W., von Lüpke, B., Muth, M., Schuck, J., Seifert, T., Spiecker, H., 2007: Eiche im Pfälzerwald. Mitteilungen der Forschungsanstalt für Waldökologie und Forstwirtschaft Rheinland-Pfalz 63. 150 pp.
31. Prida, A., Ducousso, A., Petit, R., Nepveu, G.,Puech, J.L., 2007: Variation in wood volatile compounds in a mixed oak stand: Strong species and spatial differentiation in whisky-lactone content, Annales of Forest Science 64: 313-320.
32. Rérat, B., 2008: Französischer Holzmarkt wirft viele Fragen auf. Holz-Zentralblatt 134: 1254.
33. Sachsse, H., 1984: Einheimische Nutzhölzer. Verlag Paul Parey. Hamburg und Berlin. 160 pp.
34. Sanz, M., Cadahía, E., Esteruelas, E., Muñoz, Á.M., Fernández de Simón, B., Hernández, T., Estrella, I., 2010: Phenolic compounds in chestnut (*Castanea sativa* Mill.) heartwood. Effect of toasting at cooperage, Journal of Agricultural and Food Chemistry 58: 9631–9640.
35. Scalbert, A., 1992: Tannins in woods and their contribution to microbial decay prevention, Basic Life Sciences 59: 935-952.
36. Scalbert, A., 1991: Review article number 63 – Antimicrobial properties of tannins, Phytochemistry 30(12): 3875-3883.
37. Scalbert, A., Monties, B., Janin, G., 1989: Tannins in Wood: Comparison of Different Estimation Methods, Journal of Agricultural and Food Chemistry 37(5): 1324 – 1329.
38. Scheffer, T.C., Lachmund, H.G., Hopp, H., 1944: Relation between hot-water extractives and decay resistance of Black locust wood, Journal of Agricultural Research 68(11): 415-426.
39. Schmidt, S., Denzel, K., Schilling, G., Gross, G., 1987: Enzymatic sysnthesis of 1,6-Digalloylglucose from b-Glucogallin by b-Glucogallin:b-Glucogallin 6-0-Galloyltransferase from oak leaves, Zeitschrift für Naturforschung 42c: 87-92.
40. Singleton, V.L., Rossi, J.A., 1965: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, American Journal of Enology and Viticulture 16: 144-158.

41. Tillmanns, H.J., 1957: Die natürliche Dauerhaftigkeit einiger Kernhölzer in Beziehung zur Lage im Stamm. Dissertation. Universität Göttingen, 57pp.
42. Trendelenburg, R., 1955: Das Holz als Rohstoff. Hanser-Verlag. München, 499 pp.
43. Viriot, C., Scalbert, A., Hervé du Penhoat, C., Moutounet, M., 1994: Ellagitannins in woods of sessile Oak and Sweet chestnut dimerization and hydrolysis during wood ageing, Phytochemistry36(5): 1253-1260.
44. Viriot, C., Scalbert, A., Lapierre, C.L, Moutounet, M., 1993: Ellagitannins and lignins in aging of spirits in oak barrels, Journal of Agricultural and Food Chemistry 41: 1872–1879.
45. Vivas, N., 1997: Manuel de tonnelerie à usage des utiliteurs de futaille. ÉditionFéret. Bordeaux. 165 S.
46. Wagenführ, R., 2007: Holzatlas. Fachbuchverlag Leipzig im Carl Hanser Verlag. München, 242-243 pp.
47. Wambsganß, W., Eichhorn, S., Hapla, F., 2013: Vermarktung der Edelkastanie (*Castanea sativa* Mill.) in der Region Haardt, AFZ-DerWald (16): 15-17.

Simon Eichhorn
Georg-August-University Göttingen
Faculty of Forest Sciences and Forest Ecology
Section Wood Biology and Wood Products
Büsgenweg 4
37077 Göttingen
Germany

Sebastian Erfurt
Georg-August-University Göttingen
Faculty of Forest Sciences and Forest Ecology
Section Wood Biology and Wood Products
Büsgenweg 4
37077 Göttingen
Germany

Tamás Hofmann*
University of Sopron
Institute of Chemistry
Bajcsy-Zsilinszky 4
9400 Sopron
Hungary
Corresponding author: hofmann.tamas@uni-sopron.hu

Stefan Seegmüller
Rhenish-Palatine Research
Institute of Forest Ecology and Forestry (Fawf)
Hauptstrasse 16
67705 Trippstadt
Germany

Róbert Németh
University of Sopron
Institute of Wood Sciences
Bajcsy-Zsilinszky 4
9400 Sopron Hungary

František Hapla
Georg-August-University Göttingen
Faculty of Forest Sciences and Forest Ecology
Section Wood Biology and Wood Products
Büsgenweg 4
37077 Göttingen
Germany