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Investigation of the effect of gelatine, egg albumin and cross-flow microfiltration on the phenolic composition of Pinotage wine

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ABSTRACT

The effect of fining and cross-flow microfiltration on the phenolic composition of red wine was investigated. Both gelatine (G) and egg albumin (EA) fining decreased the mean degree of polymerisation (mDP) of tannin significantly by 26.4% and 25.2%, respectively, compared to the control (C). Cross-flow microfiltration (CF) also decreased the mDP significantly by 25%. Thus, the fining agents and cross-flow microfiltration selectively removed the highly polymerised phenols. After 3.5 months of bottle ageing, differences between the different treatments and the control decreased. CF had the most significant effect on the flavan-3-ol and polymeric phenol (tannin) content of the wines compared to the control followed by G fining. CF and EA treatments significantly decreased the total pigment content compared to C. CF was also the only treatment that could be distinguished from the other treatments by sensory analysis. All treatments improved clarity of the wines with cross-flow microfiltration having the largest effect.

1. Introduction

Phenolics are important contributors to wine quality, ageing potential and sensory characteristics. Phenolic compounds are responsible for the differences observed between white and red wine and are extracted from grape skins and seeds during winemaking. The concentration of phenols can be influenced by grape variety, viticultural practices and winemaking processes and transformation that can take place during wine ageing (Castillo-Sanchez et al., 2008).

The main flavonoid compounds present in grapes and wine are anthocyanins, flavan-3-ols and their polymerised products (Aron & Kennedy, 2007). Flavan-3-ols can be present as monomers, oligomers and polymers in red wine, also known as proanthocyanidins or condensed tannins (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006).

Anthocyanins originate from skins in grapes and are responsible for the red colour of red grapes and wine (Harbertson, Picciotto, & Adams, 2003). Polymeric pigments are more stable to pH changes and bisulphite bleaching than monomeric pigments (Ribéreau-Gayon et al., 2006). Thus, monomeric anthocyanins and polymeric pigments can be distinguished by these characteristics (Harbertson et al., 2003). During the first year of storage rapid changes take place in the colour composition of the wine. The purple–red colour of a young red wine changes to orange–red with aging. These changes occur when the monomeric pigments are replaced with oligomeric forms which are more stable through direct and indirect polymerisation reactions (Cheynier et al., 2006).

Fining agents are used to eliminate or reduce undesirable substances in wine. The main purpose of using a protein based fining agent in particular is to soften the wine and to reduce wine astringency through complexation of the proteins with the phenolic compounds in the wine and removal thereof through precipitation (Sarni-Manchado, Deleris, Avallone, Cheynier, & Moutounet, 1999). Protein fining agents differ in several physico-chemical ways. The different characteristics are molecular weight distribution, isoelectric point and the surface charge density (Cosme, Ricardo-Da-Silva, & Laureano, 2009).

Fining agents such as egg albumin, casein, gelatine and PVPP reduces the phenolic content of wines and may decrease the colour of some wines (Castillo-Sanchez, Mejuto, Garrido, & Garcia-Falcon, 2006; Castillo-Sanchez et al., 2008; Cosme, Ricardo-Da-Silva, & Laureano, 2008; Cosme et al., 2009; Sarni-Manchado et al., 1999). Several authors found that there is increased interaction between proteins and more polymerised proanthocyanidins and proanthocyanidins esterified with gallic acid than less polymerised proanthocyanidins (Cosme et al., 2009; Ricardo-Da-Silva et al.,1991; Sarni-Manchado et al., 1999). Cosme et al. (2009) stated that egg albumin and low molecular weight gelatine only

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removed 20–28% of the condensed tannins with a 4.9 mean degree of polymerisation (mDP). All gelatine molecular weight fractions consistently removed more low molecular weight proanthocyanidins than egg albumin. Egg albumin also removed the least amount of colour.

Cross-flow microfiltration is a relatively new technique and only a few studies have been published. Lopez and co-workers (López et al., 2005) did a study on the influence of cross-flow microfiltration on different vinegars (white, rosé and red). They determined that sterilisation and clarification happens simultaneously during cross-flow microfiltration. There was a reduction in turbidity for all the vinegars, but total acidity and pH were not affected. Additionally, there was a 37% reduction in modified colour intensity of red vinegar, although no significant influence on the total phenol or polymeric phenol content.

Phenolic compounds play an important role in the quality of the final wine product and contribute significantly to the sensory properties of red wine. They contribute to colour, bitterness and astringency as well as other mouth-feel properties of wine (Gawel, 1998; Oberholster, Francis, Iland, & Waters, 2009). Although flavan-3-ols are both bitter and astringent, astringency increases faster than bitterness with increased polymerisation. This explains the concept of harsh tannins in young wine and softer tannins in an aged wine, due to more polymeric flavanols in aged wines than in young wine with more monomers. The perception of astringency is described in many subtle forms such as 'roughing', 'drying' and 'puckering'. Gawel and co-workers (Gawel, Oberholster, & Francis, 2000) developed a mouth-feel wheel to describe the different mouth-feel sensations and astringent sub-attributes perceived when tasting wine. Sensory changes take place when anthocyanins and tannins interact with each other and could explain the differences in mouth-feel properties perceived between a white and a red wine (Oberholster et al., 2009).

