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Phenol content of grape skins and the loss of ability to make anthocyanins by mutation

by

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Der Phenolgehalt in der Haut der Traubenbeere und der Verlust der Anthocyanbildung durch Mutation

Zusammenfassung. — Da die Größe der Traubenbeeren beträchtlichen Schwankungen unterliegt, kann der Gesamtgehalt der aus der Beerenhaut extrahierbaren Phenole — bezogen auf die Einzelbeere oder das Beerengewicht — bei Rebsorten mit weißen Beeren größer sein als bei roten Sorten. Wurden die Phenolgehalte (ausgedrückt als Gallussäure-Äquivalente, GAE) auf die Einheit der Beerenoberfläche bezogen, so lagen die Werte repräsentativer weißer *Vitis-vinifera*-Sorten stets niedriger (0,042—0,122 mg GAE/cm^2) und außerhalb des Schwankungsbereiches repräsentativer roter Sorten (0,132—0,424 mg GAE/cm^2). Diese Befunde werden dahingehend interpretiert, daß die für den Verlust der Anthocyanbildung verantwortlichen Mutationen nicht auch eine Umwandlung der Präcursoren in andere Phenole nach sich ziehen.

Introduction

Grapes in the wild are usually black or reddish-black, barring occasional mutants, and contain appreciable, usually large amounts of anthocyanins in the berry skin. The white grapes of great commercial importance must, therefore, represent the result of one or more "loss" mutations — the loss of the ability to make anthocyanins. This loss is ordinarily expressed throughout the vine, as would be expected. White-fruited varieties do not ordinarily exhibit red leaf coloration in the autumn, for example. Flavonoids are not translocated, but apparently are synthesized in the cells where they are found. Therefore study of white compared to black grape berry skins should be as meaningful as any other tissue from a biochemical viewpoint and more important from a practical one.

When an enzyme is lost by mutation from a biochemical sequence, it is a tenet of biochemistry that the immediate substrate of that enzyme should then accumulate unless there are alternate synthetic routes or regulatory mechanisms to prevent it. SINGLETON and ESAU (1969) reported similar content in total phenol estimated as gallic acid in pulp, juice, or seeds of white or black grapes. The skins, on the other hand, averaged 1859 mg/kg fresh berries from black grapes versus half as much, 904 mg/kg, from white. The difference was about the same magnitude as the average anthocyanin content of the black grapes.

This suggested that white grapes did not accumulate other phenols when the ability to make anthocyanins was lost. This report investigates this idea further.

Material and methods

The grapes were harvested at commercial ripeness in connection with other studies utilizing grapes from the University vineyards at Davis and Oakville, California.

Representative samples of 200—500 berries were snipped between the torus and the berry without damaging the skin. Skins were separated by hand to ensure no seed entrainment and employing several persons to hasten the separation. The skins were squeezed by hand in cheesecloth to remove excess juice and then extracted by grinding several times with aqueous ethanol in a manner previously shown to be essentially quantitative in recovery of extractable phenols (SINGLETON 1966). The extracts were analyzed for total phenols in terms of gallic acid equivalents (GAE) by an automated Folin-Ciocalteu method (SLINKARD and SINGLETON 1977). Average berry weight and juice Brix by refractometry were recorded. Skin surface was calculated from the volume determined from the berry weight and specific gravity. The berry surface was calculated as spherical, ellipsoidal, or ovoid depending on the characteristic shape of the berries of the variety. The specific gravity was calculated from juice Brix plus a small constant factor accounting for the higher density of seeds and skins (SINGLETON *et al.* 1973).

Results and discussion

The table presents data obtained from 1977 harvests for 8 white and 4 red (ranging from low to high anthocyanin content) *Vitis vinifera* varieties of commercial importance in California. Complementary but less complete data were obtained from other vintages. Note that phenols mg/g would be more useful in comparing wine content per

