Investigating the Phenolic Composition of Merlot and Shiraz Grape Extracts and Wines Produced from Grapes With Different Seed-to-skin Ratios

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This study explored how Shiraz and Merlot grape extracts and wines made with different skin-to-seed ratios using various extraction methods differ in terms of phenolic composition. These methods included the Iland, Glories and machine-crushed techniques. Each method varied in extraction solvent, pH, extraction time and grape-processing techniques. The Iland method showed no significant differences between Shiraz and Merlot grapes in terms of anthocyanin concentration and colour density for all treatments. However, tannin concentrations and the total phenolic index varied significantly, with higher tannin levels generally observed in treatments with more seeds. Machine-crushed and microwave extraction did not significantly affect anthocyanin levels in the extracts, but showed differences in tannin concentrations, especially in Merlot grapes. The Glories method showed higher potential anthocyanin levels in the seedless treatments for both cultivars compared to those with seeds. However, the method's evaluation of seed tannin contribution requires reassessment due to interesting findings in the seedless treatments. In winemaking, treatments with more seeds exhibited the highest anthocyanin and tannin levels, affecting the colour density and total phenolic index over time, especially with extended maceration time. Analysing tannin composition via phloroglucinolysis revealed that seed presence affected tannin molecular weight and composition, with notable differences observed between seedless and seeded treatments in the grape extracts and corresponding wines. Overall, the study underscores the intricate relationship between grape seed ratios, extraction methods and phenolic composition. The findings contribute to understanding how these factors affect wine phenolic composition and could contribute to future research on optimising phenolic extraction in winemaking.

INTRODUCTION

The phenolic composition and properties of red wine grapes have been investigated extensively, with several reports indicating the presence of high levels of phenolic compounds (Rockenbach *et al*., 2011). The phenolic content in grapes is affected by several factors that include, but are not limited to, cultivar, maturity, vintage or location within the berry (Shi *et al*., 2003). Wine producers strive to harvest grapes at optimal ripeness, which is determined by many factors, including technological and phenolic composition. To determine the harvest date, the phenolic content of the skins, pulp and seeds is thus also often assessed (Ferrer-Gallego *et al*., 2012).

Several methods have been reported that can be used to determine phenolic levels in red grapes (Iland *et al*., 2000; Lee & Rennaker, 2011). These methods aim to facilitate phenolic extraction from the solid tissues of the berries. Phenolic extraction methods are classified into conventional, i.e., solid-liquid extraction, and non-conventional, i.e., microwave-assisted and ultrasound-assisted extraction methods. Standard protocols have been established for the latter approaches (Caldas *et al*., 2018). Some of the most common solvent-based methods to analyse grape phenolics are the Iland (Iland *et al*., 2000), Glories (Glories, 1984a, 1984b) and ITV standard methods (Cayla *et al*., 2002), which quantify anthocyanins and the total phenolic content of extracts from grape homogenates. The Glories method, in addition to the total content of the most relevant phenolic parameters, provides information on the contribution of grape seeds and skins to the tannin content. The homogenisation of grapes used in these methods causes the crushing of the seeds, providing different extraction conditions to those taking place in a conventional alcoholic fermentation with crushed berries. To overcome this issue and mimic fermentation extraction conditions, extraction methods that rely on hand crushing of the grapes to quantify the phenolic content have been proposed (Bindon *et al*., 2014b). While solvent-assisted extraction can yield high phenolic compound recovery, the use of large amounts of organic solvents can pose a health and safety risk for researchers and be hazardous to the environment. As a result,

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several alternative methods for extracting phenolics from grapes that limit the use of solvents have been attempted (Xia *et al*., 2010). Among these are microwave-assisted and ultrasound-assisted extraction methods. In comparison to other extraction methods, microwave-assisted extraction is reported to be more efficient, as it heats the matrix internally and externally without a thermal gradient and, as such, functional compounds can be extracted efficiently. In addition, some of the benefits of this method are reduced extraction times and reduced solvent volumes (Wang & Weller, 2006; Li *et al*, 2011; Sommer & Cohen 2018). The extraction efficiency is therefore dependent and differs for each method due to the different extraction media and the grape preparation procedure.

Proanthocyanidins or condensed tannins are among the most important phenolic classes in grapes because they contribute to the astringency and mouthfeel properties of red wines (Smith *et al*., 2015). Tannins are mainly present in grape skin and seeds, and differences in terms of concentration and composition have been reported (Gambuti *et al*., 2009; Mattivi *et al*., 2009; Bautista-Ortín *et al*., 2014; Ren *et al*., 2020; Rousserie *et al*., 2020). Briefly, skin tannins consist of long polymeric chains and a large proportion of prodelphinidins to procyanidins, whereas seed tannins are comparatively small, but with large proportions of galloylated units (Smith *et al*., 2015). Overall, tannin levels are higher in seed than in skin tissue which can lead to higher proportions of seed tannins present in wine (Cerpa-Calderón and Kennedy 2008), depending on factors such as maceration time and pressing regimes. Considering the implications of tannins for wine mouthfeel properties, it is understandable that a need exists for suitable methods that provide information on grape phenolic composition at harvest.

Studies indicate that climatic conditions can affect the total number of seeds or proanthocyanidin levels per berry (Ewart & Kliewer, 1977; Del Rio & Kennedy, 2006). Different skin-to-seed ratios during alcoholic fermentation have been shown to affect the phenolic composition of red wines (Bautista-Ortín *et al*., 2014; Ren *et al*., 2020). Further investigating the effects of different seed-to-skin ratios on grape extracts for grape phenolic analyses, and how this reflects in the corresponding wines, is thus of importance. However, to our knowledge it has not yet been investigated. Furthermore, the suitability of different methods of grape phenolic analysis has also not been investigated in this regard. The main aims of the study were thus to investigate the suitability of different extraction methods to quantify the phenolic content of Shiraz and Merlot grape extracts with different seed-to-skin ratios, as well as to assess the phenolic composition of wines made from these.

MATERIALS AND METHODS

Procedure to obtain grapes and ferments with different skin-to-seed ratios

Two crates containing approximately 18 kg of Shiraz and Merlot grapes each were collected from two Stellenbosch vineyards and immediately frozen at -20°C. Frozen grapes (2 500) from both cultivars were collected from the crates and mixed homogenously. The seeds of 2 000 of these frozen grapes of each cultivar were manually removed using a surgical blade. This was done by cutting each frozen berry in half and removing the seeds. The remaining 500 berries of each cultivar were used for phenolic analyses, as indicated in the following section. The skins and seeds were individually mixed to ensure homogeneity before being equally divided into 9 x 350 mL coffee jars with plungers. The skins and juice were equally divided by weight between the coffee jars (240 ml juice:90 g skins in each plunger, which was the juice-to-skin yield originally found in the crushed grapes). The seeds were then added by weight according to the required treatments. Gombau *et al*. (2020) reported that the main determining factor of tannin concentration and astringency in red wines is the seed weight percentage in respect to berry weight. The following treatments were thus each carried out in triplicate: seedless, normal seed-to-skin ratio (referred to as "1 x seeds"), and twice the normal seed-to-skin ratio (referred to as "2 x seeds") (see Fig. 1).

