

A simple method for demonstrating macroscopically the location of polyphenolic compounds in grape berries

by

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Eine einfache Methode zur makroskopischen Lokalisation polyphenolischer Verbindungen in Weinbeeren

Zusammenfassung. — Schnitte von Beeren verschiedener *Vitis vinifera*-Sorten wurden mit HCl, Vanillin-HCl oder FeCl₃ angefärbt, um makroskopisch zu zeigen, daß der Hauptanteil der polyphenolischen Verbindungen in allen Entwicklungsphasen in der Schale, den Samen und den Leitbündeln der Weinbeere lokalisiert ist.

Although it is known that the skins, seeds and vascular tissue contain the bulk of the polyphenolic compounds present in ripe grape berries (5), a simple chemical staining technique for these compounds in grapes has not been described adequately. Such a technique could be useful for studying the location of polyphenols in new cultivars at various stages of development. We have now treated slices of developing and mature berries of several cultivars of *Vitis vinifera* L. with staining reagents. Brief written references have been made to staining banana (7) and sultana fruit (2) with vanillin-HCl.

Transverse and longitudinal freehand slices (1 mm or less in thickness) of berries and stems were rinsed in water for 1 minute and then transferred to either concentrated HCl, 1% vanillin in concentrated HCl, or 5% aqueous ferric chloride. The varieties of grapes tested are shown in Table 1.

When slices of berries were placed in vanillin in HCl an intense red colour appeared within a few minutes around the periphery of the berries, in the seeds and in the vicinity of the central and peripheral conducting tissue. At all stages of development the pericarp tissue stained only lightly or not at all. Stem sections stained

Table 1
Varieties of *Vitis vinifera* tested for polyphenolic compounds

Variety	Type	Parts of plant tested
Sultana (syn. Sultanina, Thompson Seedless, Kishmish)	White, seedless	Mature berries, stems, leaves.
Sultana, Bruce's sport	White, seedless	Mature berries, stems, leaves.
Rhine Riesling	White, seeded	Young, medium and mature berries, stems, leaves.
Muscat Gordo Blanco	White, seeded	Young, medium and mature berries, stems, leaves.
Cabernet-Sauvignon	Red, seeded	Young, medium and mature berries, stems, leaves.

intensely in the bark and medullary rays while much lighter staining was observed in the pith and wood. Fig. 1 shows the location of the stain in berries of the variety Muscat Gordo Blanco at three stages of development, and in the stem. Essentially similar results were obtained with all varieties tested. Leaves of all varieties of

