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RAPID COMMUNICATION

# A simple technique for the detection of red wine adulteration with elderberry pigments

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HPLC analysis of anthocyanins in a red wine with added elderberry extract, showed an extra peak in the chromatogram, due to cyanidin 3-sambubioside-5-glucoside from elderberry. Similarly, an enhanced peak from additional cyanidin 3-glucoside and co-eluting cyanidin 3-sambubioside was also found. These two additional peaks, in a wine chromatogram, may be used as evidence of elderberry addition.

## INTRODUCTION

Elderberries (*Sambucus nigra*), are a rich source of anthocyanins. They are used for juices, wines and jams and as a potential food colourant source (Francis, 1989). The phenolic composition has been investigated by several authors. The anthocyanins are all cyanidin-based, being the 3-sambubioside-5-glucoside, 3-sambubioside and 3-glucoside (Harborne, 1963). Additionally, cyanidin 3,5-diglucoside was found by Bronnum-Hansen & Hansen (1983). Cyanidin 3-sambubioside-5-glucoside acylated with *p*-coumaric acid was identified in fruits of *S. racemosa* (Lamaison *et al.*, 1979) and also in *S. canadensis* by Johansen *et al.* (1991). NMR studies of this latter species (Nakatani *et al.*, 1995) also identified a second variant of this pigment acylated with *p*-coumaric acid and a new *p*-coumaric acid acylated derivative of cyanidin 3-sambubioside. Qualitatively and quantitatively, the distribution of the pigments is dependent on the variety studied (Mazza & Miniati, 1993).

Information on the non-coloured phenolics of elderberry is sparse, but the main flavonols reported are kaempferol, myricetin and quercetin (Starke & Herrmann, 1976).

Elderberry pigment extracts may be used for colouring foodstuffs and wines, particularly port wines (Amerine, 1954). The addition of elderberries is believed to accelerate wine fermentation and has also been used to produce a red wine from white juice (Saller & De Stefani, 1960). The addition of elderberry juice to grape wine is illegal and has been detected by paper chromatography (Schneider & Epp, 1959). Other authors have used paper chromatography to detect the addition of elderberry juice in blackberry juice (Fitelson, 1970) and HPLC to detect the adulteration of blackcurrant juice with elderberry juice (Pfannhauser & Reidl, 1983). In this report, we use HPLC to detect and characterise the extra anthocyanins in a red wine, arising from elderberry addition.

## MATERIALS AND METHODS

A wine was made in Portugal from *Vitis vinifera* var. Roriz grapes. Elderberries were from Portugal.

### Extraction of anthocyanins from red wine

Wine (1 ml) was passed through a $C_{18}$ Sep-Pak cartridge (Waters Associates). The retained pigments were washed with 1% formic acid and eluted with methanol containing 1% formic acid, vacuum concentrated and dissolved in HPLC solvent—0.6% perchloric acid: methanol (8:2) (0.5 ml) and filtered (0.45 $\mu$m).

### Extraction of anthocyanins from elderberries

Elderberries (3 g) were soaked (10 min) in 3 ml of a mixture of methanol (90 ml)-water (7 ml)-conc. HCl (3 ml). The filtered extract was evaporated to dryness