The coffee plungers were moved to a 25°C fermentation room, and 30 mg/L SO_2 was added using a 2% SO_2 solution. *Saccharomyces cerevisiae* Lalvin ICV D21® (Montreal, Canada), which was rehydrated and inoculated into the must according to the instructions of the manufacturer, were used for the alcoholic fermentation.

The fermentations were carried out under similar conditions for both grape varieties, with variation in the maceration time. Shiraz was fermented for seven days on the skins, while Merlot underwent an extended maceration time of 14 days. Punch-downs were performed three times per day for the first seven days, using a spoon due to the small-scale ferments. Samples were collected every day after the last punch-down (17:00) in 2 mL microfuge tubes until the last day of skin contact, when the skins were pressed using the plunger and the pomace was removed. All samples were stored at -20°C until required for analysis.

Grape phenolic extraction methods

For the grape phenolic analyses, a sub-sample of 500 berries (200 berries for the homogenate methods and 300 for the machine-crushed method) from the previously mentioned 2 500 Shiraz or Merlot berries were used for three grape extraction methods (Fig. 1). Each extraction method was performed in triplicate. The 500 berries were manually deseeded in the same manner as previously indicated, and the skins and seeds were divided by weight according to the required skin-to-seed ratio treatment (no seeds, "Seedless", normal seed to skin ratio (referred to as "1xSeeds") and twice the normal seed to skin ratio (referred to as "2xSeeds")). The Iland (1 h extraction of grape homogenate in 50% ethanol) (Iland et al., 2000), Glories (4 h extraction of grape homogenate in aqueous solution at pH 1 and pH 3.2 solution) (Cristea, 2014), and a machine-crushed method with microwaved treatment proposed inhouse method (machine-crushed and microwave-heated grapes with 3 h extraction in 50% ethanol) were performed on each of the treatments.

Glories grape phenolic extraction

The Glories grape phenolic extraction method reported by Vivas *et al*. (1998) was utilised, with modifications (Cristea, 2014). Triplicate skin-to-seed grape treatments (SL, 1 x seeds

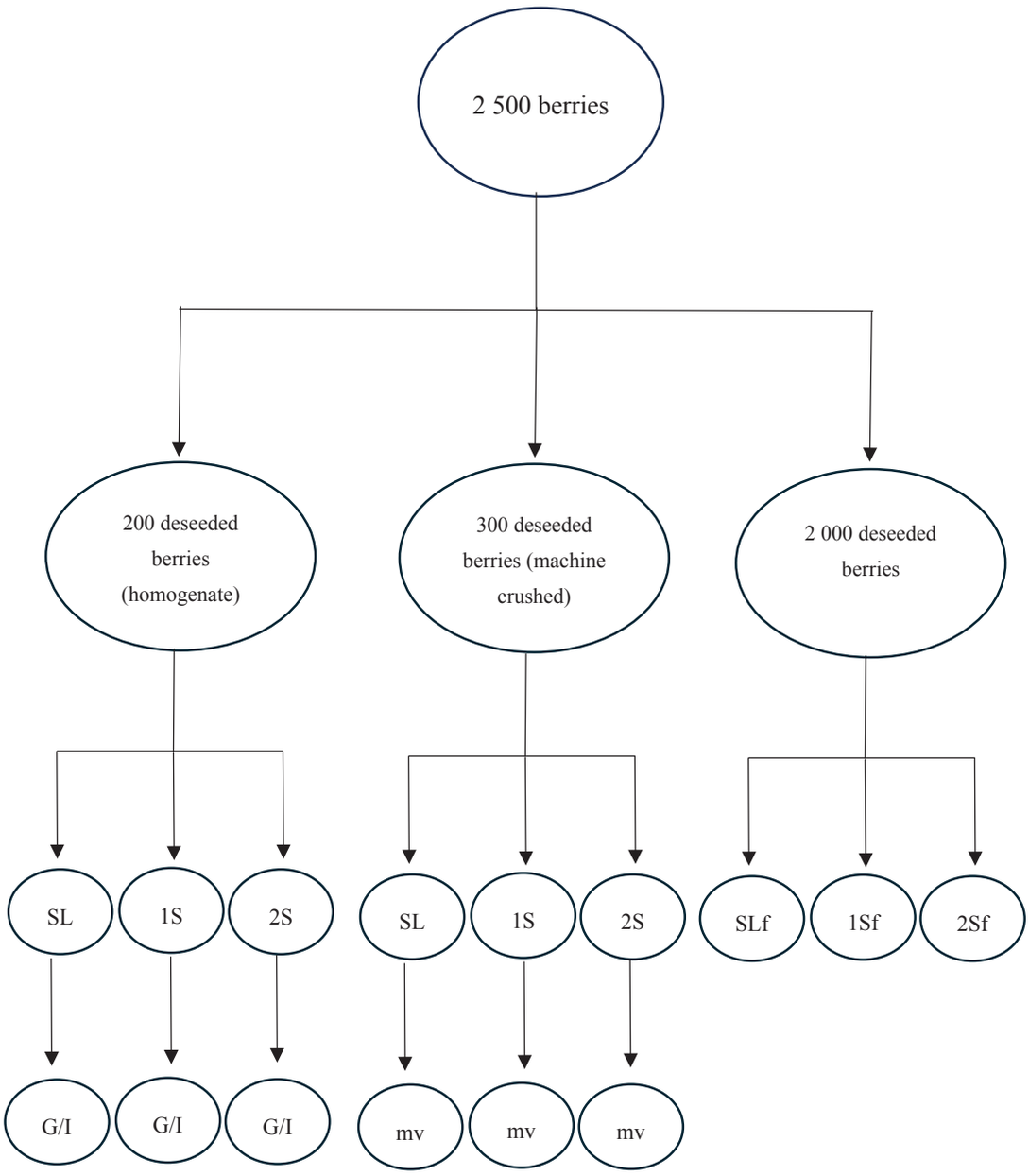

FIGURE 1
Diagram indicating the distribution of berries and treatments used in the Shiraz and Merlot grape phenolic analyses and wine ferments. SL - seedless, 1 x S – 1 x seeds, 2 x S – 2 x seeds, f - ferments, G - Glories, I - Iland, mv - microwave

and 2 x seeds) were homogenised for 4 min using an IKA T18 basic Ultra Turrax (Hamburg, Germany) homogeniser. Two 20 g samples of the homogenate for each replicate were weighed, and 20 mL of a pH 1 solution (0.1 N HCL) was added to one sample, and 20 mL of a pH 3.2 solution (22 mL of 1 N NaOH and 5g w/v tartaric acid, pH adjusted with NaOH in 1 litre of distilled water) was added to the other sample. The samples were subjected to extraction using a shaker table (New Brunwick Scientific) at 25°C for 4 h. After 4 h, the samples were centrifuged for 5 min at 10 000 rpm (Eppendorf 5415 D, Hamburg, Germany) and the clarified supernatants were analysed (see "Chemical analysis" for details).

