# STORAGE AND ACCUMULATION OF NONSTRUCTURAL CARBOHYDRATES IN TRUNKS OF *FAGUS SYLVATICA* L. IN RELATION TO DISCOLOURED WOOD

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## ABSTRACT

Discoloured wood in living trees is formed extensively in ripewood species, one of the best known examples is *Fagus sylvatica* L. The usability of beech wood is strongly influenced by discolouration which represents a serious defect originating in the living stem. The formation of discoloured wood has a profound influence on the distribution of soluble sugars. The radial variation of the quality and quantity of dissoluble carbohydrates has been compared in beech stems with and without red heartwood. We have examined the total dissoluble sugar contents with OPLC and ion-chromatography, identified and quantified glucose, fructose, sucrose, raffinose and stachyose in the wood tissues. In the beech stems without red heartwood a smaller change of concentrations could be detected in a wide tissue range. Lower levels of sucrose have been found in the red-heartwooded beech, than in the stems without red heartwood. The biochemical transformations of the sugars in the red-heartwooded beech take place at the colour boundary, in a narrow tissue range, resulting a significant decrease in the concentrations of these compounds. The soluble sugars may function as precursors for the formation of extractives which are deposited during the process of discolouration.

Keywords: beech, heartwood formation, red heartwood, non-stuctural carbohydrates, glucose, fructose, sucrose, raffinose, stachyose, OPLC, ion-chromatography.

## INTRODUCTION

The red heartwood is the most damaging structural and colour anomaly of living beech causing a considerable loss of value of the stands and significantly influencing the profitability of beech cultivation. The molecular processes being carried out during the formation of red heartwood provide important data to elucidate the physiology of the discolouration. The significant decrease of the moisture content in a narrow range in front of the colour boundary is peculiar to the formation of red heartwood [1]. Several measurements have proved that intensive chemical and biochemical reactions take place in these tissues. Acidity decreases [2], the pH rises [3], the concentration of the phenoloids increases [4], large amounts of dissoluble carbohydrates accumulate [5], and the activities of the enzymes oxidizing phenols (e.g. peroxidase: E.C. 1.11.1.7. and polyphenol-oxidase: E.C. 1.10.3.1.) increase steeply [6]. These results suggest that the polymerisation of the quinons forming in the enzymatic oxidation of the phenoloids (flavonoids) lead to the formation of red chromophores of the heartwood [7] [8]. Nonstructural carbohydrates play an important role in the chemical reactions being responsible for the formation of the red heartwood. As precursors the carbohydrates supply carbon atoms to the synthesis of the phenoloids. Their condensation with flavonoids to glycosides increases the solubility of the derivatives in aqueous medium and makes the transport of these compounds possible. In case of red heartwood tissues, the alteration of concentration of dissoluble carbohydrates have been investigated in radial direction. Five nonstructural

carbohydrates could be identified (glucose, fructose, succrose, raffinose and stachyose) and radial alterations of their concentrations have been measured. Comparative investigations have proved that the radial alterations of the concentrations of the carbohydrates in red-heartwooded beech and in the beech without red heartwood significantly differ from each other.

## EXPERIMENTAL

**Wood samples** The investigated beech trees originated from the forest district of Sopron (Hungary) and were felled in January months of 2002 and 2003. The ages of trees were between 90-100 years. The sample discs were taken from from the stems at a height of 3 meters and their diameter varied between 30-50 cm. Three red-heartwooded and three non red-heartwooded discs were investigated in January of 2002 and one of each type in January of 2003. The discs were taken to the laboratory immediatelly after collection, sectioned, rasped and extracted within one day. Concerning the division of discs into some parts Fig. 1. and Fig. 2. show the positions of the sampling and the letter marks belonging to them.

