Distribution, Quantification, and Identification of Tannins in Acorns from Red and White Oak Trees

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ABSTRACT

A common assumption in studies of seed predation is that seeds survive attack and are dispersed only when animals fail to find seeds, drop undamaged seeds, or fail to recover seeds after they are cached. Previous studies have demonstrated that many acorn consumers consistently eat only a portion of the cotyledon of several species of acorns and thereby permit embryo survival. This study aimed to ascertain the distribution, relative quantity and identity of the tannins that are present in the acorns to determine if there was selectivity in expression in addition to distribution throughout the cotyledon. LC/ESI/MS was performed on the basal, middle, and apical segments of acorns from two species of red oak trees: *Q. rubra* (Northern red oak) and *Q. palustris* (pin oak), and from two species of white oak trees: *Q. macrocarpa* (bur oak) and *Q. muhlenbergii* (chinkapin oak). As previously reported the amount of total tannins increased from basal to apical segments in all species of oak investigated with higher amounts of tannins expressed in red oak acorns as compared to white oak acorns. While the total amounts of quantified tannins were consistently higher in the apical segments, there were variations within the individual tannins with respect to the distribution and degree of expression.

INTRODUCTION

Tannins, as preservers of animal skins, were in commercial use long before there was any clue about their natural function. Their astringent properties, how they affect taste, may have an important role in their natural function. Their role in the taste of wines and other spirits is of major concern to the alcohol producing industry. In recent times, the operational definition of a vegetable tannin has been broadened to include ". . . water soluble phenolic compounds having molecular weights between 500 and 3,000 and besides giving the usual phenolic reactions have special properties such as the ability to precipitate alkaloids, gelatin, and other proteins." The role of plant tannins at the ecological level is almost certainly a mixed function, involving a defense mechanism against living-plant enemies and a delay in decomposition when plant tissue becomes litter, either in the soil proper, as in roots, or on the soil surface, as in leaves. There is no compelling evidence that tannins have any role in plant physiological processes.

SAMPLE PREPARATION FOR MASS SPECTROSCOPY

Cotyledons and embryos of *Quercus palustris* (Pin Oak), *Quercus rubra* (Red Oak), *Quercus macrocarpa* (Bur Oak), and *Quercus muehlenbergii* (Chinkapin Oak) were treated with the following method. The cotyledons and embryos were removed from the seed coat, allowed to dry at 37ºC and then pulverized with a mortar and pestle. 500mg of the dry material was added to 5mL of a solution of methanol/water (80:20 v/v) containing 0.8mM NaF to prevent sample oxidation. The solution was shaken on a Glas-Col bench top shaker for one hour and allowed to settle. The supernatant was removed and filtered with a 0.2 µm hydrophilic nylon membrane filter. The filtered extract was analyzed using LC/ESI/MS and LC/EI/MS.

INSTRUMENTATION – HPLC/DAD/ESI-MS/MS Analyses

LC/ESI/MS/MS experiments were performed on an Agilent MSD XCT ion trap mass spectrometer (Palo Alto, CA) equipped with an electrospray ionization (ESI) interface, 1100 HPLC, a DAD detector, and Chemstation software. The column used was a 150 x .5 mm i.d., Zorbax XDB – C18 3.5 µm (Agilent, Palo Alto, CA). Flow rate was 5.00 µL/min, injection volume was 0.5 µL, and column temperature was 25ºC. The ESI parameters were as follows: nebulizer, 15 psi; dry gas (N_2), 5.00 L/min; dry temperature, 325ºC; trap drive, 78.0; skim 1, -40V; lens 1, 5.00V;octopole RF amplitude, 200.0 Vpp; capillary exit, -200V. The ion trap mass spectrometer was operated in negative ion mode scanning from m/z 50 to m/z 2200 at a scan resolution of 13000 amu/s. Trap ICC was 70000 units and maximal accumulation time was 2000000 µs. MS-MS was operated at a fragmentation amplitude of 1.0V, and threshold ABS was 20000 units.

LIQUID CHROMATOGRAPHIC SEPARATION

The constituents were separated using a water (A) and methanol (B) gradient (each containing 0.1% formic acid). Initial conditions were 3% methanol increasing to 25% methanol at 6 minutes increasing to 35% at 25 minutes increasing to 90 % at 35 minutes holding at 90% to 40 minutes and returning to starting conditions at 45 minutes. The detection wavelength was 254nm.

CONCLUSIONS

Our quantification of the tannins throughout the acorns did support the literature with regards to the increase of tannin levels from basal to apical segments of the acorn. Our observations were that while the amounts of tannins increased, the tannins varied in identity. As reported in the literature we were able to confirm that red oak acorns contain more total tannins than white oak acorns. However, these reports only considered the total polyphenolic compounds. We examined the amounts of ellagitannins and gallotannins and discovered that within the red oak acorns the concentration of gallotannins is much greater than ellagitannins, whereas the white oak acorns were much higher in ellagitannins.

