INVESTIGATION OF THE EFFECT OF GELATINE AND EGG ALBUMIN FINING AND CROSS-FLOW MICROFILTRATION ON THE PHENOLIC COMPOSITION OF PINOTAGE RED WINE•

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1. INTRODUCTION

Phenolic compounds and more specifically the polymeric pigments and phenols are important contributors to the quality of red wine. They are mainly responsible for the mouth-feel and astringency characteristics of a wine (Gawel, 1998; 2000). The effect of fining agents such as polyvinylpolypyrrolidone (PVPP), egg albumin, gelatine and casein on the colour and proanthocyanidin fraction of red wines has been investigated (Maury *et al.*, 2003; Cosme *et al.*, 2009). However, little research is available on the influence of cross-flow microfiltration on the phenolic content of red wines. López *et al*. (2005) investigated the effect of cross-flow microfiltration on vinegar and found that it decreased the colour density and total phenols (mg L^{-1} gallic acid equivalents by Folin-Ciocalteu) of red vinegar. Previous studies on the effects of fining reported a decrease in colour intensity, anthocyanin and total phenol concentrations in wine with fining compared to the control (Sims *et al.*, 1995; Sarni-Manchado *et al.,* 1999). In this study the effects of gelatine and egg albumin fining as well as cross-flow microfiltration on the phenol composition of Pinotage red wine were investigated.

2. MATERIALS AND METHODS

A young Pinotage wine (2010 vintage) from Koopmanskloof winery (Stellenbosch, South Africa) was fined with gelatine (Gecoll Supra, Laffort, dosage 100 mL hL^{-1}) and egg albumin (Laffort, 10 g hL^{-1}) after malolactic fermentation (MLF). The wines (20 L/replicate) were settled for 10 days at 15 °C before sampling. The wine was also treated with cross-flow microfiltration. All treatments were done in five replicates except for cross-flow microfiltration where 200 L of wine was filtered and stored in 20 L x 3 containers.

The phenol composition of each wine was determined by RP-HPLC (Peng *et al*., 2002). The polymeric phenol composition was analysed by isolating the polymeric fraction from the wine using Toyopearl TSK HW 40-F (Sigma-Aldrich, Johannesburg, 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 acid (TFA). After 2 mL of wine was

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loaded, the column was washed with 45 mL of the equilibration solution and the polymeric phenols (tannin) eluted with 30 mL of acetone/water (60/40) containing 0.05 % trifluoroacetic acid. All of the above chemicals were purchased from Merk (Pty) Ltd, South Africa. Eluents were dried and resolubilised in 1 mL MeOH (chromasolv, Sigma-Aldrich, South Africa) before phloroglucinolysis. The phloroglucinolysis protocol of Drinkine *et al*. (2007) was used and the cleavage products were analysed by RP-HPLC with a method adapted from Kennedy and Taylor (2003). The average molecular mass (MM) of the isolated wine tannin was determined by gel permeation chromatography (GPC) (Kennedy, Taylor, 2003). Total phenols and wine colour measurements were also performed on the treatments (Somers, Evans, 1977).

3. RESULTS

Both gelatine and egg albumin fining decreased the mDP of the wine tannin isolated by respectively 32 and 28 % compared to the control (tab. 1). This is in agreement with the findings of other researchers (Maury *et al*., 2003; Cosme *et al*., 2009). Cross-flow microfiltration also decreased the mDP of the wine tannin compared to the control although to a lesser extent (22 %). Thus, the fining agents and cross-flow microfiltration selectively removed the highly polymerised phenols, but contrary to the findings of Maury *et al.* (2003), the galloylated tannins were not specifically removed. The average MM of the wine tannin determined by GPC correlated with the results obtained by phloroglucinolysis with a decrease in MM with fining and cross-flow microfiltration (tab. 1).

Tab. 1 - Phloroglucinolysis and GPC results of the isolated wine tannin.

Treatment[a]	Extension				Terminal	
	% EGC-P	% C-P	% EC-P	% ECG-P	% C	% EC
C	39.54±0.46	1.57±0.02	50.57±00.46	2.68±0.03	4.16±0.04	1.46±0.05
G	40.00±0.44	1.71±0.02	48.12±0.48	1.93±0.06	6.03±0.05	2.20±0.05
EA	40.64±0.40	1.58±0.01	47.86±0.48	2.18±0.03	5.74±0.05	2.01±0.05
CF	41.51±0.34	1.20±0.02	48.56±0.69	1.52±0.11	5.48±0.04	1.73±0.04

Treatment[a]	mDP[b]	% Galloyl[c]	MM[d]
C	17.87	1.47	3023.73
G	12.14	1.93	2941.72
EA	12.87	2.12	2960.74
CF	13.90	1.52	2857.33