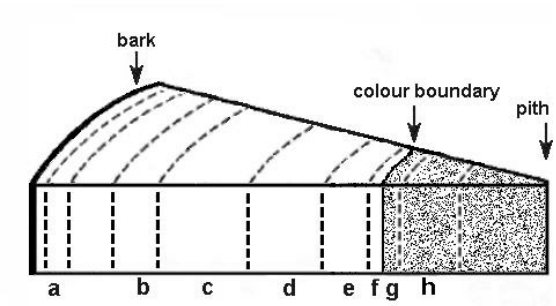


**Fig.1. The graphic description of the wood samples in the red-heartwooded beech disk. a: outer sapwood, b: inner sapwood, c: transitional zone, d: outer heartwood, e: inner heartwood, f: red heartwood boundary white, g: red heartwood boundary red, h: inner red heartwood**

### Materials and methods

***Chemicals:*** All applied chemicals were of analytical grade, and were purchased from Reanal (Budapest, Hungary), Sigma (Deisenhofen, Germany) and Merck (Darmstadt, Germany).

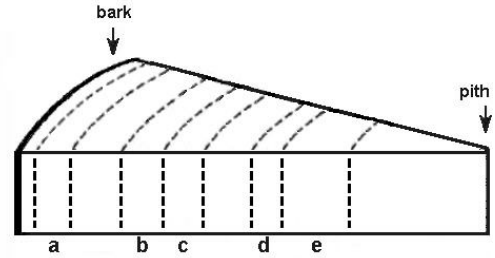


**Fig.2. The graphic description of the wood samples in the beech disk without red heartwood. a: outer sapwood, b: inner sapwood, c: transitional zone, d: outer heartwood, e: inner heartwood**

***Equipment:*** Scanning Spectrophotometer (Shimadzu UV 3101 PC); Optimum-Performance Laminar Chromatograph (Personal OPLC NIT Basic System 50, CAMAG TLC Scanner 3 densitometer, winCATS 1.2.3 softver); Extractor (Dionex ASE 200); ion-chromatograph (HPIC Dionex CHROMELEON Version 6.40).

### Extraction

***a.) Dissoluble total carbohydrates determination.*** 0.25g of wood grist fractions were extracted with 25ml 80% aqueous methanol for 6 hours using Variomag Poly15 magnetic stirrer. The extracts were filtered through Whatman GF/A grade glass fibre filter.

***b.) Dissoluble carbohydrates* I.** 0,05g of wood grist fractions were homogenized with 1ml 80% aqueous methanol. Samples were extracted for 10 minutes by sonication (Elma T570), and then centrifuged for 15 minutes (3000/min).

***c.) Dissoluble carbohydrates* II.** 0,2 g of freeze-dried wood grist fractions were extracted with 75% aqueous methanol for 2x30 minutes in 40 °C, pressure: 50 bar, using Dionex ASE 200 extractor. The extracts were inwashed with 75% aqueous methanol in the 25 ml measuring flask. 15 ml of solution was evaporated to dryness in 40°C. The rest was dissolved in 5 ml distilled water and was filtered through 0,2 µm filter.

### Analysis

***a.) Dissoluble total carbohydrates.*** Dubois-method [9], using glucose as standard.

***b.) Dissoluble carbohydrates I.*** The supernatant was analysed on TLC silica gel by Personal OPLC NIT BASIC SYSTEM 50 (optimum-performance laminar chromatography, pressure:50 bar, mobile phase: 85:15 acetonitrile:water) applying one dimensional overrun development. Visualization by spraying the plates with reagent (composition: 4 g diphenyl-amine, 4 ml aniline, 20 ml o-phosphoric acid (86%), dissolved in 200 ml acetone) and by heating at 112$^{o}$C for 5 minutes. Quantitaive evaluation with CAMAG TLC Scanner 3 Densitometer operating with winCATS software, in absorption mode, $\lambda$=540nm.

***c.) Dissoluble carbohydrates II.*** In case of the samples from ***c*** extraction the analysis of dissoluble carbohydrates have also been carried out by ion chromatography. Investigation of the content of total carbohydrate and OPLC measurements and ion chromatographic separations have been carried out in Institute for Cemistry, University of West Hungary and Federal Research Centre for Forestry and Forest Products, Hamburg and Department of Genetics, Szent István University.

