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Evaluation of Strawberry Cultivars for Ellagic Acid Content

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Abstract. **Ellagic acid in tissue extracts of green and red-ripe strawberries *(Fragaria* × *ananassa* Duch.) was detected and quantified by HPLC. Ellagic acid content of green fruit pulp ranged from 1.32 to 8.43 mg·g^{-1} of tissue dry weight (mean 3.36 mg·g^{-1}) and in achenes of green fruit from 1.32 to 20.73 mg·g^{-1} (mean 7.24). Ellagic acid content of red fruit pulp at one location for 35 cultivars and selections ranged from 0.43 to 4.64 mg·g^{-1} of dry weight (mean 1.55) and from 0.43 to 3.47 mg·g^{-1} (mean 1.45) for 15 clones at another location. Achenes from red-ripe fruit ranged from 1.37 to 21.65 mg·g^{-1} (mean 8.46) for 34 clones at one location and from 2.81 to 18.37 mg·g^{-1} (mean 8.93) for 15 clones at another location. Leaf ellagic acid content ranged from 8.08 to 32.30 mg·g^{-1} of dry weight (mean 14.71) for 13 clones examined. Large differences in ellagic acid content were found among cultivars, but tissue values were not consistent within cultivars. Values from one tissue type did not correlate consistently with values of the other tissues. Sufficient variation was found among cultivars to suggest that increased ellagic acid levels may be achieved in progeny from crosses with selected parental material.**

Ellagic acid, a naturally occurring phenolic constituent of many plant species (Bate-Smith, 1961; Daniel et al., 1989), has shown promising antimutagenic and anticarcinogenic activity against at least three classes of chemical carcinogens. Mechanisms of chemical carcinogen inhibition and the roles of ellagic acid in plants have been reviewed (Maas et al., 1991).

The objective of the study reported herein was to determine cultivar variability of ellagic acid content of strawberry fruit and other plant parts. This work was undertaken in response to increased interest in this naturally occurring plant phenol as a potential antimutagenic and anticarcinogenic constituent of food that is commonly consumed by a large portion of the U.S. population (Daniel et al., 1989).

Fruit samples were taken from plants of 36 clones (Table 1) (cultivars and advanced breeding selections) in one planting [North Farm (NF)] and from plants of 15 other clones (Table 2) in a second planting [East Farm

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Table 1. Ellagic acid content of strawberry tissues, North Farm planting, Beltsville, Md.[z,y]

	Ellagic acid (mg·g^{-1} dry wt)				
	Fruit pulp		Achenes		
Clone[x]	Green	Red	Green	Red	Leaves
Allstar	3.29	0.83	5.63	12.26	...
Arking	8.00	4.64	4.46	5.70	...
Blakemore	8.43	2.33.	3.88	2.09	15.41
Cesena	5.26	1.88	8.54	7.88	17.90
Dana	8.51	1.72	4.47	9.38	12.73
Delite	2.81	1.12	1.58	5.01	20.05
Eadibelle	1.89	2.26	4.90	---	...
Earliglow	3.15	0.82	2.69	19.54	8.08
Fairfax	3.80	2.00	12.16	4.50	...
Honeoye	2.46	0.43	5.12	10.42	12.95
Kent	3.83	1.59	5.06	21.65	...
Lateglow	3.10	1.14	9.70	12.71	...
Lester	1.63	1.20	10.06	6.96	8.89
Marlate	...	0.51	---	10.01	...
Micmac	2.22	2.65	10.31	8.76	...
Midland	4.68	2.46	2.98	4.61	...
Midway	'2.98	1.78	8.57	6.96	8.36
Redchief	2.61	1.78	20.73	5.45	12.35
Scott	2.90	0.75	2.84	9.93	...
Sparkle	...	1.04	...	7.18	...
Sunrise	...	1.02	. .	2.04	...
Tangi	3.77	1.49	2.92	1.37	...
Tribute	3.22	1.54	3.08	8.53	32.30
Tristar	3.71	1.70	4.18	10.39	...
Vesper	4.86	2.38	7.02	12.27	...
US 4588	1.32	1.45	1.37	3.56	16.01
US 5086	4.31	1.85	6.86	7.67	...
US 5146	2.35	1.36	16.33	10.02	14.41
US 5279	---	1.59	...	16.56	...
US 5288	2.10	1.04	3.97	6.21	...
US 5393	2.18	1.05	12.16	3.64	...
US 5395	3.23	...	10.21	...	..-
US 5455	3.59	1.32	8.47	7.77	11.73
US 5456	2.87	1.49	9.05	2.34	...
US 5517	2.58	1.20	9.50	18.96	...
US 5524	1.59	0.96	12.82	5.13	...
Mean	3.54	1.55	7.24	8.46	14.71
SD	1.82	0.77	4.45	5.03	6.39