Iland grape phenolic extraction method

The grape phenolic extraction method reported by Iland *et al*. (2000) was used to extract and analyse samples for phenolic compounds. Triplicate skin-to-seed grape treatments samples were homogenised for 4 min using an IKA T18 basic Ultra Turrax (Hamburg, Germany) homogeniser. One gram of each homogenised sample was weighed into a 15 mL Falcon tube, and 10 mL of the extraction solvent (50% v/v ethanol solution at pH 2, pH adjusted with 1 M HCl) was added to the sample. The samples were placed in a Branson 5510 sonicator (Danbury, USA) for one hour and manually shaken at 15-minute intervals. After 1 h, the samples were centrifuged for 5 min at 10 000 rpm (Eppendorf 5415 D, Hamburg, Germany), and the clarified supernatant was analysed as indicated under "Chemical analysis".

Machine-crushed and microwaved grape phenolic extraction method

A new grape phenolic extraction protocol using a custom-made electric small-scale crusher was assessed. This method is based on the hand-crushed method of Bindon *et al*. (2014b). The custom-made crusher was used with the aim to ensure better repeatability during the sample processing, thereby minimising the potential variability introduced during the manual hand-crushing procedure of the grape berries. The skins and juice of 300 berries were equally divided, and the seeds were divided according to weight for each seed-to-skin treatment (in triplicate), weighed with a laboratory balance, vacuum sealed in plastic bags and machine crushed using the custom-made machine crusher (Fig. 2). The rollers in the machine tore the skins with the seeds remaining undamaged. The samples were transferred to glass jars and microwaved for 35 sec on medium-high heat (Hisense H20MOWMG microwave oven, 700W, China) until a temperature of 85°C was reached in the sample. After this, the equivalent of 1 mL of a 50% v/v ethanol solution/gram of grape tissue was added to each sample. The samples were subjected to extraction using a shaker table (New Brunswick Scientific)) for 3 h at 25°C. After 3 h, the samples were centrifuged for 5 min at 10 000 rpm (Eppendorf 5415 D, Hamburg, Germany) and the clarified supernatant was analysed, as indicated in the following section.

Chemical analyses

Chemical reagents

Hydrochloric acid ACS reagent (37%), ammonium sulphate, methyl cellulose and sodium hydroxide were obtained from Sigma-Aldrich (Burlington, MA, USA). Ethanol, rectified at 96.4%, was obtained from Kimix (Cape Town, South Africa).

Phenolic parameters according to the Glories method

For the analyses of extractable and potential anthocyanins, 95 µL of an ethanolic HCl solution (prepared by diluting 0.1 mL HCl in 100 mL of 96.4% v/v ethanol) and 1.81 mL of a 2% v/v HCl solution were added to 95 µL of the clarified supernatant of each sample (pH 1 and 3.2) in a 2 mL microcentrifuge tube. These are referred to as the extraction solvent samples. An aliquot of 570 µL of 15% w/v SO_2 solution was added to 1.43 mL of the extraction solvent sample for each pH sample (sulfured sample). An aliquot of 570 µL of distilled water was added to a second sample of 1.43 mL of the extraction solvent for each pH (control sample). The samples were left in a dark cupboard for 20

FIGURE 2
Custom-made machine crusher used in machine-crushed phenolic extraction method.

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min and measured at 520 nm (100 µL on 96-well microtiter plate) using a UV-vis spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Inc., Waltham, MA, USA), with distilled water as a blank. The potential and extractable anthocyanins were calculated using the following equations (Sommer & Cohen, 2018):

ApH 1=(Abs control sample-Abs sulfured sample)×875

ApH 3.2=(Abs control sample-Abs sulfured sample)×875

The anthocyanin extractability index and the percentage seed tannin contribution were calculated using the following equations:

EA (%)=((A pH 1-A pH 3.2))/(A pH 1)

MP (%)=(OD 280-(A PH 3.2*40))/(OD 280)×100

where OD 280 is the optical density at 280 nm absorbance, multiplied by the dilution factor (DF = 100) of the A pH 3.2 sample. For the calculation of the percentage seed tannin contribution (MP%), the method uses the shared location of skin tannins and anthocyanins in the grape skin cells as a reasoning to calculate the amount of skin tannin present. Based on experimental data from Rib*é*reau-Gayon *et al*. (2006), a constant factor of 40 was proposed after the ratio between OD 280 nm (as an estimation of the total tannin content) and the anthocyanin content at pH 3.2 (as an estimation of the skin tannin) was obtained for several samples. The A pH value of 3.2 was therefore multiplied by 40 and subtracted from the OD of 280 nm value to obtain the percentage seed tannin contribution.

Total anthocyanin and total phenolic content

A method reported by Iland *et al*. (2000) was utilised to quantify total anthocyanin concentration and total phenolic content in samples extracted with the Iland and machine-crushed methods, as well as in the wines. A sample of 100 µL of grape extract was diluted with 1.9 mL of 1 M HCl in a 2 mL microcentrifuge tube (dilution factor = 20) and allowed to stand for 1 h in a dark room. For the wine, a 40 µL sample was diluted with 1.96 mL using 1 M HCl in a 2 mL microfuge tube (dilution factor = 50) and allowed to stand for 1 h in a dark room. After 1 h, the samples were measured at 280 nm and 520 nm (200 µL in a 96-well microtiter plate) using a UV-vis spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Inc., Waltham, MA, USA), with 1 M HCl as blank. The following equations were used to calculate the total anthocyanin content of the grape extracts

Anthocyanins(mg/g)= (A520nm×DF×final extract (mL)×1000)/(500×100×homogenate weight (g))

and wines as malvidin-3-glucoside equivalents (Iland, 2000):

Anthocyanins (mg/L)=A520 nm×MW×DF /ε×L

where A520 nm refers to absorbance at 520 nm, MW refers to the molecular weight of malvidin-3-*O*-glucoside (529 *g/mol*), DF is the dilution factor, Ɛ is the extinction coefficient (28.000 L/(cm ·mol)) of malvidin-3-*O*-glucoside, and L refers to the standard 1 cm pathlength.

The total phenolic index (TPI) was calculated as follows:

Total phenolics index (TPI)=A280 nm×DF

where A280 nm is the absorbance at 280 nm and DF is the dilution factor.

