EVALUATION OF METHODS USED FOR THE ISOLATION AND CHARACTERIZATION OF GRAPE SKIN AND SEED AND WINE TANNINS•

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1. INTRODUCTION

Tannins and/or proanthocyanidins comprise a significant portion of the phenolic material in red wine grapes and wines and consequently have an important influence on the quality of red wine. Tannins are the main contributors to the mouth-feel and astringency characteristics of red wine (Gawel, 1998; 2000). Due to the possible interference of anthocyanins, grape skin extracts and wine tannins are often purified with different solid-phase extraction (SPE) methods before further tannin analyses such as phloroglucinolysis (Cortell *et al*., 2005; Obreque-Slier *et al*., 2010). Phloroglucinolysis of crude and purified tannins from grape seeds and skins and wine samples was investigated to develop a standard protocol for tannin analysis in our laboratory.

2. MATERIALS AND METHODS

The skins and seeds from 'Cabernet sauvignon' grapes (17 °Brix, Elgin vineyards, South Africa) were separated from the pulp and extracted with 70 % (v/v) acetone (Merck (Pty) Ltd, South Africa) containing 0.1 % (v/v) ascorbic acid (Sigma-Aldrich, South Africa) (1 g skins or seeds L^{-1} extraction solvent). Extracts were concentrated under reduced pressure at 35 °C to remove acetone and then lyophilized to a dry powder. A young Pinotage wine (2010 vintage) made from grapes from Welgevallen vineyards (Stellenbosch, South Africa) were also used for isolation of wine tannin.

The crude proanthocyanidin extracts and wine were further purified using Toyopearl TSK HW 40-F (Sigma-Aldrich, South-Africa) size exclusion columns (60 mm x 14.5 mm). The columns were equilibrated with 30 mL ethanol/water (55/45) containing 0.05 % trifluoroacetic

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acid (TFA) (Sigma-Aldrich, South Africa). After the extract (2 mL grape skin and wine or 1 mL grape seed) was loaded, the column was washed with 45 mL of the equilibration solution and the proanthocyanidins eluted with 30 mL of acetone/water (60/40) containing 0.05 % TFA. Eluents were dried and resolubilised in 1 mL MeOH (Sigma-Aldrich, South Africa) before phloroglucinolysis. The phloroglucinolysis protocol of Drinkine *et al*. (2007) was implemented and the cleavage products were analysed by RP-HPLC using a method adapted from Kennedy and Taylor (2003).

3. RESULTS

Packed SPE cartridges were used multiple times for the purification of wine tannin with an average recovery of 74 ± 11 % for the polymeric phenols. Recovery decreased to 50 ± 3 % when the packed cartridges were used more than four times.

Phloroglucinolysis results of grape seed and skin and wine tannin had excellent repeatability and intermediate precision with less than 3 % standard deviation for mean degree of polymerisation (mDP) determination. RP-HPLC separation of extension and terminal units had very good selectivity (fig. 1).

Phloroglucinolysis was also performed on grape skin and seed extracts without prior purification by SPE as this sample preparation procedure is not always possible due to the increase in sample numbers and preparation time. Repeatability and selectivity of separations were the same as when tannin fractions were purified (fig. 2). In the case of grape skin tannin analysis, crude and purified fractions gave similar results with a <5 % standard deviation in the determination of the mDP. But the difference for crude *vs* purified grape seed tannins was more substantial with a mDP respectively of 6.6 and 4.3 for the purified and crude extracts. This may be due to the fact that SPE recovery of the polymeric phenol fraction according to RP-HPLC analysis (Peng *et al*., 2002) was only 40 %, although it increased significantly with sequential use of the cartridge (±100 %). The lower molecular weight proanthocyanidins may have been removed during tannin isolation, increasing the mDP of the tannin fraction.