Total phenol content of grape skin extracts

Gesamtphenolgehalt von Beerenhautextrakten

	° Brix	mg GAE/ berry	mg GAE/ g of berry	mg GAE/ 10 cm^2 skin
Muscat of Alexandria	18.8	0.469	0.126	0.42
Emerald Riesling	19.8	0.446	0.265	0.70
White Riesling	21.2	0.421	0.275	0.70
French Colombard	21.8	0.816	0.408	1.12
Thompson Seedless	22.1	0.619	0.499	1.12
Chardonnay	23.9	0.633	0.465	1.12
Chenin Blanc	19.6	0.777	0.478	1.22
Sémillon	23.8	1.083	0.392	1.22
Mean (white grapes)	21.4	0.658	0.364	0.95
Grenache	21.0	0.918	0.491	1.32
Pinot Noir	25.2	0.900	0.738	1.73
Cabernet-Sauvignon	21.5	1.810	1.631	3.71
Zinfandel	19.3	3.478	1.455	4.24
Mean (black grapes)	21.8	1.777	1.079	2.75

unit of harvested grapes and mg/berry more useful in studying biochemical changes such as ripening. These values are useful in comparing varieties, but they do not give a clear answer to the question posed. For example, Sémillon's rather large skin phenol content per berry is seen to be the result of large berries when converted to mg/g. The total phenol per unit of skin surface, however, clarifies the point at issue. The amount of GAE phenol per unit of berry surface averaged less than 1 mg/10 cm^2 for white and more than 2 mg/10 cm^2 for red. The ranges for these and other similar data did not overlap. This gives strong support for the hypothesis that when the ability to synthesize anthocyanins is lost the precursors are not shunted to extra production of colorless phenols.

The Grenache berries were notably low in anthocyanin content, inherent in the variety and accentuated in the hot Davis climate. They approached white grapes, but were still higher. Pinot Noir, also in our vineyards not a high-colored variety, was low relative to the well-colored Cabernet-Sauvignon and Zinfandel.

The lack of a compensating increase in other flavonoids or phenols upon the loss of the ability to synthesize anthocyanins (which ordinarily represent about 500 mg/kg in whole black wine grapes) not only indicates independent control of the precursor pool and phenolic products, but also indicates any functions of the berry anthocyanins in the life of the plant are not substituted for by other flavonoids. Furthermore, it is clear that anthocyanins are not vital to the plant, at least under conditions of cultivation. It may be significant that white grapes as a group ripen (i.e., reach minimally acceptable sugar content) before red grapes. Dispensing with anthocyanins and production of no replacement phenols would spare the diversion of at least the photosynthate sugar necessary to produce the anthocyanins. It is also notable that the single important black variety widely noted for early ripening in cool climates, Pinot Noir, is also noted for relatively low and unusual (unacylated) anthocyanins. Another harvest of Chardonnay from a different vineyard had 1.13 mg GAE/10 cm^2 of skin, but owing to different average berry size much less constant skin phenol per berry or per gram. The content per unit surface is found to be a better characteristic to judge differences among varieties.

This pattern, loss without diversion to extra synthesis of other phenols, seems to persist in less complete loss mutations in the anthocyanins of grapes. Weakly colored black varieties of grapes seem generally to retain the more "primitive" cyanidin derivatives and lose the more "advanced" trihydroxy and methylated malvidin and delphinidin (SINGLETON and ESAU 1969). This is shown by generally simpler chromatographic patterns from lighter colored grapes such as Flame Tokay, Emperor, Red Malaga, and even Pinot Noir. This is true even when the samples are concentrated to the point that total applied pigment is similar (AKIYOSHI *et al.* 1963, RANKINE *et al.* 1958). The biochemical sequence implies malvidin and petunidin are made by methoxylation of delphinidin and are the preferred pigments in *V. vinifera.* Cyanidin appears to be a prerequisite to delphinidin. There are no grapes with a dilute blue color which would probably require delphinidin derivatives present and other anthocyanins absent. Rather than causing the production of extra phenols when anthocyanin synthesis is lost, there is some evidence for the reverse. Myricetin derivatives (trihydroxy B-ring) may be absent in white grapes (RIBEREAU-GAYON 1964).

Summary

Because berry size varies so much, the total content of phenols extractable from skins can be higher for some varieties with white berries than for some with red. When

recalculated as mg of extractable phenol per unit of berry surface, values for representative white *Vitis vinifera* varieties were invariably lower (0.042—0.122 mg GAE/cm^2) and outside the range for representative red varieties (0.132—0.424 mg GAE/cm^2). This is discussed as evidence that the mutation losing the ability to make anthocyanins in grapes does not lead to diversion of precursors to other phenols.

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