Colour density: The colour density of all the grape extracts and wines was obtained by calculating the sum of 420 nm, 520 nm and 620 nm absorbances, as reported by Li *et al*. (2017). Briefly, 50 µL of the grape extracts and wines were pipetted into a 96-well microtiter plate and the absorbances were measured using a UV-vis spectrophotometer (Multiskan GO Microplate Spectrophotometer, Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Methylcellulose tannin assay: The method for tannin quantification, adapted by Mercurio *et al*. (2007) to a high throughput format was used in grape samples extracted with the Iland and machine-crushed methods, as well as for the wines. The reagents required for this method are 0.04% w/v methyl cellulose solution and a saturated ammonium sulphate solution (Mercurio *et al*., 2007). To prepare the control sample, 50 µL of the wine samples were pipetted into a 2 mL microfuge tube, followed by 400 µL of saturated ammonium sulphate solution and 1 550 µL of distilled water. The treatment sample was prepared by adding 600 µL of methyl cellulose solution to a 50 µL sample of wine in a 2 mL microcentrifuge tube and vortexed. After a 2 min to 3 min waiting period, 400 µL of saturated ammonium sulphate and 950 µL of distilled water were added to the treatment sample. Thereafter, the samples were left to stand for 10 min. Both the control and the treatment samples were then centrifuged at 10 000 rpm for 5 min (Eppendorf 5415 D, Hamburg, Germany). The absorbances of the controls and treatment supernatants were measured at 280 nm in a 96-well microplate using a UV-vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the differences between samples were calculated. This value was used to calculate the presence of tannins by converting the difference in absorbances into epicatechin equivalents using a standard calibration curve and multiplying by a dilution factor of 40.

The MCP tannin assay was modified for the grape extracts (Aleixandre-Tudo *et al*., 2018), for which 200 µL of the extract were used and the volume of water was adjusted to a dilution factor of 10. The following equation was used to calculate the tannin content of the grape extracts:

tannins (mg/g)=tannin extracts(mg/L)×volume extract(L)/ weight homogenate (g)

Polymeric pigments: The modified Somers assay adapted by Mercurio *et al*. (2007), which builds on the work originally reported by Somers and Evans (1977), was used to quantify polymeric pigments in the wines. Briefly, 200 µL of a wine sample were diluted with 1.8 mL of buffer solution (12% v/v ethanol and 0.5% w/v tartaric acid, pH 3.4 adjusted with 1 M NaOH solution and 0.375% w/v sodium metabisulfite) in a 2 mL microfuge tube. The samples were left to stand at room temperature for one hour. The absorbance was measured

at 520 nm and the concentration of polymeric pigments in mg/L was calculated using the following equation:

Polymeric pigments (mg/L)=A520 nm×MW×DF /ε×L

Phloroglucinolysis: The wine samples and different grape extracts (only the extract at pH 1 in the case of the Glories method) for each treatment were analysed by acid-catalysed cleavage in the presence of excess phloroglucinol, using a method adapted from Kennedy and Taylor (2003) and reported by Kuhlman *et al*. (2022). Briefly, 100 µL each of the grape extracts and wine samples were added to a 2 mL microcentrifuge tube and reacted 1:1 with the phloroglucinol reagent. Samples were incubated at 50°C for 20 min. After 20 min, the cleavage reaction was stopped by adding 1 mL sodium acetate. The samples were pipetted into HPLC vials, capped, and analysed by RPLC-DAD at 280 nm.

Statistical analysis

One-way analysis of variance (ANOVA) and post-hoc tests were conducted on the grape and wine phenolic data using the Newman-Keuls test with TIBCO Statistica.

RESULTS AND DISCUSSION

Effect of different seed ratios on phenolic composition of grape extracts

The contributions of seeds to the phenolic levels in grape extracts prepared by different extraction methods (Iland, Glories and machine-crushed methods) were investigated. The extraction solvent, extraction time pH and grape processing differ among these methods. Tables 1 to 3 show the phenolic composition per grape extraction method for the different seed-to-skin ratio treatments and cultivars. Table 1 presents the phenolic parameters obtained with the Iland extraction method for Shiraz and Merlot grapes. *S*ignificant differences among the three treatments were not evident in the total anthocyanin concentration and colour density for both cultivars. However, significant differences in tannin concentrations and total phenolic index were observed between the treatments in both cultivars. The 2 x seeds treatment showed the highest tannin levels. Shiraz had lower total phenolic values in the seedless treatment than in the 1 x seeds and 2 x seeds, with significant differences not apparent between them. However, the seedless and 1 x seeds treatments were not significant for total phenolic index in Merlot, with the 2 x seeds treatment having a higher total phenolic content than the former treatments.

The machine-crushed and microwaved extraction methods, which used crushed berries and undamaged seeds during extraction with microwave assistance, also showed no significant differences between anthocyanin concentrations and colour density for both the Shiraz and Merlot cultivars (Table 2). Interestingly, the tannin concentrations of Shiraz were not affected. This contrasts with a study reported by Li *et al*. (2011), where seed polyphenols of Cabernet Sauvignon, Shiraz, Sauvignon Blanc and Chardonnay were extracted much more rapidly with microwave assistance compared to ultrasound-assisted and solvent extractions. In contrast, significant differences were found in tannin concentration for Merlot, with 2 x seeds having a higher tannin content than the seedless treatment. However, no significant differences were found in the total phenolic content of both cultivars.

The anthocyanin and colour density values (not significant) found using the Iland and machine-crushed methods (Table 1 and 2) could be explained with the use of a similar juice-to-skin ratio and extraction medium (50% v/v ethanol solution). The treatments with and without seeds probably followed similar anthocyanin extraction patterns, indicating the null effect of seeds in the anthocyanin extraction. These results agree with Bautista-Ortín *et al*. (2014), who reported that the absence of seeds does not lead to a preferential extraction of anthocyanins from the skins.

The Glories method is one of the most popular methods to analyse grapes for anthocyanin and phenolic content (Fragoso *et al*., 2010). The method provides information on the anthocyanin extractability (EA%) and the percentage contribution of seed tannins to the total phenol content. The results show that the highest levels of potential anthocyanins (A pH 1) in the seedless treatment were obtained for both cultivars (Table 3). A similar trend was seen in the extractable anthocyanins (A pH 3.2). The 1 x seeds treatment had higher EA% compared to the 2 x seeds in Shiraz. In contrast, the EA% for all treatments in Merlot were not significantly different. The seedless treatments showed a percentage of tannins derived from seeds (MP %), although no seeds were present in this treatment. However, there were no significant differences in the percentage contribution of tannins from seeds for the 1 x seeds and 2 x seeds treatments for both cultivars, although a trend was seen in both Shiraz and Merlot.

TABLE 1
Phenolic levels of the different treatments for the Iland grape-extraction method.

