CHAPTER 12

DETERMINATION OF SOLUBLE CARBOHYDRATES

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1. INTRODUCTION

Leaf litter and other plant detritus consist primarily of structural polysaccharides and lignin, neither of which are readily accessible to stream invertebrates (Chapters 11, 12 and 17). However, more easily digestible soluble carbohydrates, such as sucrose or glucose are also present in notable concentrations. Initially, these compounds may account for up to 16% of total dry mass (e.g. in hickory leaves; Suberkropp et al. 1976), while leaching can reduce this value by ≥80% within a few days (Gessner 1991). The rate of leaching may be significantly influenced by treatment of the leaves before immersion in the stream (Chapter 5; Gessner 1991, Bärlocher 1997).

During decomposition, microbial enzymes attack detrital polymers, releasing a mixture of oligomeric and monomeric carbohydrates, which again are more accessible to invertebrates than the original polymers (Bärlocher & Porter 1986). In addition, fungi colonizing leaves (which can account for up to 17% of detrital dry mass at intermediate stages of decay; Gessner 1997) contain soluble carbohydrates in their mycelia.

Analysis of total soluble carbohydrates facilitates the quantification of nutritionally valuable carbon fractions of leaf material. Identification of the individual compounds, combined with analyses of hydrolyzed polysaccharides allows characterization of the course of enzymatic breakdown of these leaf constituents. The same methods can also be modified to measure activities of selected degradative enzymes present in microorganisms or invertebrates (Chapter 32).

Two approaches can be taken to analyze soluble carbohydrates. One involves determining total available carbohydrates (Method A) and ignores their composition.
The procedure we present follows a modified method of White & Kennedy (1986). The second approach (Method B) is more specific. It quantifies individual monosaccharides, which can then be used to calculate the total amount of soluble carbohydrates present in a sample (Mansfield et al. 1997). Both methods require that the soluble sugars first be extracted from the lignocellulosic material; the procedure described below follows a modified protocol of Guy et al. (1984).

2. EQUIPMENT, CHEMICAL AND SOLUTIONS

2.1. Equipment and Material

- Freeze-drier
- Analytical balance
- Spectrophotometer
- Rotavap evaporator
- Desiccators (containing phosphorus pentoxide)
- Mortar and pestle
- Test tubes
- Acid-washed glass test tubes (10 ml; wash with 10% nitric acid overnight, then rinse thoroughly with distilled water)
- Test tube rack
- Hot water bath or heated test tube reactor
- Thermometer
- Ice water bath
- Vortex
- Freezer (−20 °C)
- Micropipettors
- Cuvettes (disposable ones are suitable)
- Spectrophotometer (set at 540 nm)
- Laboratory timer or stop watch
- Separatory funnel
- Aluminium foil
- High Performance Liquid Chromatograph (HPLC) with electrochemical detector using pulsed amperometry
- HPLC filters (0.45 µm pore size)
- HPLC vials and caps
- Disposable syringes

2.2. Chemicals

- Glucose
- Sucrose
- Fructose