## RESULTS AND DISCUSSION

1. The radial distribution of the concentration of total dissoluble carbohydrates have been measured in red-heartwooded beech. In this analysis the samples of 2002 have been investigated. Three different extraction methodes have been applied. The results are presented in Table 1. In case of method **b** and **c** the amount of the total dissoluble carbohydrates has been calculated by summing the quantities of each separated sugar component.

2. The data in Table I. shows the effectiveness of the different extraction methods in decreasing order of **c**>**a**>**b** in all non-discoloured wood samples of red-heartwooded beech.

3. The radial alteration of the total carbohydrate levels measured with method **a** and **b** are similar to each other. The efficiency of the subcritical solvent in extraction **c** varies, substantialy according to the different structural properties of the wood samples (sample **c** and **f**). The steep of the amount of total carbohydrate was bigger next to the colour boundary than in front of its white side and a decrease in the red range after this boundary could be detected in case of all extraction methods (Fig. 3.). This phenomena could not be experienced in the non red-heartwooded beech.

**TABLE I. THE RADIAL ALTERATION OF THE TOTAL CARBOHYDRATE CONTENTS [µg/g wood] MEASURED WITH DIFFERENT EXTRACTION METHODS IN THE RED-HEARTWOODED BEECH DISK.**

| | a | b | c | d | e | f | g | h |
|---|---|---|---|---|---|---|---|---|
| a) extraction | 32305,60 | 28438,40 | 23917,40 | 22490,50 | 18729,10 | 19317,50 | 5905,11 | 4804,96 |
| b) extraction | 26147,06 | 19491,25 | 14619,04 | 10987,41 | 8988,48 | 8917,79 | 0 | 0 |
| c) extraction | 48924,29 | 45237,40 | 29844,83 | 32676,76 | 37741,64 | 44657,75 | 1643,52 | 1111,53 |

**STANDARD DEVIATION:**

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| a) extraction | 1696,50 | 989,02 | 1678,78 | 1165,74 | 897,73 | 1831,19 | 605,49 | 950,16 |
| b) extraction | 4644,57 | 2822,90 | 1313,06 | 2087,71 | 387,29 | 1656,27 | 0 | 0 |
| c) extraction | 3949,56 | 3073,04 | 1706,13 | 3676,48 | 11677,42 | 5122,69 | 526,16 | 547,40 |

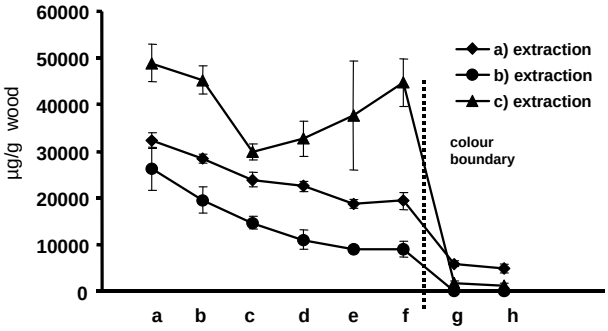


**Fig.3. The radial distribution of the total carbohydrate contents extracted with different methods from the red-heartwooded beech samples.**

Using optimum performance laminar chromatography (OPLC) five different non-structural carbohydrates could be separated from the samples collected in January of 2003: fructose, glucose, sucrose, raffinose and stachyose (Fig. 4.). These compounds could also be detected in the stems containing no discolouration. The radial levels of glucose, fructose and sucrose (Fig. 5.) are commensurable in tendency to the concentration of the total dissoluble carbohydrate (Fig. 3.). The concentration all of the three sugars increase before the coloured wood boundary and decrease steeply afterwards. This was also the trend with raffinose and stachyose being present in much lower concentrations in beech wood. In the stems, which did not contain red heartwood, none of the above sugar levels showed up an increase in the ripewood. Significant differences could be found in the levels and the radial alteration of the sugar components. In case of beech with and without redheartwood glucose, fructose and sucrose are the components being present in the highest concentrations. The concentration of these carbohydrates decreased from the transition sone (sample **c**). In the beech containg no red heartwood this decrease is continual and monotonic.