		Anthocyanins (mg/g)	Tannins (mg/g)	Total phenolics	Colour density
Shiraz	Seedless	1.20 ± 0.02	3.30 ± 0.18^{a}	8.18 ± 0.18^{a}	3.12 ± 0.23
	1 x seeds	1.19 ± 0.06	5.31 ± 0.16^{b}	11.01 ± 0.45^{b}	3.08 ± 0.06
	2 x seeds	1.07 ± 0.13	6.42 ± 0.62^{c}	12.51 ± 1.53^{b}	3.09 ± 0.30
Merlot	Seedless	1.47 ± 0.36	2.44 ± 0.15^{a}	10.53 ± 1.98^{a}	3.70 ± 0.85
	1 x seeds	1.39 ± 0.23	5.47 ± 0.49^{b}	14.63 ± 0.77^{a}	4.25 ± 0.61
	2 x seeds	0.85 ± 0.03	6.84 ± 0.63^{c}	16.63 ± 2.61^{b}	3.51 ± 0.12

Means and standard deviations with different letters are significantly different ($p < 0.05$).

TABLE 2
Phenolic levels of the different treatments for the machine-crushed grape-extraction method.

		Anthocyanins (mg/g)	Tannins (mg/g)	Total phenolic index	Colour density
Shiraz	Seedless	0.72 ± 0.04	0.92 ± 0.01	47.06 ± 3.04	15.97 ± 1.11
	1 x seeds	0.74 ± 0.04	0.98 ± 0.01	50.17 ± 2.92	16.59 ± 1.30
	2 x seeds	0.74 ± 0.02	1.13 ± 0.01	50.96 ± 1.66	18.02 ± 1.23
Merlot	Seedless	0.71 ± 0.05	0.69 ± 0.07^{a}	40.51 ± 2.84	16.05 ± 0.64
	1 x seeds	0.72 ± 0.08	0.81 ± 0.04^{ab}	44.93 ± 4.74	18.64 ± 2.50
	2 x seeds	0.69 ± 0.03	0.86 ± 0.02^{b}	44.59 ± 1.31	17.96 ± 3.62

Means and standard deviations with different letters are significantly different ($p < 0.05$).

TABLE 3
Phenolic levels of the different treatments for the Glories grape-extraction method.

		A pH 1 (mg/L)	A pH 3.2 (mg/L)	EA (%)	TPI	MP (%)
Shiraz	Seedless	1 039.3 ± 34.04^{a}	635.40 ± 8.96^{a}	37.05 ± 2.61^{ab}	44.54 ± 1.57	32.85 ± 2.51^{a}
	1 x seeds	935.88 ± 2.42^{b}	561.20 ± 15.88^{b}	40.04 ± 1.59^{b}	43.87 ± 0.23	41.53 ± 1.61^{b}
	2 x seeds	741.95 ± 20.05^{c}	492.32 ± 11.53^{c}	33.61 ± 1.93^{a}	41.13 ± 1.47	45.17 ± 3.24^{b}
Merlot	Seedless	1 406.46 ± 104.82^{a}	691.34 ± 53.95^{a}	50.73 ± 3.40	41.02 ± 6.28^{a}	21.02 ± 14.1^{a}
	1 x seeds	1 029.96 ± 24.47^{b}	477.41 ± 39.11^{b}	53.57 ± 4.45	46.74 ± 6.82^{ab}	52.71 ± 5.03^{b}
	2 x seeds	828.69 ± 29.29^{c}	428.38 ± 46.76^{b}	48.31 ± 5.20	59.29 ± 2.53^{b}	66.87 ± 4.12^{b}

Means and standard deviations with different letters are significantly different ($p < 0.05$). ApH 1 – potential anthocyanins, ApH 3.2 – extractable anthocyanins, EA (%) – extractable anthocyanin index, TPI – total phenolic index, MP (%) – % seed tannin contribution.

The Glories method to quantify the percentage contribution of tannins from seeds needs to be reassessed, as the seedless treatment, despite the absence of seeds, indicated the "contribution of seeds" to tannins. The manner in which percentage seed tannin contribution (MP%) is calculated is the cause of this result. The skin tannin contribution is calculated from the extractable anthocyanin content (AU at 520 nm, pH 3.2 solution) multiplied by a constant dilution factor of 40. The seed tannin content is then obtained after the skin tannin content is subtracted from the total phenolic content (280 nm absorbance) (see equation and additional explanation in the Materials and Methods section, "Phenolic parameters according to the Glories method") (Ribéreau-Gayon et al., 2006). In the case of the seedless treatment, the total phenolic content was accounted for mainly by the skin tannin content. A constant factor should account for the total content of tannins, which should only be affected by the skins in the case of the seedless treatment, but this is not the case where seeds were not present. Extractable anthocyanins and the factor of 40 should thus not be used when extracts containing only skins are assessed.

Considering the above, the total phenolic content in Shiraz grapes was not significantly different between the three seed treatments in the Glories index (Table 3), which might indicate a low contribution of seed tannins to the total tannin content of the phenolic extract obtained after grape homogenisation. However, significant differences in tannin concentrations were found with the MCP method where the Iland extracts were used (Table 1). The reason for this discrepancy is not clear and warrants further investigation. It might be due to the ethanol addition in the latter method, which is not used in the Glories method and which could result in improved tannin extraction. In contrast, the Merlot grapes showed statistical differences in tannin levels, indicating the contribution of seeds to the total phenolics present in the extracts where the Glories method was used. Further studies are required to compare/assess published phenolic extraction methods in terms of extraction conditions, extraction time and solvents used. The extent of the tannin extraction seems to be cultivar dependent, but with variables such as grape and seed ripeness levels, growing conditions for a specific location and vintage effect most probably contribute to the extent of the seed tannin diffusion into the phenolic extract. Limited information on this topic is currently available in the literature, but the morphology of the seeds seems to change during fermentation, which would improve flavanoid extraction.

Tannin concentrations from the different extraction methods were affected by the absence/presence of seeds, as treatments with seeds had higher tannin levels for both cultivars in the case of the Iland extract (Table 1), and for Merlot in the case of machine-crushed extract (Table 2). The contribution of seed tannins to total phenolic content for both cultivars in the case of the Glories extract (Table 3) also increased with more seeds. However, the highest tannin levels where more seeds were added were not seen in the machine-crushed extracts

of Shiraz (Table 2). This may be due to the seeds being intact during the extraction, while homogenised grapes are used in the Glories and Iland methods. It has been reported that homogenisation methods help break up seeds, which improves the extraction of tannins (Cynkar *et al*., 2004). Grape homogenisation and seed crushing therefore might be limiting factors when the grape phenolic measurements are used as an indication of the wine phenolics using conventional winemaking conditions; the seeds remain intact and are not crushed during the winemaking process.

Influence of different seed ratios on phenolic composition of wines

The phenolic composition of the different wines is reported in Table 4. It can be observed that the anthocyanin levels of both cultivars were not significantly different after seven days. Highest anthocyanin levels were recorded in treatments with seeds on day 14 in Merlot wines. Kontoudakis *et al*. (2010) reported that Cabernet Franc and Mourvèdre require seven days or more to reach the maximum anthocyanin levels, while others need three days to reach that point. Other studies have reported that the maximum anthocyanin levels are reached at about five to six days of fermentation (Budić-Leto *et al*., 2003). Significant differences were not evident in the colour density for both cultivars at day 7, and at day 14 for the Merlot.

