# Testing different methods for the extraction and purification of leaf and phloem sugars for oxygen isotope analysis

Melanie Egli [1], Marco M. Lehmann [2], Nadine Brinkmann [1], Roland A. Werner [3], Matthias Saurer [2] & Ansgar Kahmen [1]

[1] *Department of Environmental Sciences – Botany, University of Basel, Basel, Switzerland*
[2] *Forest Dynamics, Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland*
[3] *Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland*

## 1. Introduction

Stable oxygen isotopes are key players in environmental sciences. When analysed in plant sugars, they enable tracing those molecules through different plant tissues. Because carbohydrates exchange oxygen atoms with their environment, they enable invaluable insights into how, when, and where they interact with their environment. Hence follows that it is crucial to understand which steps (during sampling or in the laboratory) might unintentionally alter stable oxygen isotopes.

### Aims

- ⚠ **Highlight** potentially **problematic work steps** when analysing **oxygen stable isotopes** – from **sampling leaf + phloem tissue** to **obtaining** their **sugars' final $\delta^{18}O$ ratios**
- ⚠ Analyse **leaf + phloem sugars' $\delta^{18}O$** and **quantify** the **effect** of **different techniques** used in the **laboratory**

## 2. Experimental

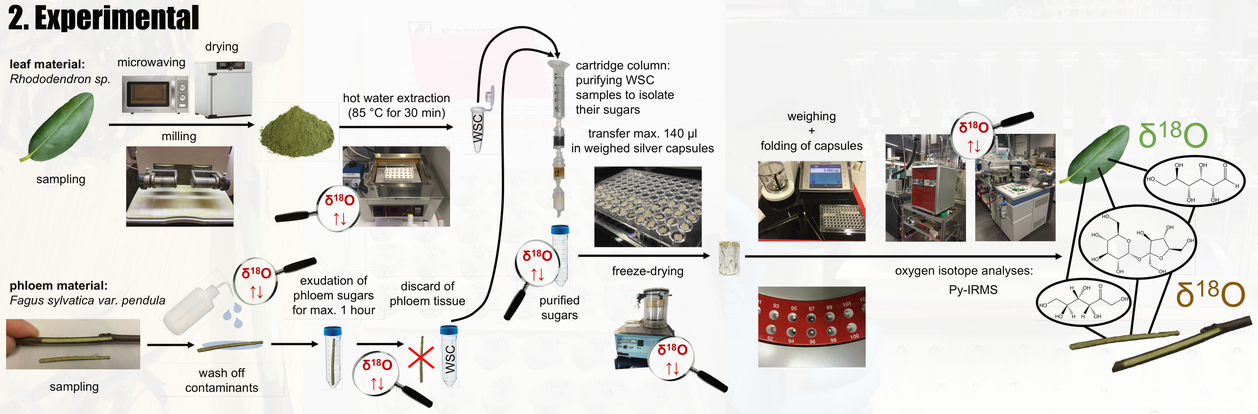


**Figure 1:** Schematic illustrating the process of oxygen isotope analysis for leaf and twig phloem sugars. Potential 'pitfalls' which might unintentionally offset the sugars' oxygen isotopic composition are highlighted by the magnifying glasses and investigated further in the figures 2-7 below. WSC stands for water-soluble content which consists of amino acids, organic acids, phenolic compounds, and sugars. Purification of the sugars is achieved using SPE (solid phase extraction) whereby three consecutive cartridges with sorbents retain phenolic and ionic compounds.

## 4. Conclusions

- ✓ **Freeze-drying** seems **more reliable** than oven-drying as it provided **more consistent** oxygen isotope signatures **($\delta^{18}O$)** in sucrose + phloem sugars.
- ✓ Overall, **less dissolution + drying steps** are **advisable**, otherwise **$^{18}O$-depletion** in leaf sugars might occur.
- ✓ For the **extraction** of **WSC** (= water-soluble content), **temperature** (85 °C) + **duration** (30 min) appear to **not affect** the **$\delta^{18}O$ values** of **sucrose**.
- ✓ **Purification** by **SPE cartridges** is **essential** to remove unwanted compounds (incl. EDTA to increase sugar yields). **Leaf + phloem sugars** were **successfully isolated** – **purified phloem sugars' +** pure **sucrose's $\delta^{18}O$ values** seem **unaltered**.
- ✓ The **most consistent $\delta^{18}O$ values** for **bulk phloem sugars** were received after **1 hour exudations** in **pure water**.
- ✓ With **increasing time,** the **sugar concentration decreased**, while the **individual compounds' carbon isotopic compositions** were **inconsistent**.

## 3. Results

### 3.1 Drying methods

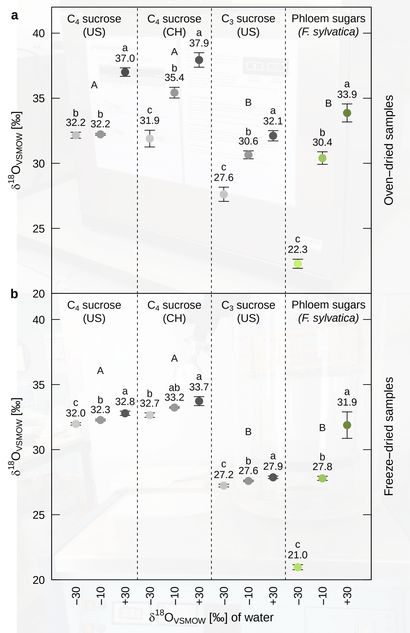


**Figure 2:** Comparison of oxygen isotope values between oven- (a) vs freeze-dried (b) sucrose samples and phloem sugars from a beech (*Fagus sylvatica*) twig (means ± SD, n = 4). The samples were dissolved in different waters with their own distinct oxygen isotopic composition.

