Analysis of carbon isotope composition of plant carbohydrates

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Starch is the main carbon storage carbohydrate in plants. It is considered as one of the most abundant organic compound on earth, second only to other carbohydrates such as cellulose. The carbon isotope composition of starch has been studied with regard to different photosynthetic pathways and the water-use efficiency of plants mainly in the 1970s and 80s. Recently, the carbon isotope composition of plant carbohydrates has attracted renewed interest, since these compounds are thought to be major substrates of autotrophic respiration. Therefore, the knowledge of the isotopic signature of these compounds is a prerequisite to determine the contribution of heterotrophic respiration to the terrestrial carbon flux.

At present, several methods for the isolation and determination of carbon isotope composition of starch are available. However, a comparison of these methods has shown that the methods yield very different results for both amount and carbon isotope composition. We here report on an inter-laboratory comparison aiming (1) comparing the available methods on both an "artificial" plant material (a mixture of isotopically defined, commercially available plant constituents) and a range of different plant tissues, (2) identifying possible sources of errors for the different methods and (3) recommending a reference method to enhance the comparability of isotope data in this field.

All methods for starch determination, and for isolation of starch from plant material for isotopic measurements, is based on a three step procedure: the removal of soluble sugars by excessive washing with aqueous media, the hydrolysis of starch by acid or enzymatic means, and the purification of the hydrolysate by precipitation of hydrolyzed starch (acid hydrolysis) or removal of enzyme by dialysis or ultrafiltration (enzymatic hydrolysis).

Both approaches, i.e. acids and enzymatic hydrolysis, are prone to several (potential) problems. First, suitable reference materials and reference methods for quantification, and particularly for isotope composition in starch, are lacking. Second, incomplete removal of soluble sugars and other low-molecular weight compounds prior to starch hydrolysis can contaminate of the starch hydrolysates. Third, the methods should selectively hydrolyze starch but leave other high-molecular weight compounds unchanged. Contamination can derive from hydrolysable organic carbon compounds such as hemicelluloses, cellulose, gums

(mucilages, pectins) and condensed polyphenols but also from proteins. Fourth, complete hydrolysation of starch has to be ensured since starch granules may be isotopically inhomogeneous.

From the experiments we conclude:

(1) the methods based on acid hydrolysis and on enzymatic hydrolysis of starch are not comparable with regard to carbon isotope composition and content.

(2) The specificity (selectivity) of the methods based on the acidic hydrolysis was low, and we therefore suggest terming these preparations as HCl-hydrolysable carbon, rather than starch.

(3) The methods based on enzymatic hydrolysis at the moment provide the only feasible way for a compound-specific analysis of isotopes in starch.