The high levels of condensed tannins in seeds are well documented (Kyraleou *et al*., 2017; Blancquaert *et al*., 2019). Differences in tannin concentration between the treatments were observed in both varieties at day 7, with the 2 x seeds treatment having the highest levels. However, no significant differences were observed between the seedless and 1 x seeds treatment for the Shiraz at day 7 (Table 4). Shiraz and Merlot wines followed an extraction pattern in which larger differences were seen in tannin concentration between the seedless and seeded treatments, especially after three to four days of fermentation (Figs 3 and 4). These results are in agreement with studies by Hernández-Jiménez *et al*. (2012), Casassa and Harbertson (2014) and Zhang *et al*. (2015), who reported intense extraction of seed phenolics after three days of fermentation, reaching a maximum after about two to three weeks of maceration. Extended maceration thus favours the extraction of seed phenolics and condensed tannins, which is reflected in the significant increase in the total phenolic index and tannin concentration in Merlot grapes from day 7 to day 14 (Fig. 4). The literature also reports that extended maceration time favours the extraction of tannins from seeds (Gambuti *et al*., 2009; Hernández-Jiménez *et al*., 2012). This increase may be due to increased ethanol concentrations, which aid in degrading the lipid coat of seeds. Increased maceration times can lead to increased hydration of the seeds, leading to intense extraction (Hernandez-Jiménez *et al*., 2012), as well as morphological changes to the seeds that enhance extraction. However increased tannin extraction from seeds during extended maceration can increase the perceived astringency of wines (Harbertson *et al*., 2009), therefore tannin concentrations should be monitored during maceration.

The Merlot wines had overall higher tannin concentrations than the Shiraz wines at day 7 (Table 4). Under similar winemaking conditions, the proportion of proanthocyanidins in the skins and seeds may differ among grape cultivars (Harbertson *et al*., 2002, Gambuti *et al*., 2009). Shiraz and Merlot wines at day 7 and day 14, respectively, showed similar trends in terms of tannin levels as those observed for the grape extracts obtained with the Iland and Glories methods (Table 1 and Table 3, respectively). In the latter case, increased tannin levels were also observed in treatments with additional seeds. This agrees with work by Canals *et al*. (2008), who reported that seed addition resulted in a notably high proanthocyanidin content in wines. Other studies have also found that increased levels of seeds enhance the proanthocyanidin content in wines (Bautista-Ortín *et al*., 2014; Pascual *et al*., 2016). Furthermore, Bindon *et al*. (2014a, 2014c) reported that the presence of tannins from seeds enhanced the colour and pigmented polymer

TABLE 4
Phenolic levels of the different Shiraz and Merlot wines produced with different seed-to-skin ratios.

		Anthocyanins (mg/L)	Tannins (mg/L)	Polymeric pigments (mg/L)	Total phenolics	Colour density
Sh	SL	381.59 ± 46.39	1 062.27 ± 46^{a}	29.21 ± 7.77	32.66 ± 3.50^{a}	14.62 ± 1.85
	1xS	396.53 ± 5.59	1 276.23 ± 19. 81^{a}	31.92 ± 2.75	36.27 ± 0.80^{ab}	13.76 ± 0.22
	2xS	417.69 ± 12.44	1 404.30 ± 0.80^{b}	31.63 ± 5.6	40.64 ± 1.23^{b}	15.08 ± 0.16
M7	SL	541.05 ± 18.45	1 578.37 ± 104.81^{a}	27.15 ± 0.82^{a}	51.38 ± 2.65^{a}	25.01 ± 1.52
	1xS	539.19 ± 17.80	2 099.18 ± 2.32^{b}	28.60 ± 1.37^{ab}	68.12 ± 1.32^{b}	25.84 ± 1.24
	2xS	556.83 ± 10.21	2 425.23 ± 120.23^{c}	30.32 ± 0.68^{b}	84.25 ± 3.91^{c}	23.26 ± 1.51
M14	SL	555.54 ± 14.7^{a}	1 839.45 ± 277.05^{a}	46.98 ± 1.59^{a}	61.56 ± 2.89^{a}	26.07 ± 0.91
	1xS	628.29 ± 13.90^{b}	2 763.56 ± 77.74^{b}	56.25 ± 2.71^{b}	85 ± 2.91^{b}	27.19 ± 1.75
	2xS	624.21 ± 39.02^{b}	3 816.64 ± 277.05^{c}	61.07 ± 1.26^{c}	99.57 ± 2.47^{c}	26.92 ± 0.42

Means and standard deviations with different letters indicate significance ($p < 0.05$). SL – seedless, 1xS – 1 x seeds, 2xS – 2 x seeds. Sh. – Shiraz after seven days of skin maceration; M7 – Merlot wines after seven days of skin maceration; M14 – Merlot wines after 14 days of skin maceration.

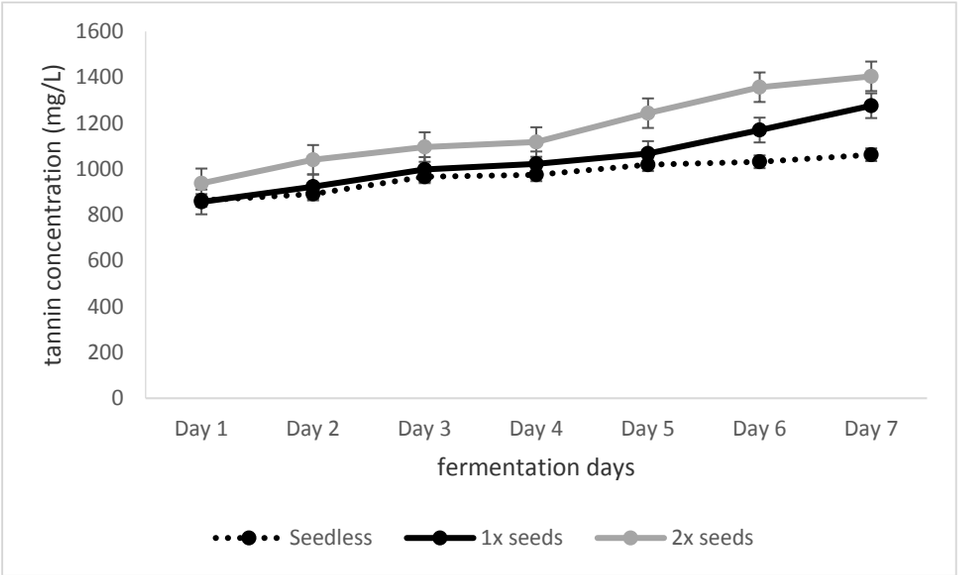

FIGURE 3
Tannin concentrations (mg/L) of fermenting must and wines made from Shiraz grapes with the three different seed-to-skin ratios, from day 1 to day 7 of alcoholic fermentation.

