CHAPTER 14

TOTAL PHENOLICS

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1. INTRODUCTION

Phenolics are a heterogeneous group of natural substances characterized by an aromatic ring with one or more hydroxyl groups. The number of these compounds identified to date exceeds 100,000 (Waterman & Mole 1994). Phenolics may occur as monomers with one hydroxyl group (e.g. ferulic acid). Compounds with several phenolic hydroxyl substituents are referred to as polyphenolics. Among these, tannins (subdivided into phlorotannins, hydrolyzable and condensed tannins) are of particular interest because of various demonstrated or posited ecological effects (Zucker 1988; Chapter 15). In particular, phenolics play a major role in the defence against herbivores and pathogens (Waterman & Mole 1994, Lill & Marquis 2001). In addition, some phenolics such as anthocyanins may prevent leaf damage resulting from exposure to excessive light (Gould & Lee 2002). Since the bulk of phenolics remains present during leaf senescence and after death, these compounds may also affect microbial decomposers (Harrison 1971) and therefore delay microbial decomposition of plant litter (Zucker 1988, Salusso 2000). The amount of phenolics in plant tissues varies with leaf species, age and degree of decomposition. Values for selected plants are summarized in Table 14.1.

A first step in many studies assessing the ecological effects of phenolics is an estimate of the total concentration of phenolic hydroxyl groups. The most commonly used assay for that purpose was originally designed to quantify the phenolic amino acid tyrosine (Folin & Denis 1912). Folin & Ciocalteu (1927) made the assay more sensitive and less prone to formation of precipitates. Preparation of the Folin-Denis or Folin-Ciocalteu reagent is relatively time-consuming, but these reagents are now commercially available (Waterman & Mole 1994). Here we present the procedure introduced by Folin & Ciocalteu (1927).

M.A.S. Graça, F. Bärlocher & M.O. Gessner (eds.), Methods to Study Litter Decomposition: A Practical Guide, 97 – 100.

Table 14.1. Phenolics concentrations in terms of tannic acid or ferulic acid equivalents for selected plant tissues, including senescent leaves (s), live (l) and yellow-green to brown-dead grass leaves (y)

Species	*Common name*	*Phenolics (% leaf dry mass)*	*Reference*
Spartina alterniflora (y)	Smooth cordgrass	0.4—1.5	1
		0.2—1.2	2
Alnus glutinosa (s)	Alder	2.7	3
		6.6	4
		6.8—7.6	5
Sapium sebiferum (l, s)	Chinese tallow tree	3.0	6
Eucalyptus globulus (s)	Eucalyptus	6.4	3
		9.8	4
Fagus sylvatica (s)	Beech	8.0	7
Carya glabra (s)	Hickory	9.1	8
Quercus alba (s)	Oak	16.2	8
Acer saccharum (s)	Sugar maple	15	7

1 = Graça et al. (2000); 2 = Bärlocher & Newell (1994); 3 = Pereira et al. (1998); 4 = Bärlocher et al. (1995); 5 = Gessner (1991); 6 = Cameron & LaPoint (1978); 7 = Graça & Bärlocher (1998); 8 = Suberkropp & Klug (1976).

2. EQUIPMENT, CHEMICALS AND SOLUTIONS

2.1. Equipment and Material

- Eppendorf pipettes
- Vortex
- Refrigerator
- Dried leaves
- Mill or mortar and pestle
- Analytical balance (±0.1 mg precision)
- Eppendorf tubes
- Centrifuge
- Spectrophotometer

2.2. Chemicals

1. Tannic acid standard
2. Acetone
3. Deionized water
4. 2% Na_2CO_3
5. 0.1 N NaOH
6. Folin-Ciocalteu reagent (e.g. Sigma F-9252; diluted 1:2 with deionized water)

3. EXPERIMENTAL PROCEDURES

3.1. Calibration

1. Prepare a stock solution of 25 mg tannic acid in 100 ml of acetone (30% water, 70% acetone).
2. Transfer 0, 0.2, 0.4, 0.6, 0.8, 1.0 ml of the stock solution into 6 Eppendorf tubes and add 1.0, 0.8, 0.6, 0.4, 0.2 and 0 ml of distilled water, respectively. Mix with vortex.
3. Add 5 ml of 2% Na_2C0_3 in 0.1 N NaOH and mix.
4. After 5 min, add 0.5 ml of Folin-Ciocalteu reagent and mix.
5. After 120 min, read absorbance at 760 nm.
6. Plot tannic acid concentration vs. absorbance. The relationship should be linear.

3.2. Measurement

1. Grind up dried leaves. Use powder passing trough a 0.5-mm mesh size screen.
2. Weigh out approximately 100 mg portions of the ground leaves and transfer to Eppendorf tubes.
3. Extract phenolics in 5 ml of 70% acetone for 1 h at 4 °C.
4. Centrifuge (10,000—20,000 *g*, 10—20 min).
5. Take 0.5 ml of the supernatant (or another value between 0.1 and 0.8), make up to 1 ml with distilled water as above.
6. Add Na_2CO_3 and Folin-Ciocalteu reagent as above.
7. After 120 min, read absorbance at 760 nm.
8. Based on the standard curve, determine tannic acid equivalents per mg of leaf powder. Remember that in Step 5, only a fraction (0.5 ml) of the sample was used.

4. REFERENCES

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