FUTURE WORK

We plan to look at the individual tannins to examine the possibility that certain tannins may be responsible for predator aversion towards acorn species and aversion to portions of individual acorns.

REFERENCES

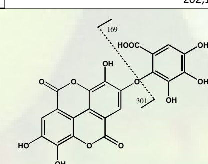
1. Phenolic Compounds and Fatty Acids From Acorns (Quercus Spp.), The Main Dietary Constituent of Free-Ranged Iberian Pigs. Emma Cantos, Juan Carlos Espin, Clemente Lopez-Bote, Lorenzo De La Hoz, Juan A. Ordonez, and Francisco A Tomas-Barberan. J. Agric. Food Chem. 2003, 51, 6248-6255.
2. Tannins: Does Structure Determine Function? An Ecological Perspective. William V. Zucker. The American Naturalist, 1983, Vol. 121, Issue 3, 335-365.

RESULTS

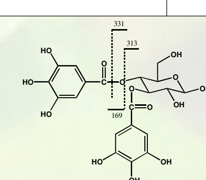
		Tannin										
Tree	Section	*331*	*433*	*447*	*463*	*469*	*481*	*483*	*615*	*633*	*783*	*785*
Chinkapin Oak	*Top*	X	44.5	X	41.5	X	5.9, 7.6, 8.5	X	X	20.8, 23.2, 23.5	17.5, 17.9, 19.5	X
	Middle	X	44.5, 44.9	X	41.6	X	6.0, 7.8, 8.5	X	X	19.3, 20.1, 23.4	17.5, 17.8, 19.8	X
	Bottom	X	44.6	45.2	41.7	X	5.8, 7.8, 8.6	X	43.7	19.0, 20.4, 23.3	17.7, 19.7	X
Bur Oak	*Top*	X	44.0	44.7	40.5	X	7.5, 8.9	X	X	X	6.2, 16.7, 17.3, 18.7, 18.9, 19.2, 19.6	X
	Middle	X	44.0	44.7	40.4	X	7.8, 9.0	24.6	X	X	6.3, 16.9, 17.4, 17.7, 18.7, 19.2, 19.7	X
	Bottom	X	43.7	44.4	39.9	X	8.0, 8.8	24.0	X	X	6.1, 16.8, 17.8, 18.7, 18.9, 19.5, 20.0	X
N. Red Oak	*Top*	12.1	X	X	X	38.0, 43.0	X	18.6	46.5	11.8, 14.7, 16.8	X	19.4, 20.0, 24.3, 25.5
	Middle	11.7	X	X	X	37.9, 43.2	X	15.1, 18.6	X	12.0, 14.9, 16.9	X	19.3, 19.9, 23.5, 24.2, 25.4
	Bottom	12.0	X	X	X	37.7, 43.4	X	15.0, 18.6	X	11.9, 14.6, 16.7	X	18.5, 24.1, 25.2, 27.0
Pin Oak	*Top*	X	X	X	X	38.8, 43.5	X	18.7	X	12.3, 18.6	X	17.0, 19.3, 20.2, 20.8, 21.4, 21.8, 22.4, 24.1, 25.3, 26.5
	Middle	X	X	X	X	38.2, 43.1	X	18.8	X	12.4, 17.0	X	17.2, 18.2, 18.6, 19.1, 20.1, 20.6, 22.0, 22.6, 23.8, 25.3, 26.6
	Bottom	X	X	X	X	38.5, 43.2	X	18.8	X	12.2	X	17.1, 18.1, 18.6, 19.2, 20.2, 21.5, 21.9, 22.6, 23.8, 24.8, 26.2