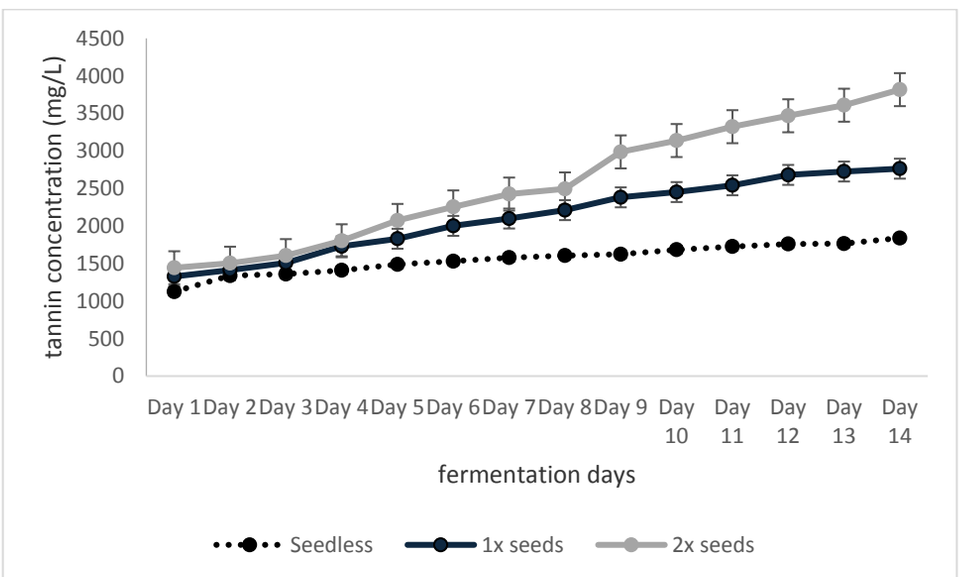

FIGURE 4
Tannin concentrations (mg/L) of fermenting must and wines made from Merlot grapes with the three different seed-to-skin ratios from day one to day 14 of alcoholic fermentation.

concentrations of Cabernet Sauvignon wines, in comparison to tannins from skins or without tannin fractions from either seeds or skins.

During fermentation, non-bleachable pigments are formed by the reaction of anthocyanins with tannins (Cheynier *et al*., 2006; Bindon *et al*., 2008). These pigmented products are more stable than monomeric anthocyanins at wine pH and are more resistant to sulphur dioxide bleaching. The polymeric pigment concentrations in the Shiraz wines were not significantly different between treatments. However, more pigments were formed in the Merlot 2 x seeds treatment for both day 7 and day 14 compared to the seedless treatment.

The total phenolic index of the 2 x seeds treatment was significantly higher than that of the seedless treatment in the Shiraz wines. Moreover, significant differences were found in total phenolic index in the Merlot wines at day 7 and day 14, where the 2 x seeds treatment showed high levels of total phenolics (Table 4).

Condensed tannin and monomer composition in the different seed-ratio treatments using HPLC-phloroglucinolysis

The assessment of the tannin composition by phloroglucinolysis revealed that the mean degree of polymerisation

(mDP) of tannin (Tables 5 and 6) was affected by the presence of seeds. A decreased mDP was sometimes observed in the grape extracts and the wines of both grape cultivars in the 1 x seeds and 2 x seeds treatments, although these trends were clearer for Merlot grape homogenates and wines. In contrast, high mDP was observed in the 2 x seeds treatment for the Iland grape extracts. The mDP was not calculated in the Iland seedless extract of Shiraz, as the extension and terminal subunits were not detected. The seedless treatments of the Glories and machine-crushed grape extracts at day 7 and day 14 in Merlot wines and the machine-crushed and day 7 wines for Shiraz had high mDP (Tables 5 and 6). This may be

TABLE 5
HPLC-phloroglucinolysis tannin characterisation of Shiraz grape extracts and wines

	mDP	**%Galolyll**	**Av MM**
Glories SL	1.83 ± 0.02^{ab}	7.78 ± 0.28^{a}	550.07 ± 7.91^{a}
Glories 1xS	2.02 ± 0.08^{b}	12.11 ± 0.97^{b}	621.64 ± 24.12^{b}
Glories 2xS	1.85 ± 0.07^{a}	11.16 ± 0.71^{b}	565.41 ± 19.29^{a}
Iland SL	-	-	-
Iland 1xS	2.30 ± 0.02^{a}	6.20 ± 0.12^{a}	684.02 ± 5.98^{a}
Iland 2xS	2.45 ± 0.02^{b}	5.99 ± 0.01^{a}	728.48 ± 6.76^{b}
Machine-crushed SL	2.72 ± 0.25^{a}	8.94 ± 1.09	822.73 ± 69.91^{a}
Machine-crushed 1xS	2.10 ± 0.09^{b}	11.18 ± 084	643.72 ± 25.33^{b}
Machine-crushed 2xS	2.10 ± 0.03^{b}	9.33 ± 0.26	622.91 ± 8.22^{b}
Wine SL	2.70 ± 0.05^{a}	8.70 ± 0.15	813.46 ± 13.65^{a}
Wine 1xS	1.88 ± 0.05^{b}	11.65 ± 1.67	575.83 ± 18.15^{b}
Wine 2xS	1.75 ± 0.04^{c}	9.66 ± 0.49	529.69 ± 10.90^{c}

Means and standard deviations with different letters are significantly different ($p < 0.05$). mDP – mean degree of polymerisation, %gallolyl – percentage gallolylation, AvMM – average molecular weight. SL – seedless, 1xS – 1 x seeds, 2xS – 2 x seeds.

TABLE 6
HPLC-phloroglucinolysis tannin characterisation of Merlot grape extracts and wines