[a]Treatments and percent composition of wine tannin subunits (in moles) with the following subunits and abbreviations: C, control; G, gelatine; EA, egg albumen; CF, cross-flow microfiltration. ;- P, phloroglucinol adduct of extention subunit; EGC, (-)-epigallocatechin; C, (+)-catechin, EC, (-)-epicatechin, ECG, (-)-epicatechingallate. [b]mDP, mean degree of polymerisation; [c]% Galloyl, percentage galloylated units (ECG and ECG-P) of the total. [d]MM, molecular mass based on 50% mass elution by GPC. $N = 5 \pm$ standard deviation, except CF, $N = 3 \pm$ standard deviation.

Although GPC results indicated, contrary to phloroglucinol results, a larger decrease in wine tannin MM by cross-flow microfiltration. These differences are due to the fact that phloroglucinolysis only determine the composition of the proanthocyanidin part of wine tannin. RP-HPLC analysis showed that fining and cross-flow microfiltration had no effect on the flavan-3-ol content (monomeric and dimeric proanthocyanidins) of the wines (fig. 1).

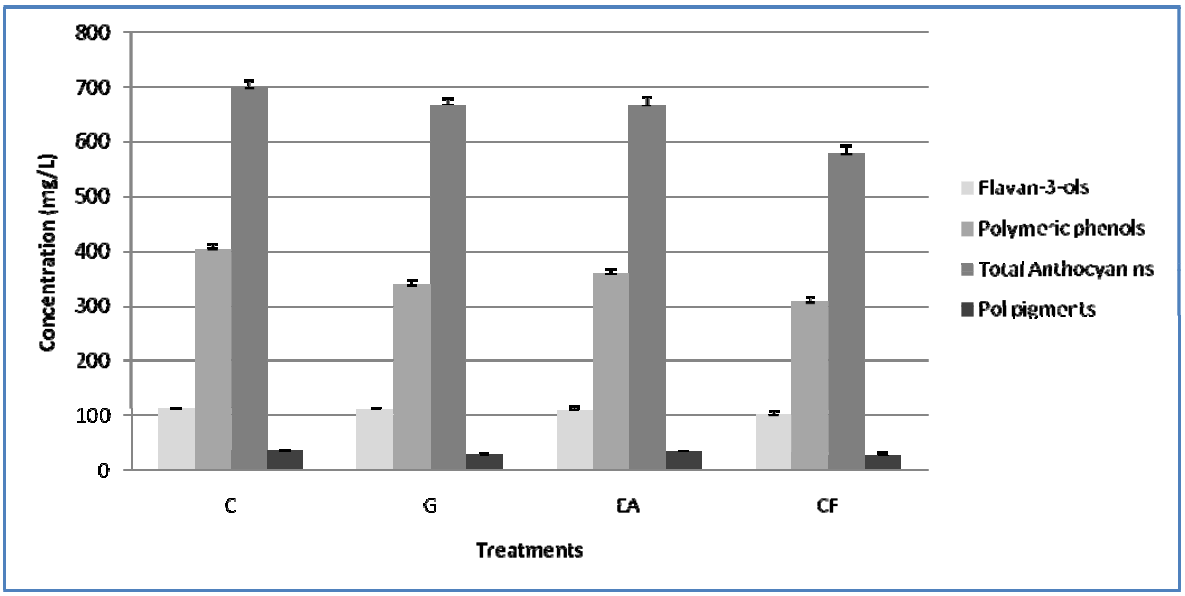

Fig. 1 -Phenol composition of wine treatments determined by RP-HPLC analyses.

Fining and cross-flow microfiltration did however decrease the anthocyanin and polymeric phenol content. Cross-flow microfiltration removed the most red colour in addition to the polymeric phenol content, followed by gelatine fining when compared to the control. Spectrophotometric results (data not shown) support these findings with a decrease in colour density (520 nm + 420 nm), total potential colour (520 nm, pH 1) and total phenols of treated wine compared to the control. This is similar to what other researchers found (Sims *et al*., 1995; Sarni-Manchado *et al*., 1999).

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Abstract

Results indicated that cross-flow microfiltration removed similarly to fining treatments the most astringent tannins, but cross-flow microfiltration also removed up to 14 % more colour. RP-HPLC and spectrophotometric results showed that egg albumin is a softer fining treatment compared to gelatine and cross-flow microfiltration. All treatments will be analysed again after four months of bottle aging to determine the longer term effects. These findings also need to be supported by

sensory analysis, which is the next step of this study. All the wines will be evaluated sensorially using the developed mouth-feel wheel (Gawel *et al*., 2000).

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