



Distribution, Quantification, and Identification of Tannins in Acorns from Blackjack, Sawtooth and Texas Live 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. Previously published 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 is a continuation of previous work in our laboratory 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 three species of oak trees: *Quercus acutissima*, *Quercus marilandica*, and *Quercus virginiana*. *Q. acutissima* (sawtooth oak) is native to Korea and Japan and is an introduced oak species to the United States. *Q. marilandica* (blackjack oak) and *Q. virginiana* (live oak) are native to the United States and the acorn samples were collected in Texas. The amount of total tannins increased from basal to apical segments in two of the three species of oak investigated. In support of our previous work, higher amounts of tannins were expressed in red oak acorns as compared to white oak acorns. There were variations within the individually identified tannins with respect to the distribution and degree of expression throughout the acorn.

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 marilandica* (Blackjack Oak), *Quercus virginiana* (Live Oak), and *Quercus acutissima* (Sawtooth Oak) were treated with the following method. The cotyledons and embryos were removed from the seed coat, allowed to dry at 37°C and then pulverized with a mortar and pestle. A ratio of 100mg of the dry material was added to 1mL 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.

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 0.5 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 25°C. The ESI parameters were as follows: nebulizer, 15 psi; dry gas (N₂), 5.00 L/min; dry temperature, 325°C; 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

In previous work on 2 species of red oak and 2 species of white oak, it became obvious that the red oak species did possess much greater total amounts of tannins than white oak. However, the types of tannins expressed favored gallotannins in red oaks and ellagitannins in white oaks. This trend was observed and held true for the three oaks investigated in this study as well. The Sawtooth Oak was not classified as red or white in the literature, but falls into the class of a white oak according to our mass spectral characterizations of the different species (Table 4). While there are typically 8-10 different tannins in each acorn, the oaks seem to produce one or two tannins in great excess over the others with amounts approaching 95% in some cases (Table 3). Although the two white oaks originated in two very dissimilar countries, they both contain a tannin with M/Z 433, which appears to be derived from ellagic acid. The Sawtooth Oak does contain several tannins that are not found in the oaks native to North America.

FUTURE WORK

Work in our laboratory continues to progress towards elucidating the role of the tannins in the acorns, leaves, and bark of the North American Oaks. We are also beginning an investigation into the tannins present in the leaves of Mexican Oak trees and plan to characterize their identities along with our ongoing research.

REFERENCES

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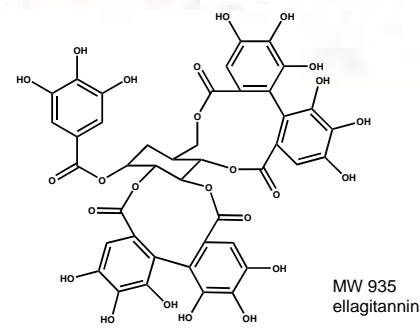
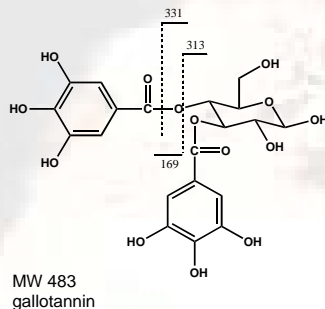


TABLE 1. TANNINS CATEGORIZED by M/Z AND LOCATION IN ACORN

Tree	Section	Tannin															
		331	403	433	447	463	469	481	483	585	609	615	625	633	783	785	935
Black Jack	Top	X	X	X	X	X	35.9, 39.3	X	X	X	46.0	X	X	19.2, 21.5	X	22.9, 25.9	X
	Middle	X	X	X	X	X	36.7, 39.3	X	X	X	X	X	X	19.2, 21.4	X	22.8	X
	Bottom	X	X	X	X	X	35.8	X	X	X	X	X	X	19.3, 21.4	X	22.9	33.3
Live Oak	Top	X	X	42.8	X	X	X	X	X	X	X	X	X	X	X	X	X
	Middle	X	X	42.9	X	X	X	X	X	X	X	X	X	X	X	X	X
	Bottom	X	X	42.9	X	39.3	X	X	X	45.0	X	X	X	X	X	X	X
Sawtooth	Top	X	24.4	43.0	X	39.0	X	X	23.9	45.3	X	X	44.1	X	17.3, 18.9	X	X
	Middle	X	24.3	42.6	X	38.2	X	X	X	44.9	X	X	43.7	X	18.7	X	X
	Bottom	X	24.4	42.5	X	38.6	X	X	X	44.8	X	X	43.6	X	16.9, 18.8	X	X

TABLE 2. PROPOSED IDENTITIES of TANNINS by RETENTION TIME

No.	RT	Structure	LC/MS (M - H) m/z	MS/MS m/z	Oak Tree
1	16.9, 17.3, 18.8	dihexahydroxydiphenoyl-glucose	783	763, 481, 301	Sawtooth
2	19.2	galloyl-hexahydroxydiphenoyl-glucose	633	613, 421, 301	Black Jack
3	21.5	galloyl-hexahydroxydiphenoyl-glucose	633	613, 465, 301	Black Jack
4	22.9	digalloyl-hexahydroxydiphenoyl-glucose	785	765, 633, 483, 301	Black Jack
5	23.9	digalloylglucose	483	423, 271, 169	Sawtooth
6	24.4	Unknown Tannin	403	313, 169	Sawtooth
7	25.9	digalloyl-hexahydroxydiphenoyl-glucose	785	765, 631, 451, 301	Black Jack
8	33.3	galloyl-di-hexahydroxydiphenoyl-glucose	935	783, 583, 451, 301	Black Jack
9	35.8, 36.7	Unknown Tannin	469	305, 169	Black Jack
10	38.2	ellagic acid-glucose	463	301	Sawtooth
11	39.3	ellagic acid-glucose	463	418, 301	Live Oak
12	39.3	Unknown Tannin	469	425, 261, 169	Black Jack
13	42.5, 43.0	ellagic acid-glucose	433	301	Live Oak and Sawtooth
14	43.6, 44.0	Unknown Tannin	625	555, 301	Sawtooth
15	45.0	Unknown Tannin	585	537, 415, 301	Sawtooth
16	45.0	Unknown Tannin	585	517, 356, 301	Live Oak
17	46.0	Unknown Tannin	609	547, 469, 263, 169	Black Jack

TABLE 3. PERCENTAGE OF TANNINS IN EACH SPECIES

	parent	count	%total	parent	count	%total	parent	count	%total
Black Jack	469	13320798	94.76	Live Oak	433	1329079	403	942903	0.98
	609	341361	0.24		463	79024	433	57352808	59.37
	633	3654522	2.60		585	10116	463	22412685	23.20
	785	3022756	2.15				483	61680	0.06
	935	344148	0.24				585	6280096	6.50
							625	2348609	2.43
							783	7198470	7.45

TABLE 4. CHARACTERISTIC MS/MS FRAGMENTATION ELLAGITANNIN (301) AND GALLOTANNINS (169)

Acorn	301	169	total
Black Jack			
Top	8757186	68323781	77080967
Middle	5924811	68318982	74243793
Bottom	7225010	1540395	8765405
	21907007	138183158	
Live Oak			
Top	3069283	1056	3070339
Middle	141670	1102	142772
Bottom	746654	0	746654
	3957607	2158	
Sawtooth			
Top	34944072	375161	35319233
Middle	31401352	436664	31838016
Bottom	41493680	665013	42158693
	107839104	1476838	