[z]Fruit samples were taken in May and June, depending on the ripening period of each clone. Leaf samples were taken following the last harvest date in mid-June.
[y]Dashes indicate that data were not available.
[x]US = U.S. Dept. of Agriculture advanced selection.

Table2. Ellagic acid content of strawberry fruits, East Farm planting, Beltsville, Md.[z]

	Red-ripe fruits (mg·g^{-1} dry wt)	
Clone	Pulp	Achenes
Allstar	0.80	9.56
Annapolis	0.43	4.35
Arking	2.93	11.21
Atlas	2.06	7.45
Cornwallis	1.67	8.84
Guardian	1.68	18.37
Hood	1.28	6.32
Mrak	0.82	7.23
Muir	1.05	9.71
NCH-87-08Y	1.32	7.89
Oso Grande	1.20	11.79
Parker	3.47	2.81
Sumas	1.68	6.16
Totem	0.83	8.14
Yolo	0.47	14.14
Mean	1.45	8.93
SD	0.86	3.88

[z]Fruit samples were taken in May and June, depending on the ripening period of each clone.
[y]NCH = North Carolina advanced selection.

(EF)] at Beltsville, Md. Fruit samples (100-200 g) were taken from green and red-ripe berries. Leaf samples (50-100 g) were taken from 13 clones in mid-June, following the last fruit harvest. All tissue samples were freeze-dried, pulverized, and kept frozen at − 70C until extractions were made. We found it necessary to separate achenes from fruit pulp to accurately quantify ellagic acid content of bulked samples. Modified phenol extraction, HPLC, and spectrophotometric methods (Daniel et al., 1989) were used to determine ellagic acid content of tissue samples (Wang et al., 1990). Fruit pulp (1.0 g), achene (0.5 g), and leaf (0.5 g) subsamples were extracted three times under N with aqueous acetone (1:4). Extracts were combined and then hydrolyzed with trifluoroacetic acid at 100C for 1 h. Cooled samples were passed through an 8 mm × 10 cm C18 Radial-pack column with 5-µm-sized particles (Millipore Corp., Millford, Mass.) preconditioned with methanol. Proper column preconditioning significantly affected ellagic acid retention (Wang et al., 1990). Solvent systems consisted of (A) 10 mM ammonium phosphate buffer and (B) 30 mM ammonium phosphate and methanol (1: 1) at pH 3.0. Retention time and purity of ellagic acid in samples were compared with commercial standards (ellagic acid dihydrate, 97% purity; Aldrich Chemical, Milwaukee, Wis.). Ellagic acid retention time was between 32 and 34 min and absorption was at 253 nm for optimum detection. A Waters 600E System Controller coupled with Waters 990 Photodiode Array Detector and a Waters 990 Plotter/Integrator system with Waters software were used to detect and quantify ellagic acid in samples (Millipore Corp.). Each cultivar or selection tissue sample was quantified at least once, except for samples of 'Al1star' and 'Arking'. The latter were run in duplicate to test intersample variation and reproducibility of extraction, detection, and quantification procedures.

Ellagic acid was extracted from strawberry fruit receptacle tissue, achenes, and leaf tissue. More ellagic acid was found in achenes than in fruit pulp on a dry-weight basis and, most of all, in leaf tissue (Tables 1 and 2). These values varied greatly among the cultivars and clones examined.

The ellagic acid content of green fruit pulp was higher than in red fruit pulp in 90% of the clones from the NF planting (Tables 1 and 3). This trend was not evident with achenes from green or red fruit (Table 4). Leaf values averaged nearly twice as high as achene values and 4.4 to 9.5 times higher than green and red pulp, respectively.