No.	*RT*	Structure	UV Type	LC/MS (M - H) m/z	MS/MS m/z	Oak Tree
1	5.9	hexahydroxydiphenoyl-glucose	B	481	421, 317, 301	Chinkapin Oak
2	6.2	dihexahydroxydiphenoyl-glucose	B	783	763, 481, 301	Bur Oak
3	7.5-8.0	hexahydroxydiphenoyl-glucose	B	481	421, 301	Chinkapin Oak and Bur Oak
4	8.5-9.0	hexahydroxydiphenoyl-glucose	B	481	421, 377, 301	Chinkapin Oak and Bur Oak
5	11.7-12.1	galloyl-glucose	B	331	271, 169	Northern Red Oak
6	11.9-12.3	galloyl-hexahydroxydiphenoyl-glucose	B	633	614, 271, 169	Northern Red Oak and Pin Oak
7	14.6-14.9	galloyl-hexahydroxydiphenoyl-glucose	B	633	614, 301	Northern Red Oak
8	15	digalloyl-glucose	B	483	331, 169	Northern Red Oak (middle and bottom)
9	16.4-16.8	dihexahydroxydiphenoyl-glucose	B	783	763, 481, 301	Bur Oak
10	16.8-17.0	galloyl-hexahydroxydiphenoyl-glucose	B	633	614, 481, 463, 301	Northern Red Oak and Pin Oak (middle)
11	17.1	digalloyl-hexahydroxydiphenoyl-glucose	B	785	630, 481, 301	Pin Oak
12	17.3-17.9	dihexahydroxydiphenoyl-glucose	B	783	763, 481, 301	Bur Oak and Chinkapin Oak
13	18.1-18.6	digalloyl-hexahydroxydiphenoyl-glucose	B	785	614, 483, 301	Pin Oak (middle and bottom)
14	18.6	galloyl-hexahydroxydiphenoyl-glucose	B	633	614, 421, 301	Pin Oak (top)
15	18.6-18.8	digalloyl-glucose	B	483	331, 169	Northern Red Oak and Pin Oak
16	18.7-19.2	dihexahydroxydiphenoyl-glucose	B	783	763, 613, 481, 301	Bur Oak
17	19.0-19.3	galloyl-hexahydroxydiphenoyl-glucose	B	633	614, 483, 301	Chinkapin Oak (middle and bottom)
18	19.1-19.4	digalloyl-hexahydroxydiphenoyl-glucose	B	785	614, 483, 301	N. Red Oak (top and middle) and Pin Oak
19	19.5-19.8	dihexahydroxydiphenoyl-glucose	B	783	763, 633, 481, 301	Chinkapin Oak and Bur Oak
20	19.9-20.2	digalloyl-hexahydroxydiphenoyl-glucose	B	785	633, 614, 483, 301	N. Red Oak (top and middle) and Pin Oak
21	20.1-20.4	galloyl-hexahydroxydiphenoyl-glucose	B	633	613, 481, 301	Chinkapin Oak (middle and bottom)
22	20.7	digalloyl-hexahydroxydiphenoyl-glucose	B	785	614, 483, 301	Pin Oak (top and middle)
23	21.4-22.0	digalloyl-hexahydroxydiphenoyl-glucose	B	785	633, 483, 301	Pin Oak
24	22.5	digalloyl-hexahydroxydiphenoyl-glucose	B	785	633, 483, 301	Pin Oak
25	23.4	galloyl-hexahydroxydiphenoyl-glucose	B	633	613, 463, 301	Chinkapin Oak
26	23.8-24.2	digalloyl-hexahydroxydiphenoyl-glucose	B	785	615, 483, 301	Northern Red Oak and Pin Oak
27	24.0-24.6	digalloyl-glucose	B	483	331, 301, 271, 169	Bur Oak (middle and bottom)
28	25.2-25.5	digalloyl-hexahydroxydiphenoyl-glucose	B	785	615, 483, 301	N. Red Oak and Pin Oak (top and middle)
29	26.2-26.6	digalloyl-hexahydroxydiphenoyl-glucose	B	785	631, 483, 451, 301	Pin Oak
30	37.7-38.0	Unknown Tannin	B	469	331, 307, 169	Northern Red Oak
31	39.9-40.5	ellagic acid-glucose	A	463	313, 301, 169	Bur Oak
32	41.6	ellagic acid-glucose	A	463	301	Chinkapin Oak
33	43.0-43.5	Unknown Tannin	B	469	331, 307, 263, 169	Northern Red Oak and Pin Oak
34	43.7	Unknown Tannin	A	615	569, 463, 301	Chinkapin Oak (bottom)
35	43.7-44.0	ellagic acid-pentoside	A	433	301	Bur Oak
36	44.5-44.6	ellagic acid-pentoside	A	433	301	Chinkapin Oak
37	44.4-44.7	Dihydroxybenzoic acid-ellagic acid	A	447	367, 301	Bur Oak
38	45.2	Dihydroxybenzoic acid-ellagic acid	A	447	301	Chinkapin Oak (bottom)
39	46.5	Unknown Tannin	B	615	571, 469, 169	Northern Red Oak (top)

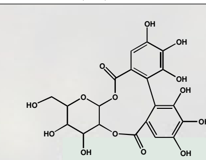
	White Oak						Red Oak					
	Burr Oak			Chinkapin Oak			Northern Red Oak			Pin Oak		
Tannin	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
168-170	1,168,589	243,306	726,660	73,718	175,914	127,050	77,308,102	84,607,114	79,786,993	90,393,225	73,689,000	87,922,495
300-302	38,371,456	32,195,068	41,092,532	18,804,638	25,525,436	46,133,816	12,613,380	13,951,542	15,878,603	10,491,639	9,378,354	16,685,546
Total												
168-170	2,515,237						493,706,929					
300-302	202,122,946						78,999,064					



MW 469
ellagitannin



MW 483
gallotannin



MW 481
ellagitannin