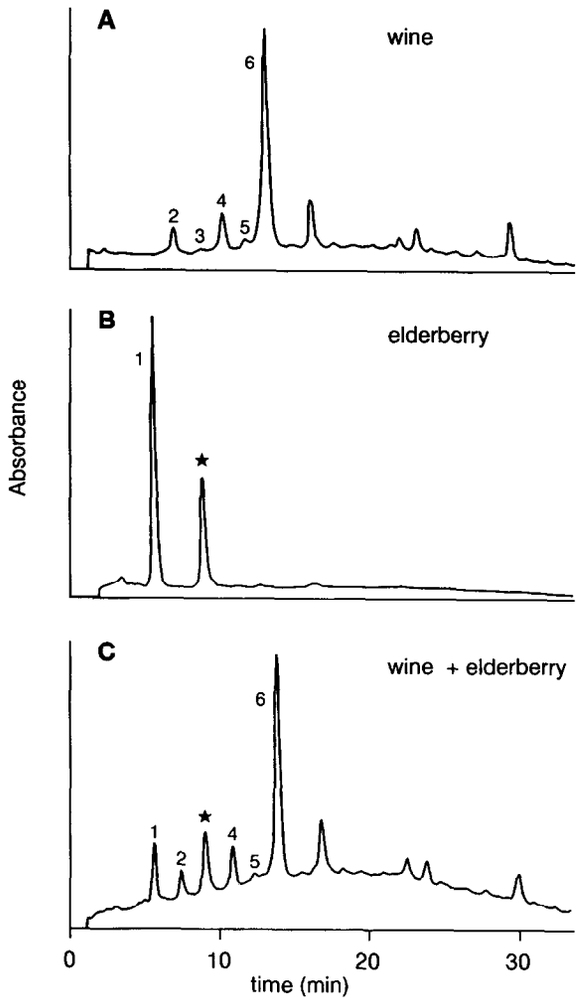


**Fig. 1.** HPLC chromatograms—520 nm. (1) Cyanidin 3-sambubioside-5-glucoside, (2) delphinidin 3-glucoside, (3) cyanidin 3-glucoside, (4) petunidin 3-glucoside, (5) peonidin 3-glucoside, (6) malvidin 3-glucoside, (★) cyanidin 3-glucoside + cyanidin 3-sambubioside.

and residue dissolved in 0.6% perchloric acid: methanol (8:2) and filtered (0.45 $\mu$m).

### Wine sample spiked with elderberries

Ten dried elderberries (500 mg) were soaked in wine (25 ml) for 48 h. A portion of the extract was treated as for red wine extraction.

### HPLC analysis

A Hewlett-Packard 1090M Series II chromatograph was used, equipped with a diode-array detector, auto-injector (25$\mu$L) and an ODS Hypersil column (100 × 2.1 mm, particle size 5 $\mu$m) at 40°C. Elution solvents were (A) acidified water (0.6% perchloric acid) and (B) methanol, flow rate 0.3 ml min$^{-1}$. Linear gradient elution was used, from 20%B increasing to 52%B over 32 min, with detection at 520nm. Anthocyanin peak identification was confirmed by comparison of retention time and spectral data with standards isolated in our laboratory.

### Thin Layer Chromatography (TLC)

A mixture of standards of the four main elderberry anthocyanins was separated in two solvent systems: acetic acid–hydrochloric acid–water (15:3:82) and *n*-butanol–acetic acid–water (6:1:2), on microcrystalline cellulose.

### Non-coloured phenolics

Analysis was described previously (García-Viguera & Bridle, 1995).

## RESULTS AND DISCUSSION

HPLC identification of the main anthocyanin monoglucosides in the Portuguese wine are shown in Fig. 1(A). The elderberries used in this work contained three anthocyanins: cyanidin 3-sambubioside-5-glucoside, cyanidin 3-glucoside and cyanidin 3-sambubioside. This identification was confirmed by TLC, since the 3-glycosides co-eluted by HPLC (Fig. 1B—peak ★).

HPLC of anthocyanins is a particularly sensitive means of detecting elderberry addition to wine. Using our conditions, a new peak in the wine chromatogram due to elderberry anthocyanin—cyanidin 3-sambubioside-5-glucoside (Fig. 1B and C—peak 1) appeared, eluting before delphinidin 3-glucoside (Fig. 1 A and C—peak 2). Furthermore, we found that during HPLC analysis, cyanidin 3,5-diglucoside co-eluted with cyanidin 3-sambubioside-5-glucoside; thus if an elderberry species also containing cyanidin 3,5-diglucoside were to be used as a wine adulterant, the new peak in the wine chromatogram would be additionally enhanced. The HPLC gradient for wine anthocyanins did not separate cyanidin 3-glucoside from cyanidin 3-sambubioside, but this was used to advantage for detecting elderberry anthocyanins in wine. Thus, the normally small cyanidin 3-glucoside peak in wine (Fig. 1A—peak 3), becomes a significant marker with the additional presence of cyanidin 3-glucoside and cyanidin 3-sambubioside from elderberry (Fig. 1C—peak ★). Thus, the combination of this peak and the early eluting cyanidin 3-sambubioside-5-glucoside peak in a red wine chromatogram, provides strong evidence for elderberry adulteration.

