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# Preliminary study of flavonols in port wine grape varieties

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**Abstract**

Seven varietal port wine grapes (Tinta Roriz, Tinta Barroca, Tinta Amarela, Tinta Cão, Touriga Francesa, Touriga Nacional and Rufete) were analysed in order to determine differences in flavonol compositions. For this purpose, a SPE-HPLC method was applied. Although no qualitative variations were found, there are quantitative differences in the individual concentrations of the phenolic compounds. In Tinta Cão and Rufete varieties, (−)-epicatechin was the major compound. All samples showed significant amounts of kaempferol 3-glucoside and isorhamnetin 3-glucoside, with the exception of Rufete variety, where these compounds were present in traces. 

*Keywords:* Port wine grapes; Phenolics; SPE; HPLC

## 1. Introduction

Despite the wealth of information on grapes in general (Cacho, Fernández, Ferreira & Castells, 1992; Cheynier & Rigaud, 1986; González-Sanjosé, Barron & Díez, 1990; Osmianski & Lee, 1990; Simón, Hernández, Estrella & Gómez-Cordovés, 1992; Somers & Ziemeli, 1985; Wulf & Nagel, 1978), there appears to be little information on phenolic compounds in varietal port wine grapes, and, more specifically, on non-coloured phenolics. Bakker and Timberlake (1985) have studied the anthocyanin compositions of several cultivars of Portuguese grapes, including Tinta Roriz, Tinta Barroca, Tinta Amarela, Tinta Cão, Touriga Francesa, Touriga Nacional and Rufete.

Flavonols in grapes and wines play an important role in the co-pigmentation with anthocyanins (Asen, Stewart & Norris, 1972), but few studies have been done on these compounds. Cheynier and Rigaud (1986) have identified several flavonols in the skins of *Vitis vinifera* var. Cinsault but, so far, nothing has been reported about their presence in varietal port wine grapes.

In the present work, the flavonols of seven varietal port wine grapes were analysed by HPLC/DAD to evaluate differences in their chemical patterns.

## 2. Material and methods

### 2.1. Grape samples and standards

Seven varietal port wine grapes (cv Tinta Roriz, Tinta Barroca, Tinta Amarela, Tinta Cão, Touriga Francesa, Touriga Nacional and Rufete) were harvested in September of 1999 in Douro, Northern Portugal, and were freeze-dried until analysis.

Syringic, *p*-coumaric and ferulic acids, myricetin, quercetin, kaempferol and isorhamnetin heterosides, (+)-catechin and (−)-epicatechin, were obtained from Sigma Chemical Co. *trans*-Caffeoyltartaric acid (*t*-CAFTA) and *trans*-*p*-coumaroyltartaric acid (*t*-COUTA) were kindly supplied by Dr. C. Garcia-Viguera [from CEBAS (CSIC), Murcia, Spain].

### 2.2. Grape extraction

The grape flavonols extraction was based on the methodologies developed by Osmianski, Ramos and Bouzex (1988) and Osmianski and Lee (1990). Each

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lyophilised grape sample (20 g) was homogenised with 80% methanol (5×100 ml) for 5 min with sonication. The extracts were filtered through a Büchner funnel, and the methanol was removed from the combined extracts with a rotary evaporator under vacuum at 40°C. The residue obtained was redissolved in 10 ml of 0.01 N HCl and passed through a C18 (EC) SPE column (Isolute, 10 g, 70 ml), preconditioned with 10 ml ethyl acetate, 10 ml methanol and 10 ml 0.01 N HCl. The loaded cartridge was washed with 6 ml 0.01 N HCl and the column was dried by passing a current of nitrogen gas for 6 min. Phenolic compounds, other than anthocyanins, retained on the sorbent, were eluted with 100 ml ethyl acetate. The solvent was removed using a rotary evaporator and the phenolic residue was redissolved in 1 ml methanol and 20 μl were injected onto an analytical HPLC unit.

### 2.3. HPLC analysis of phenolic compounds

The separation of phenolics was achieved with an analytical HPLC unit (Gilson), using a reversed-phase ODS-Hypersil (20×0.21 cm, 5 μm size particle). The solvent system used (Garcia-Viguera & Bridle, 1995; Ramos, Andrade, Seabra, Pereira, Ferreira & Faia, 1999) was a gradient of water/formic acid (19:1) (A) and methanol (B); 0–2%B and 60–62%B at a solvent flow rate of 0.3 ml/min. Detection was achieved with a diode array detector and chromatograms were recorded at 280 and 350 nm.

The compounds in each sample were identified by comparing their retention times and UV-vis spectra, in the 200–400 nm range, with those of standards. Peak purity was checked by means of the Gilson 160 Spectra Viewer software contrast facilities.

Phenolic quantifications were achieved by the absorbances recorded in the chromatograms relative to external standards of phenolics, with detection at 280 nm for (+)-catechin, (−)-epicatechin and syringic acid and at 350 nm for the others.

## 3. Results and discussion

For optimisation of the extraction procedure, methanol was tried, but the HPLC chromatogram obtained revealed a co-elution of anthocyanins and other phenolics. So a SPE procedure was applied as fractionation method. This technique was able to remove practically all anthocyanins from the phenolic extract of the port wine grapes studied, especially those which co-eluted with non-coloured phenolics.

Two kinds of C18 Octadecyl SPE columns were tried, end-capped (EC) and non-endcapped. The EC column gave a higher recovery of colourless phenolic compounds.