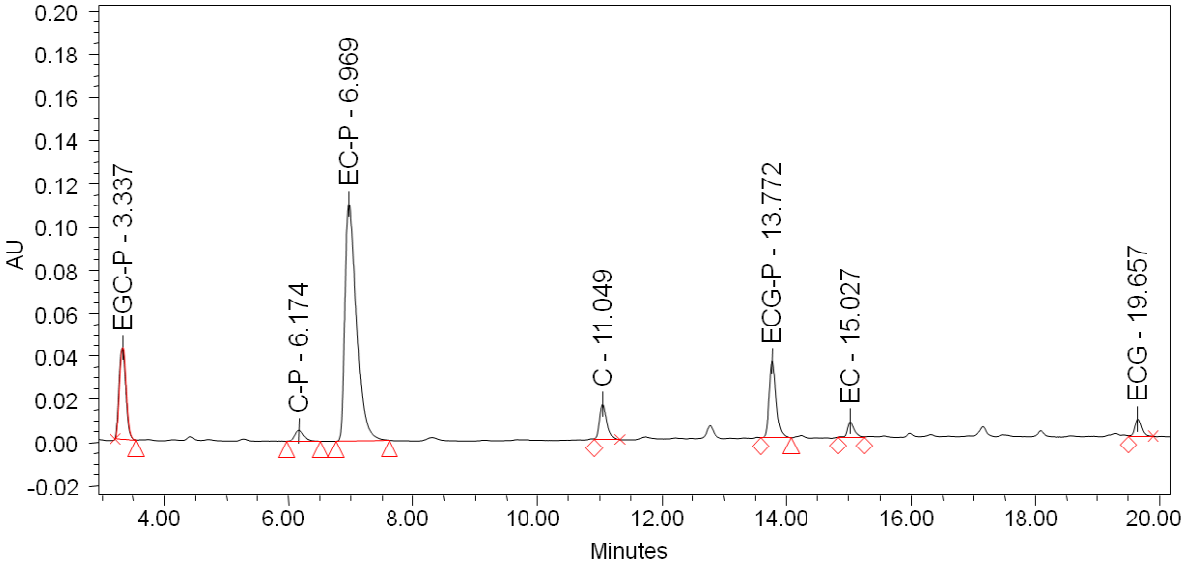

Fig. 1 - HPLC chromatogram of proanthocyanidin cleavage products from Pinotage wine tannin. Subunit abbreviations: -P, phloroglucinol adduct of extension subunit; EGC, (-)-epigallocatechin; C, (+)-catechin; EC, (-)-epicatechin; and ECG, (-)-epicatechin-3-*O*-gallate.

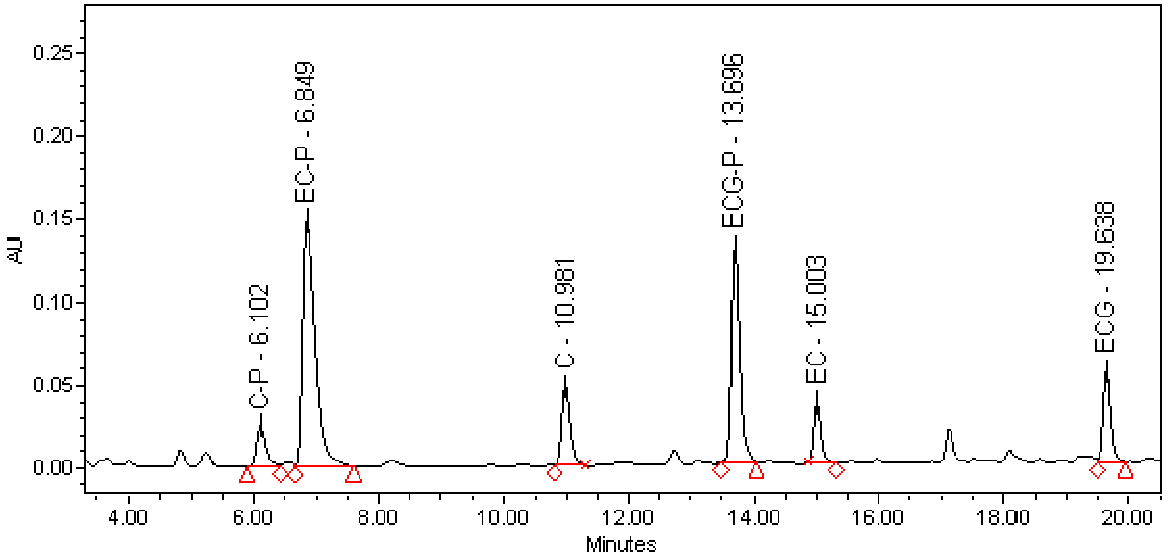

Fig. 2 - HPLC chromatogram of proanthocyanidin cleavage products from 'Cabernet sauvignon' grape seed tannin (crude fraction). Subunit abbreviations: -P, phloroglucinol adduct of extension subunit; EGC, (-)-epigallocatechin; C, (+)-catechin; EC, (-)-epicatechin; and ECG, (-)-epicatechin-3-*O*-gallate.

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Abstract

Validation of the phloroglucinolysis and RP-HPLC method showed selectivity and repeatability within acceptable limits for all investigated matrices. Recovery of polymeric phenols by SPE was also acceptable. Phloroglucinolysis on crude and purified tannin fractions was successful but in the case of grape seed tannin the results differed significantly (30 %). Generally, grape seed tannin extracts are not purified before phloroglucinolysis. These phenomena need to be investigated further.

Literature cited

Cortell J.M., Halbleib M., Gallagher A.V., Righetti T.L. Kennedy J.A. - 2005 - Influence of vine vigor on grape (*Vitis vinifera* L. cv Pinot noir) and wine proanthocyanidins. *Journal of Agricultural and Food Chemistry*, 53, 5798-5808.

Drinkine J., Lopes P., Kennedy J.A., Teissedre P.L., Saucier C. - 2007 - Ethylidene-bridged flavan-3-ols in red wine and correlation with wine age. *Journal of Agricultural and Food Chemistry*, 55, 6292-6299.

Gawel R. - 1998 - Red wine astringency: a review. *Australian Journal of Grape and Wine Research*, 4, 74-95.

Gawel R., Oberholster A. Francis I.L. - 2000 - Red wine mouth-feel terminology. *Australian Journal of Grape and Wine Research*, 6, 203-207.

Kennedy J.A. Taylor A.W. - 2003 - Analysis of proanthocyanidins by high-performance gel permeation chromatography. *Journal of Chromatography* A, 995, 99-107.

Obreque-Slier E., Pena-Neira A., Lopez-Solis R., Zamora-Marin F., Ricardo-da Silva J.M., Laureano O. - 2010 - Comparative study of the phenolic composition of seeds and skins from Carmenere and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. *Journal of Agricultural and Food Chemistry*, 58, 3591-3599.

Peng Z., Iland P.G., Oberholster A., Sefton M.A., Waters E.J. – 2002 - Analysis of pigmented polymers in red wine by reverse phase HPLC. *Australian Journal of Grape and Wine Research*, 8, 70-75.