The level of elderberry addition that may be detected will depend on various factors, including contact time, temperature and alcoholic content. However, in small scale tests with a red wine, we were able to detect anthocyanins derived from dried elderberries at a level of one berry per 20 ml wine, which translates to approximately 11 g dry berries per gallon. At this level, the effect on overall colour was hardly discernable by eye; hence the use of elderberries as a colour enhancer would need to exceed this minimum level of detection many fold, and therefore would be readily detected by HPLC.

The principal non-coloured phenolics detected in elderberries were: gallic acid, 3,4-dihydroxybenzoic

acid, rutin and quercetin. These and others are also present in wines (García-Viguera & Bridle, 1995), but the addition of elderberries to wine, did not produce sufficient differences in the phenolic chromatogram to be used as an additional diagnostic tool in this work.

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## REFERENCES

Amerine, M. A. (1954). Composition of wines I. Organic constituents. *Adv. Food Res.*, **5**, 353–510.

Bronnum-Hansen, K. & Hansen, S. H. (1983). High-performance liquid chromatographic separation of anthocyanins of *Sambucus nigra*. *J. Chromatogr.*, **262**, 385–92.

Fitelson, J. (1970). Note on paper chromatographic detection of adulteration in dark coloured fruit juices. *J. Assoc. Offic. Anal. Chem.*, **53** (3), 578–9.

Francis, F. J. (1989). Food colorants: anthocyanins. *Crit. Rev. Food Sci. Nutr.*, **28**, 273–314.

García-Viguera, C. & Bridle, P. (1995). Analysis of non-coloured phenolic compounds in red wines. A comparison of high performance liquid chromatography and capillary electrophoresis. *Food Chem.*, in press.

Harborne, J. B. (1963). Plant polyphenols IX. The glycosidic pattern of anthocyanin pigments. *Phytochemistry*, **2**, 85–97.

Johansen, O -P., Andersen, O. M., Nerdal, W. & Aksnes, D. W. (1991). Cyanidin 3-[6-(*p*-coumaroyl)-2-(xylosyl)-glucoside]-5-glucoside and other anthocyanins from fruits of *Sambucus canadensis*. *Phytochemistry*, **30**, 4137–41.

Lamaison, J. L., Guichard, J. P. & Pourrat, H. (1979). Anthocyanins from the fruits of *Sambucus racemosa* L. (Caprifoliaceae). *Plant. Med. Phytother.*, **13**, 188–91.

Mazza, G. & Miniati, E. (1993). In *Anthocyanins in Fruits, Vegetables and Grains*. CRC Press, London.

Nakatani, N., Kikuzaki, H., Hikida, J., Ohba, M., Inami, O. & Tamura, I. (1995). Acylated anthocyanins from fruits of *Sambucus canadensis*. *Phytochemistry*, **38**, 755–7.

Pfannhauser, W. & Reidl, O. (1983). Anthocyane: bildung, gewinnung und analyse natuerlicher Lebensmittelfarbstoffe. *Ernaehrung*, **7**, 560–4.

Saller, W. & De Stefani, C. (1960). Holunderzusat zu Traubenmost. *Weinberg Keller*, **7**, 45–9.

Schneider, J. & Epp, F. (1959). Paper chromatographic separation of coloured constituents of European hybrid and berry-fruit red wines. *Mitt. Rebe Wein.*, **9A**, 111–14.

Starke, H. & Herrmann, K. (1976). The phenolic compounds in fruit. VIII. Changes in flavonol contents during fruit development. *Z. Lebensm. Unters. Forsch.*, **161**(2), 131–5.