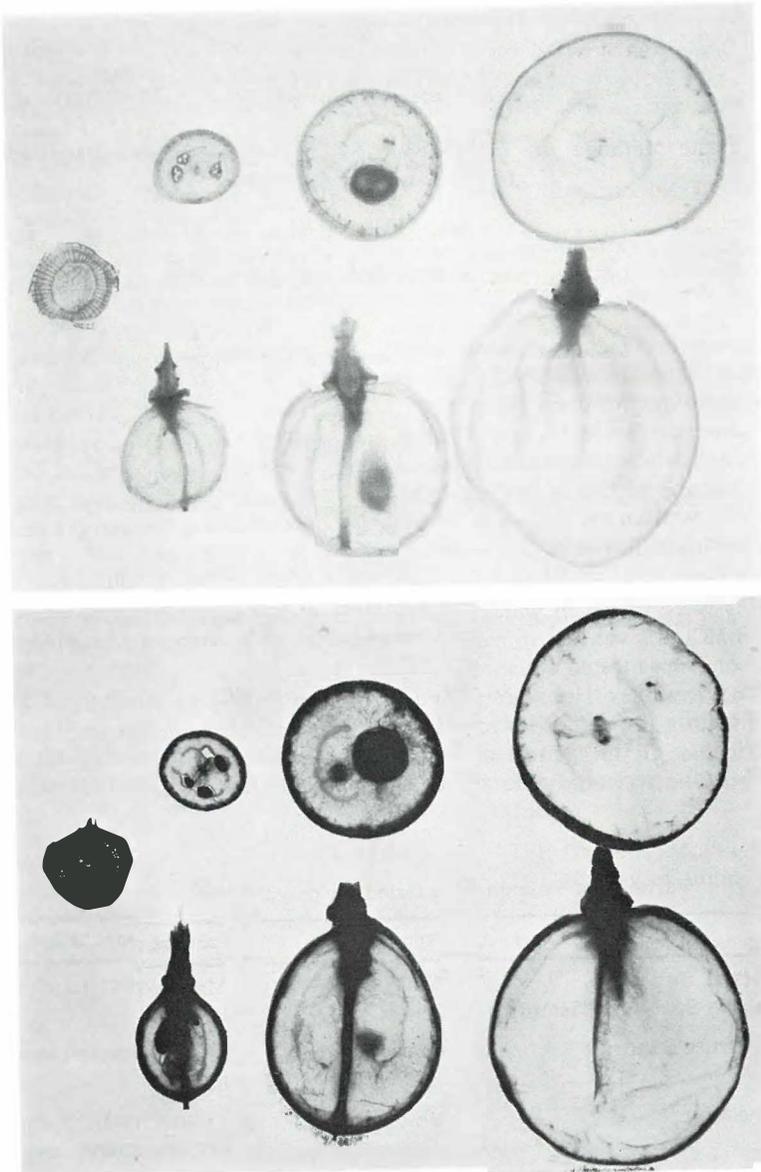


Fig. 1: Location of polyphenolic compounds in grape berries and stem. Both photographs were prepared under identical conditions.

Upper: Transverse slices of berries and stem and longitudinal slices of berries of *Vitis vinifera* c. v. Muscat Gordo Blanco. Seeds in the mature berries were avoided due to the difficulty of sectioning.

Lower: Slices as above, but after staining with 1% vanillin in HCl.

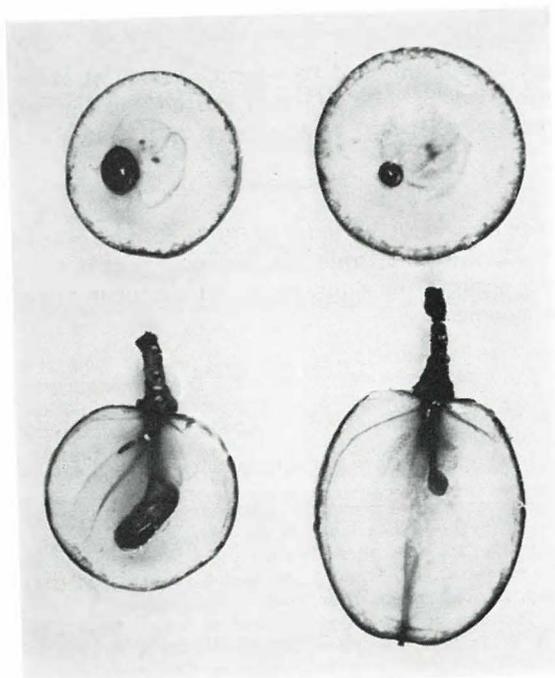


Fig. 2: Polyphenolic compounds in mature berries of Sultana (right) and a mutant of Sultana, Bruce's sport (left). Slices were stained with vanillin-HCl as in Fig. 1. Both normal and mutant berries contain small soft imperfect seeds.

grapes stained red after about one hour in vanillin-HCl whereas leaves of *Phaseolus vulgaris* remained green.

Slices of grapes treated with concentrated HCl developed, after about 24 hours, a red colour in the same regions as those coloured by vanillin-HCl. A blue colour was produced in these same areas of the berries by treatment with 5% aqueous FeCl_3 . Maximum colour development occurred in about 24 hours.

The mutant of Sultana, Bruce's sport (1), produces a lighter-coloured sultana (raisin) when dried than does the normal Sultana. Both the normal and mutant were shown to contain similar amounts of phenolic compounds by chemical analysis on extracts of the berries, but Bruce's sport contained less than 10% of the polyphenol oxidase activity of the normal Sultana (3). In the present work, staining slices of mature berries of normal and mutant Sultana showed that with all reagents used similar distribution patterns of staining occurred in both types of berry. Berries stained with vanillin-HCl are shown in Fig. 2. The intensity of staining appeared similar in each type of berry.

The three staining reagents used react with different compounds to produce coloured products. Vanillin-HCl produces a carbonium ion from compounds containing a 1:3:5 trihydroxybenzene nucleus, HCl transforms leuco-anthocyanidins to anthocyanidins and FeCl_3 is a relatively non-specific staining reagent (6). The chemical nature of phenolic compounds in grapes is complex (4, 5). Our staining results, being independent of reagent in respect of location, support the belief that the majority of polyphenolic compounds are located in the skins, seeds and

vascular tissue of grape berries and demonstrate that this distribution is established at an early stage of development.

It is concluded that staining of free-hand sections of grape berries with the vanillin-HCl reagent may be used to locate and demonstrate rapidly and reliably the distribution of polyphenols.

Summary

Slices of berries of several varieties of *Vitis vinifera* were stained with HCl, vanillin-HCl or FeCl₃ to demonstrate macroscopically that the bulk of the polyphenolic compounds occur in the skins, seeds and conducting tissue of grape berries at all stages of development.

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