In this study the effect of fining with gelatine and egg albumin, as well cross-flow microfiltration on the phenol and tannin composition, of Pinotage wine were investigated. Pinotage is well known as South Africa's signature red cultivar and is a cross between Pinot noir and Cinsaut (Hermitage). The influence of bottle ageing on these affects was also evaluated, in addition to the influence on the mouth-feel properties of the wines.

2. Materials and methods

2.1. Chemicals and fining agents

All chromatographic solvents were high-performance liquid chromatography (HPLC) grade. Ethanol, acetone, acetonitrile, methanol, *N*,*N*-dimethylformamide, phloroglucinol, sodium acetate, L-ascorbic acid, lithium chloride, caffeic acid, *p*-coumaric acid, potassium bitartrate, triethanolamine, bovine serum albumin, metabisulfite, (+)-catechin, (−)-epicatechin and quercetin were purchased from Sigma–Aldrich (Johannesburg, South Africa). Quercetin-3-glucoside and sodium dodecyl sulphate were bought from Fluka (Buchs, Switzerland), while acetic acid and trifluoroacetic acid were obtained from Riedel-de Haën (Seelze, Germany). Hydrochloric acid and sodium chloride were purchased from Merck (Pty.) Ltd., (Johannesburg, South Africa). Ferric chloride hexahydrate were obtained from Radchem (Johannesburg, South Africa). The fining agents Gecoll Supra (gelatine) and egg albumin were sponsored by Laffort (Bordeaux cedex, France).

2.2. Wine treatments

Pinotage wine (pH 4.25, titratable acidity (TA) 5.36 g/l as tartaric acid, residual sugar 0.17 g/l, free SO_2 47 mg/l) from the 2010 vintage was bought from Koopmanskloof cellar. This wine was made using normal commercial winemaking practices and was obtained after the completion of malolactic fermentation. Fining treatments were performed according to the suggestions of the manufacturers in 5 replicates. Shortly, 100 ml/hl liquid gelatine and 10 g/hl egg albumin were added to 20 l amounts of wine. Untreated wine was used as control. The fining agents were thoroughly mixed with the wine and held at 15 °C for 10 days.

Two hundred litres of wine was filtered using cross-flow microfiltration (Flavy FX3 cross-flow filter, 2 μm pore size, Bucher Vaslin SA, France). The filtered wine was stored in three separate 40 l containers for 10 days at 15 °C before bottling. All the treatments were analysed before bottling. The wines were racked and bottled in 750 ml green glass bottles, with 1 cm head space, and sealed with screw cap closures. The bottles were stored from bottling in May 2010 at 18 °C in an upright position to August 2010, when the wines were analysed. Turbidity measurements were performed using LP 2000 turbidity metre (HANNA instruments®, Inc., Michigan, USA) after 3.5 months of bottle ageing.

2.3. RP-HPLC analysis

Reverse phase high performance liquid chromatography (RP-HPLC) was performed on a Hewlett Packard Agilent 1100 series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA, USA). Data processing was performed with Chemstation software 10.02 (Hewlett Packard, Waldbronn, Germany). Separations were carried out on a polystyrene/divinylbenzene reversed phase column (PLRP-S, 100 Å, 150 × 4.6 mm, 3 μm) protected with a guard cartridge with the same packing material (PLRP-S, 10 × 4.6 mm), all purchased from Polymer Laboratories (Ltd.) (Shropshire, UK). The following mobile phases were used: solvent A, containing de-ionised water with 1.5% v/v *ortho*-phosphoric acid and solvent B consisting of 80% acetonitrile with 20% of solvent A. A linear gradient was used from 0 min, B 6%; to 73 min, B 31%; to 78 min, B 62%, staying constant for 8–86 min and then back to the starting conditions in 4–90 min, B 6%. A flow rate of 1 ml/min was used and a column temperature of 35 °C. The column was equilibrated for 15 min at the starting conditions before the next injection. This was adapted from the method of Peng, Iland, Oberholster, Sefton, and Waters (2002).

Phenols were quantified using external standards: (+)-catechin hydrate, (−)-epicatechin, gallic acid, caffeic acid, *p*-coumaric acid, malvidin-3-glucoside, quercetin-3-glucoside and quercetin. Monomeric and dimeric flavanols and polymeric phenols were quantified at 280 nm as mg/l catechin units with a quantification limit of 1.0 mg/l, and epicatechin as epicatechin with a limit of quantification (LOQ) of 1.0 mg/l. Gallic acid was also quantified at 280 nm in gallic acid units with a LOQ of 0.1 mg/l. Cinnamic acids were quantified at 316 nm and caftaric acid and caffeic acid were quantified as mg/l caffeic acid, while coutaric acid and *p*-coumaric acid were expressed as mg/l *p*-coumaric units with a quantification limit of 0.04 and 0.03 mg/l respectively. Flavonol-glycosides and flavonol aglycones were quantified at 360 nm as, respectively mg/l quercetin-3-glucoside and mg/l quercetin with a quantification limit of 0.25 mg/l for both. Anthocyanins, pigments and polymeric pigments were quantified at 520 nm as mg/l malvidin-3-glucoside with a quantification limit of 0.05 mg/l. The samples were filtered (0.45 μm) before injection. Thereafter each sample was placed in a 1.5 ml dark coloured vial. The limit of detection (LOD) was defined as a signal to noise ratio of 3 and the LOQ was defined signal to noise ratio of 7.

2.4. Spectrophotometric analysis of tannins and pigments

The tannin content of a wine can be quantified by using its capacity to bind protein and the precipitation of the resulting