Pooling of sample data showed that the ellagic acid content of red fruit tissue varied little between the two plantings (Table 3). These plantings are ≈2 km apart and were managed somewhat differently.

There seemed to be no evident relationship between levels in leaves compared to fruit pulp or achenes. 'Blakemore', for example, ranked among the top five clones (Table 1) for ellagic acid content in green and red fruit pulp and leaves, but ranked among the lowest five for its level in achenes of red fruit. 'Earliglow', however, ranked in the lowest five for red fruit pulp, green achene, and leaf values, but in the highest five for red achene values.

Separation of achenes from fruit pulp before ellagic acid extraction of fruit samples was necessary for extracting bulked samples. Achene and pulp separation, however, were more important for deriving nutritionally meaningful values for ellagic acid content in strawberries. This also would apply to other fruits having propagules that pass intact through the human digestive tract. We have no data on the availability of nutrients in strawberry achenes or, "seeds" of other small-fruit crops (raspberries, blackberries, cranberries, blueberries, grapes, etc.) to humans.

Duplicate extraction, detection, and quantification of ellagic acid from 'Allstar' and 'Arking' fruit showed none to slight variation due to procedures. Differences between duplicate 'Arking' red fruit pulp determinations among duplicate runs were 0.48 and 0.67 mg·g^{-1} dry weight for NF and EF lo-

Table 3. Summary of ellagic acid determinations of strawberry tissue.

Tissue	Clones (no.)	Ellagic acid (mg·g^{-1} dry wt) Range	Mean	SD
		North Farm		
Fruit pulp				
Green	32	1.32-8.43	3.36	1.82
Red	35	0.434.64	1.55	0.77
Achenes				
Greed	32	1.32-20.73	.7.24	4.45
Red	34	1.37-21.65	8.46	5.03
Leaves	13	8.08-32.30	14.71	6.39
		East Farm		
Fruit pulp				
Red	15	0.43-3.47	1.45	0.86
Achenes				
Red	15	2.81-18.37	8.93	3.88

Table 4. Differences in ellagic acid content of strawberry fruit as fruit ripen (based on mg·g^{-1} tissue dry weight).

Tissue	Differences in content from green to red-ripe	Pooled clone values (%)[z]
Fruit pulp	Red > green	9.7
	Green > red	90.3
Achenes	Red > green	56.7
	Green > red	43.3

[z]Percentages determined from tissue samples of 30 clones taken both in immature (full size, geen) and red-ripe stages.

cations, respectively. Differences in 'Arking' achenes from red fruit were 0.0 and 1.69 mg ellagic acid/g dry weight for NF and EF, respectively. Similarly, differences in ellagic acid content of red fruit pulp of 'Allstar' were 0.88 and 0.17 mg·g^{-1} for NF and EF, respectively. Achenes from red 'Allstar' fruit showed differences of 0.66 (NF) and 0.79 mg·g^{-1} (EF) due to procedural variables. Generally, differences that may be due to procedural variation were well within standard deviations observed among the cultivars and clones examined at both locations (Table 3).

The manner of inheritance of ellagic acid is not known. We have shown here that strawberry cultivars differ widely in tissue ellagic acid content. Other studies have determined ellagic acid content in strawberry and other fruits (Daniel et al., 1989; Wang et al., 1990), but only one study (Boyle and Hsu, 1990), with muscadine grapes, has shown cultivar variation. We presume, by analogy with other strawberry metabolizes, that cultivar differences tend to be heritable characteristics of each cultivar (Shaw, 1988) and that relative ellagic acid productivity is inherited.

Since the major portion of ellagic acid presumably is derived through a single metabolic pathway by way of gallic acid, we may find that specific combining ability is of greater importance than general combining ability in ellagic acid inheritance in strawberry, just as with ascorbic acid. The same may be true for gallic acid and geraniin (an ellagitannin) inheritance, but probably not for massed derivatives of either constituent (e.g., ''ellagitannins" as a group). If this hypothesis is correct, breeding for high (or low) ellagic acid content of fruit, or any other structure, should be highly successful. However, one unexpected observation in our examination of strawberry tissues was that no correlation was found among ellagic acid contents of fruit pulp, achenes, and leaf tissue. This lack may indicate that selection for ellagic acid content can be highly specific for tissue type; e.g., for high fruit content but low leaf content, etc.

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