THE EFFECT OF SAMPLE HANDLING AND PREPARATION ON THE IRON BINDING POLYPHENOLIC CONTENT OF BROWSE

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Abstract

The objective of this study was to determine feasible handling methods for accurate analysis of iron binding polyphenolics. Seven methods were tested with different combinations of both on-site handling and laboratory preparation. In these methods, on-site collection included freezing the samples with liquid nitrogen or dry ice or collecting the samples fresh and returning them to the lab. Once in the lab, samples were either ground immediately with liquid nitrogen, stored in the freezer, or placed in a drying oven at 60^{o}C. All dried samples were then ground in a Wiley Mill while all other samples were ground with liquid nitrogen. An iron binding assay was then used to determine total iron binding polyphenolic levels and the different processing methods were compared. Methods that involved freezing the samples tended to result in higher concentrations within most plant species versus the methods that used dry samples.

Introduction

As zoos and wildlife centers turn to incorporating browse into captive animal diets, investigating their suitability has become an important part of diet formulation. Species that are considered browsers may consume moderate to high levels of iron binding polyphenolic compounds in the wild. Research has suggested that the absence of these compounds in captive diets may cause health issues such as hemosiderosis.[4,7] Polyphenolic levels vary greatly between species of plants; therefore, sampling and analysis is an essential part of identifying browse that can be safely used in a diet.

Unlike many nutritional components, polyphenolic compounds are sensitive to many environmental factors. They may undergo chemical changes between the field and the lab, i.e. oxidation by sunlight or excessive heat.[2] Because of this, rapid preparation and minimal handling is desired. Several methods have been used to handle samples during collection. Studies have shown that freeze drying is the best preparation method due to the fact that analyzed concentrations do not differ significantly between fresh leaves.[5,8,9] However, further research has shown oven drying and air drying as comparable methods when freeze drying techniques are not available.[5,9]

Several different methods tested on-site handling and laboratory preparation. The objective was to determine which combination of both on-site handling (liquid nitrogen freezing, dry ice storage, fresh sample collection) and laboratory preparation (drying oven, freeze upon return, grind with liquid nitrogen, grind with Wiley Mill) produced the most accurate results of iron binding polyphenolics concentrations.

Methods

Plant Species

Five species of browse were tested in this study: *Carya aquatica*, *Populus deltoides*, *Quercus alba*, *Magnolia grandiflora*, and *Quercus fusiformi*. Samples were collected in July, 2002 at the Fort Worth Zoo (Fort Worth, Texas). The samples consisted of both old and new growth leaves and were randomly selected from several different sections of the plant.

On-site handling methods

Once leaves were cut and sorted to provide a representative sample of the plant they were either immediately frozen with liquid nitrogen, put on dry ice, or placed in dark storage to be taken back to the laboratory as collected fresh.

Laboratory analysis

Preparation methods included (Table 1): dry at 60^{o}C (Fisher Isotemp Oven), freeze at 4^{o}C(Amana freezer), or immediately grind. Samples were either ground into a homogenous mixture using a Wiley Mill with a 1mm sieve grate (GWM), with liquid nitrogen in a blender (Hamilton Beach Commercial), or by mortar and pestle with liquid nitrogen (Latter two methods - GLN). After this initial preparation, samples were stored in a freezer (4^{o}C) or in the dark at room temperature (18^{o}C) if ground with the Wiley Mill. Dry matters of the samples were determined by full desiccation of the samples at 60^{o}C (Fisher Isotemp Oven).

Leaves were randomly divided into 0.5 kg samples for the seven methods tested. Iron binding polyphenolic compounds were determined by the colorimetric method of Hagerman & Butler - using a Beckman DU 520 ultraviolet spectrometer and a gallic acid standard.[3]

Results and Discussion

A lack of replicated samples on a single plant species precluded statistical analysis. In general, grinding with liquid nitrogen appeared to produce higher concentrations in all instances but one (Table 2). A two to six fold difference in concentration ranges within species supported the hypothesis that handling and preparation methods effect iron binding polyphenolic content.

Numerous studies have produced variable and inconclusive results for analysis of oven-dried samples.[1,8,9] The only method shown to result in significantly greater concentrations of polyphenolic compounds is that of freeze-drying. [1,8,9] These studies validate the hypothesis that freeze drying is the optimal method of preparation. Freeze drying, however, many not always be practical or available. This study attempted to define the most favorable preparatory method/methods for accurate polyphenolic analysis if freeze drying is not an option. In the current study, trends of higher concentrations of polyphenolics were obtained from methods using liquid nitrogen to preserve the samples. Therefore, it may be beneficial to store samples in cold temperatures as well as maintain low temperatures throughout the sample processing period. Oven drying may reduce extractable phenolic content explaining the observed decreased concentrations found in treatments two, three, and five.[6]

A difficulty encountered in all polyphenolic analyses is species variation. Studies have shown treatment preparation alone is not the determining factor when measuring phenolic content.

Other factors such as species, time of year, area, condition and age of leaves sampled, and extraction method all contribute to the ability to extract polyphenolic compounds from a particular sample.[1,8,9] Cork and Krockenberger found that handling method has a significant effect on condensed tannin concentration but no significant effect on total polyphenolic concentration.[2] Yu and Dahlgren observed the exact opposite results in their evaluation of measuring polyphenols.[9] Results such as these lead to a more complex issue of polyphenolic chemistry that cannot be covered by this study alone. Further research is needed to refine differences between treatment methods. A more controlled study focusing on differences within species of browse rather than across species would be ideal.

LITERATURE CITED

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Table 1. Collection and laboratory preparation of browse species.

Method	#1	#2	#3	#4	#5	#6	#7
On-Site Collection	Fresh	Fresh	Fresh	Dry Ice	L. Nit	L. Nit	Fresh
Laboratory Prep.	Freeze	Ov. Dry	Freeze Ov. Dry	↓	Ov. Dry	↓	↓
Grinding Method	GLN	GWM	GWM	GLN	GWM	GLN	GLN

Table 2. Iron Binding Polyphenolic concentrations of five species of browse (dry matter basis).

Sample species	Handling and preparation method [a] (mgE gallic acid/g dry sample)						
	#1	#2	#3	#4	#5	#6	#7
C. aquatica	0.444[b]	0.110	0.088	0.425	0.111	0.442[b]	0.444[b]
P. deltoides	1.000[b]	0.381	0.219	0.655	0.165	0.661[b]	0.594
Q. alba	1.660	1.303	1.025	1.922[b]	1.735	1.711	2.123[b]
M. grandiflora	0.401	0.712[b]	0.173	0.428	0.159	0.358	0.653[b]
Q. fusiformi	0.323	0.135	0.115	0.327[b]	0.118	0.299	0.335[b]

[a] Methods listed in Table 1

[b] Results represent the two highest values within a species