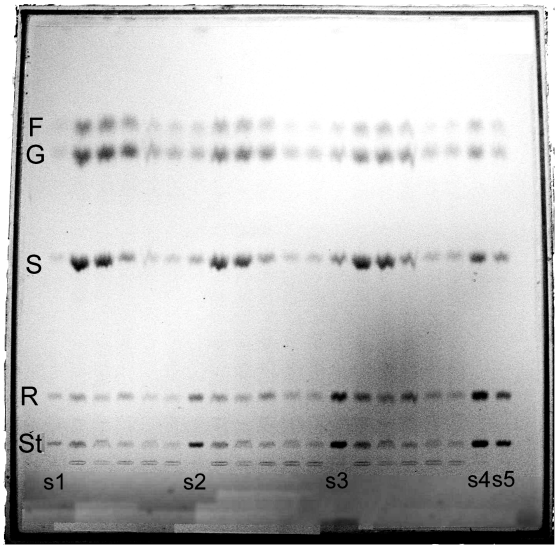


**Fig.4. The OPLC separation of the sugars extracted from red-heartwooded beech tissues. (F: fructose, G: glucose, S: sucrose, R: raffinose, St: stachyose; s1, s2, s3, s4, s5: standard tracks).**

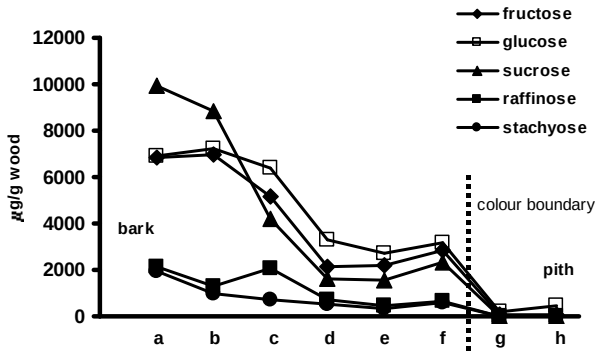


**Fig.5. The radial alteration of the soluble carbohydrates in the red-heartwooded beech.**

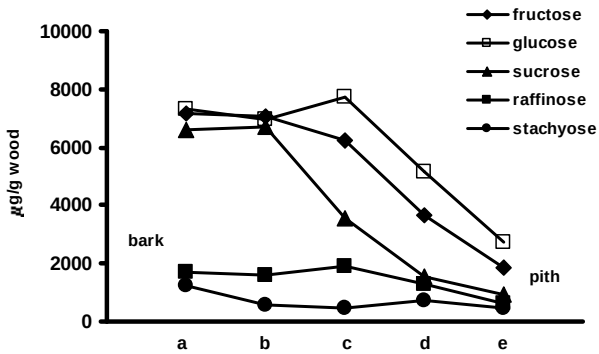


**Fig. 6. The radial alteration of the soluble carbohydrates in the beech without red heartwood.**

## CONCLUSIONS

1. Based on the amount of the total dissolved carbohydrates the effectiveness of three extraction methods have been compared. Significant differences have been found among the methods of which the subcritical method was the most effective. All the methods are suitable to describe the radial tendencies of the concetration.

2. Applying OPLC technique glucose, fructose, succrose, raffinose and stachyose have been identified and quantified in the tissues of beech both with and without red heartwood.

3. The radial alteration of dissoluble carbohydrate concentrations in red-heartwooded beech is significantly different from that of non-red-hertwooded beech. In red-heartwooded beech the amount of the total dissoluble carbohydrates rises before the colour boundary and decreases behind it sharphly. In beech containing no red heartwood a continuous and monotonic decrease can be found towards the pith.

4. The amount of the carbohydrates is negligable in the red heartwood. In the non-discoloured beech high concentrations of carbohydrates can be found next to the pith.

## ACKNOWLEDGEMENTS

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