The whole grape, peel, seeds and flesh of Touriga Nacional grapes were analysed separately.

The results demonstrated that the extract obtained from Touriga Nacional grape had the same qualitative composition as that obtained from the grape peel, being characterised by the presence of *t*-CAFTA, *t*-COUTA, syringic, *p*-coumaric and ferulic acids, myricetin, quercetin, kaempferol and isorhamnetin heterosides, (+)-catechin, (−)-epicatechin and anthocyanins. Grape seed extract contained only procyanidins and catechin derivatives while, in the extract from grape flesh, neither hydroxycinnamic acid-tartaric acid esters, procyanidins, flavonol glycosides, nor anthocyanins were detected.

Accordingly, there is no need to remove the peel from Touriga Nacional grape to define its phenolic composition. Moreover, the winemaking technology employs the whole grape.

The phenolics present in Tinta Roriz, Tinta Barroca, Tinta Amarela, Tinta Cão, Touriga Francesa, Touriga Nacional and Rufete grape samples were subjected to high performance liquid chromatography/diode-array detector (HPLC/DAD) under the conditions described. Although all the analysed grape samples showed a common phenolic profile (Fig. 1), with the identification of 12 phenolic compounds, there are differences in the concentrations of the flavonols (Table 1). Thus, in Touriga Nacional, Touriga Francesa, Tinta Barroca,

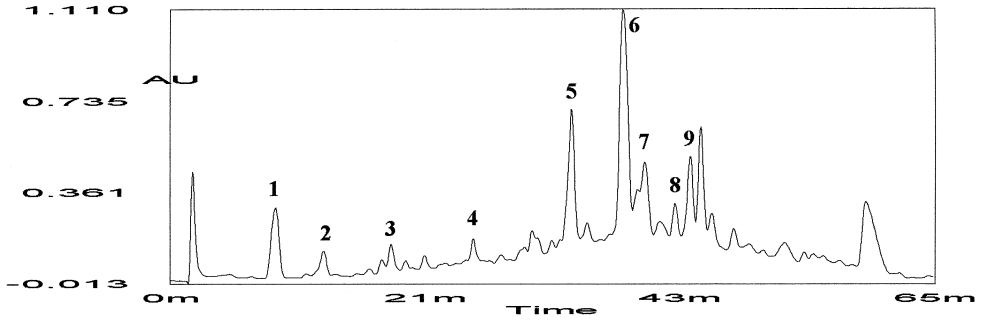


Fig. 1. HPLC chromatogram of grape sample (Detection at 350 nm). (1) *trans*-caffeoyl tartaric acid; (2) *trans*-coumaroyl tartaric acid; (3) *p*-coumaric acid; (4) ferulic acid; (5) myricetin 3-glucoside; (6) quercetin 3-glucoside; (7) kaempferol 3-rutinoside; (8) kaempferol 3-glucoside; (9) isorhamnetin 3-glucoside.

Table 1
Phenolic compounds content of port wine grape varieties (mg/kg)[a]

| Grape variety | Myricetin 3-Glucoside | Quercetin 3-Glucoside | Kaempferol 3-rutinoside | Kaempferol 3-glucoside | Isorhamnetin 3-glucoside |
|---|---|---|---|---|---|
| Touriga Francesa | 21.8±0.413 | 56.7±1.159 | 24.6±0.047 | 9.17±0.674 | 59.6±2.135 |
| Touriga Nacional | 58.1±3.360 | 159±4.152 | 23.1±1.214 | 77.3±7.336 | 283±7.434 |
| Tinta Amarela | 20.1±0.000 | 40.9±0.514 | 15.7±0.171 | 49.3±0.511 | 57.1±0.351 |
| Tinta Barroca | 38.1±5.217 | 84.0±1.852 | 31.1±0.995 | 44.8±0.198 | 139±3.061 |
| Tinta Cão | 9.96±0.405 | 8.43±0.116 | 15.6±1.541 | 25.1±1.558 | 8.83±0.758 |
| Tinta Roriz | 165±52.537 | 103±1.442 | 25.85±0.860 | 141±4.453 | 42.1±7.378 |
| Rufete | 6.59±0.472 | 32.6±1.228 | 7.75±0.953 | nq | nq |

[a] Values are expressed as mean±standard deviation of three determinations; nq, not quantified.

Tinta Amarela and Tinta Roriz varieties, the flavonol heterosides were the major phenolic compounds while, in Tinta Cão and Rufete varieties, (−)-epicatechin was the major compound instead.

Isorhamnetin 3-glucoside is the main compound in the phenolic profile of Touriga Nacional, Touriga Francesa, Tinta Barroca and Tinta Amarela grapes, while Tinta Roriz grape contained myricetin 3-glucoside as the major phenolic. In the Rufete grape variety, kaempferol 3-glucoside and isorhamnetin 3-glucoside are present in trace amounts.

Both the Touriga varieties (Nacional and Francesa) contained (−)-epicatechin in trace amounts. Touriga Francesa contained higher amounts of syringic and *p*-coumaric acids (data not shown).

As far as the authors are aware, there are no other data regarding the composition of flavonols in port wine grape varieties, so this preliminary study contributes new knowledge of the composition of the different grapes used in the production of port wines. Further studies, with a larger number of samples, are necessary to confirm the differences observed.

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