### 3.2 Dissolution & drying steps

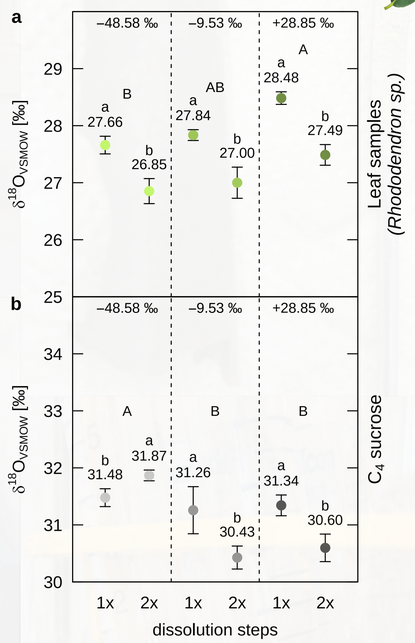


**Figure 3:** $\delta^{18}O$ values of extracted leaf sugars (a) and sucrose (b) which were once and twice dissolved in water of three distinct oxygen isotopic signatures (means ± SD, n = 4).

### 3.3 Extraction

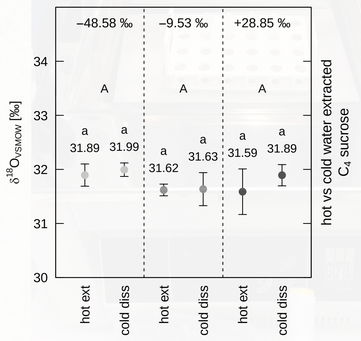


**Figure 4:** Oxygen isotopic signatures of sucrose which was extracted in hot water for 30 min at 85°C (hot ext), and its counterpart that was only dissolved in cold water (cold diss), (means ± SD, n = 4). The samples were extracted / dissolved in water with different oxygen isotopic signature.

### 3.4 Purification

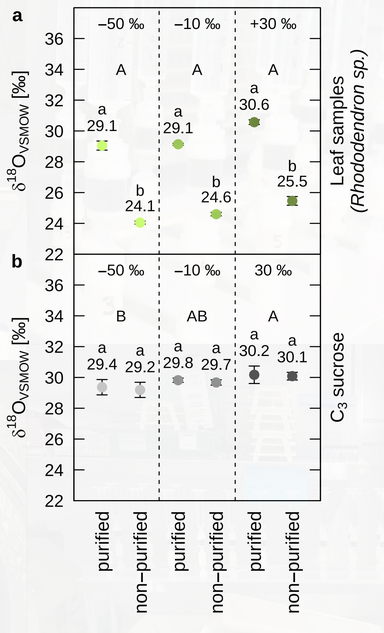


**Figure 5:** Oxygen isotope composition of purified and non-purified leaf samples (a) and sucrose (b). Samples were tested using waters of three distinct $\delta^{18}O$ values (means ± SD, n = 4).

### 3.5 Facilitators & exudation time

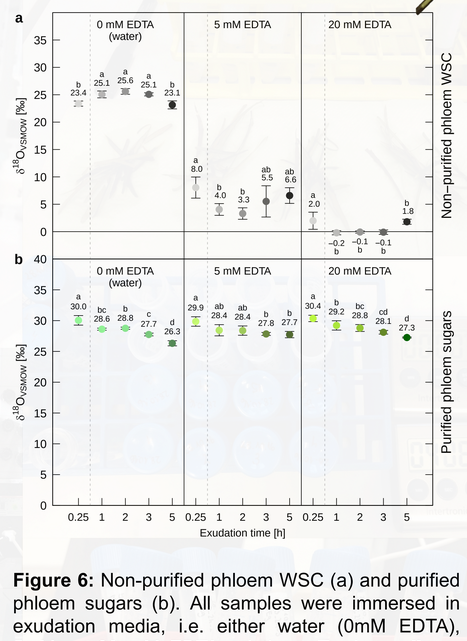


**Figure 6:** Non-purified phloem WSC (a) and purified phloem sugars (b). All samples were immersed in exudation media, i.e. either water (0mM EDTA), 5mM, or 20 mM EDTA solutions. To rinse off any contaminants prior to the sugar exudation, the phloem tissue was immersed for 15 min before they were transferred in fresh media of the same concentrations.

### 3.6 Concentration & $\delta^{13}C$

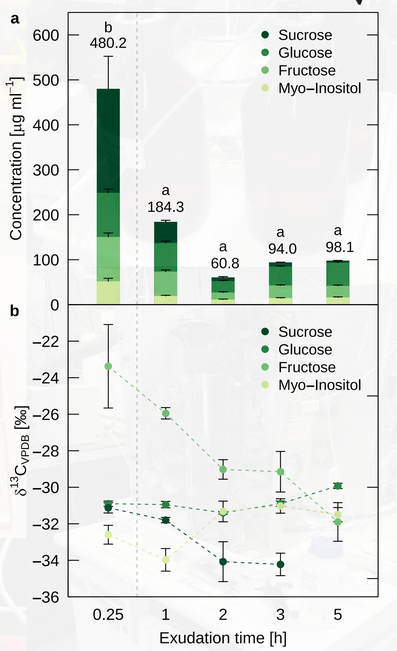


**Figure 7:** Concentration (a) and $\delta^{13}C$ values (b) of individual sugars and a sugar alcohol present in the phloem samples (n = 4). An initial immersion lasted 15 min (to wash off contaminants), followed by a transfer in fresh media for 1, 2, 3, and 5 hours (for sugar exudation).