	mDP	**%Galolyll**	**Av MM**
Glories SL	2.23 ± 0.02^{a}	19.99 ± 0.58^{a}	712.45 ± 6.10^{a}
Glories 1xS	1.66 ± 0.07^{b}	11.91 ± 10.18^{b}	508.43 ± 23.84^{b}
Glories 2xS	1.08 ± 00005^{c}	1.90 ± 0.12^{c}	316.2 ± 1.52^{c}
Iland SL	1.60 ± 0.004^{a}	6.06 ± 0.10^{a}	476.16 ± 1.11^{a}
Iland 1xS	1.92 ± 0.04^{b}	7.46 ± 0.05^{b}	576.31 ± 10.76^{b}
Iland 2xS	1.90 ± 0.02^{b}	7.06 ± 0.37^{b}	566.92 ± 5.90^{b}
Machine-crushed SL	2.28 ± 0.05^{a}	24.01 ± 1.14^{a}	711.91 ± 18.79^{a}
Machine-crushed 1xS	1.97 ± 0.01^{b}	18.67 ± 1.78^{b}	583.67 ± 44.41^{b}
Machine-crushed 2xS	1.85 ± 0.01^{c}	18.29 ± 0.30^{b}	555.90 ± 29.16^{b}
Wine day 7 SL	1.98 ± 0.02^{a}	21.15 ± 1.01^{a}	634.39 ± 8.66^{a}
Wine day 7 1xS	1.63 ± 0.03^{b}	14.69 ± 0.37^{b}	507.75 ± 9.14^{b}
Wine day 7 2xS	1.39 ± 0.03^{c}	11.6 ± 0.37^{c}	425.05 ± 9.16^{c}
Wine day 14 SL	1.99 ± 0.07^{a}	20.45 ± 0.42^{a}	637.18 ± 23.40^{a}
Wine day 14 1xS	1.57 ± 0.01^{b}	13.9 ± 0.94^{b}	487.77 ± 4.22^{b}
Wine day 14 2xS	1.3 ± 0.01^{c}	11.56 ± 0.30^{c}	397.45 ± 2.57^{c}

Means and standard deviations with different letters are significantly different ($p< 0.05$). mDP – mean degree of polymerisation, %gallolyl – percentage gallolylation, AvMM – average molecular weight. SL – seedless, 1xS – 1 x seeds, 2xS – 2 x seeds.

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explained by seed tannins typically having lower mDP than skin tannins (Kennedy *et al*., 2000; Teng *et al*., 2019). This agrees with Lee *et al*. (2008), who observed a high mDP in wines made with skins but without seeds. The addition of more seeds may result in more astringent wines, as seed proanthocyanidins have a high proportion of epicatechin-3-*O*-gallate (Vidal *et al*., 2003) and low mDP. Lisjak *et al*. (2020) reported that seed wine-like extracts of Cabernet Sauvignon had low mDP and were astringent and bitter.

The difference in percentage galloylation in Shiraz grapes and wines was non-significant in most extracts (Table 5), except for the Glories extracts, in which the seedless treatment had a significantly low percentage. In Merlot, a trend of a high percentage galloylation was observed in Glories and machine-crushed grape treatments, as well as day 7 and 14 wines in the seedless treatment (Table 6). However, the seedless treatment in the Iland extracts had a lower percentage galloylation.

Generally, the phloroglucinolysis analysis of individual monomeric flavanols (catechin, epicatechin and epicatechin 3-*O*-gallate; see Tables 7 and 8) showed that the presence of more grape seeds led to a higher content of catechin and epicatechin, as was observed in the 2 x seeds treatment of some grape extracts. This is in line with a study by Yilmaz and Toledo (2004), who found higher proportions of catechin and epicatechin in Merlot seeds than in Merlot skins. Shiraz extracts and wines showed a similar trend, where high levels of these compounds were found in the 2 x seeds treatments.

Rousserie *et al*. (2019) and Souquet *et al*. (2019) reported that seed proanthocyanidins have a higher percentage of galloylation compared to skin tannins. However, the percentage of galloylation was lower in the 2 x seeds treatment (Tables 5 and 6), in contrast to the high levels that are expected in treatments with more seeds. This may be due to the low proportion of epicatechin-3-*O*-gallate and high proportions of catechin and epicatechin subunits contributed by the seeds, indicating that seeds are releasing mainly higher levels of catechin and epicatechin than the skins (Pascual *et al*., 2016).

In the composition of the extension subunit, epicatechin-phloroglucinol (EC-P) was highest in the Iland and machine-crushed grape extracts of Shiraz in the treatments with seeds (Tables 7 and 8). A similar trend was observed in the Glories and Iland grape extracts of Merlot. The highest levels of catechin-phloroglucinol (C-P) subunits were found in the 2 x seeds treatment of Glories, Iland and machine-crushed grape extracts of Shiraz. In Merlot, the highest levels of C-P were found in the 1 x seeds treatment in machine-crushed extract. The highest levels of epicatechin gallate-phloroglucinol (ECG-P) were found with an increase in seeds in all three grape extracts of Shiraz, while the ECG-P extension subunits of the seedless treatment were not detected in any grape extracts or wines of Shiraz (Table 7). However, significant differences were not evident in the Merlot ECG-P extension subunits (Table 8).

The average molecular weight (AvMM) of Shiraz wines (Table 5) was highest in the seedless treatment of machine-crushed grape extracts and in the wines at day 7. However, the 1 x seeds treatment of the Glories grape extracts and 2 x seeds treatments of the Iland grape extracts had the highest AvMM. The AvMM of the Iland grape extracts in the seedless treatments could not be reported, as extension and terminal subunits were not detected. Finally, the AvMM of machine-crushed grape extracts between the 1 x seeds and 2 x seeds treatments was not significantly different, but lower than that of the seedless treatment. A similar trend was observed in the Iland and machine-crushed grape extracts of Merlot (Table 6). Merlot followed a trend of high AvMM in the seedless treatment and a decrease in treatments with seeds for some of the grape extracts and wines. However, the treatments with seeds of the Iland grape extracts had the highest AvMM. The AvMM of the Glories and machine-crushed grape extracts and wines decreased with an increase in seeds, with seedless having the highest AvMM in Merlot. The AvMM of Iland and machine-crushed grape extracts between the 1 x seeds and 2 x seeds was not significantly different. Overall, the AvMM followed a decreasing trend, where seeds were present for most of the grape extracts and wines in both Shiraz and Merlot. This may be explained by seed proanthocyanidins, which typically have a range of five to 20 subunits, compared to skin proanthocyanidins, which typically range from 20 to 40 subunits (Hanlin *et al*., 2010).

The quantification of procyanidin phloroglucinol degradation products under acid catalysis requires the availability of pure standards of the flavan-3-ol phloroglucinol adducts (Köhler & Winterhalter, 2005). Only catechin was used as a standard, which may be a limiting factor of the results obtained. Therefore, the results for the individual phenolic compounds obtained with phloroglucinolysis must be approached with caution. Moreover, Kennedy and Jones (2001) propose that wine contains interflavonoid bond linkages that are resistant to acid cleavage reactions. The estimation of subunits by phloroglucinolysis in wine may therefore be sub-optimal. In addition, the phloroglucinolysis of wine tannins is less efficient and precise than in grape skins and seeds because of the pigmented tannins in wine, which have complex structures – such as type A proanthocyanidins and cyclic proanthocyanidins (Arapitsas *et al*., 2021) – compared to the non-pigmented tannins found in grape skins and seeds.

CONCLUSIONS

The quality of red wine is known to be affected by phenolic compounds extracted from grape solids during maceration. Grape extraction methods can assist in providing information on the potential phenolic concentration of wines produced from grapes with different seed levels. It was found that the Iland extraction method with grape homogenate better facilitates the extraction of phenolics from the seed tissue. The Glories method gave positive results in terms of seed tannins for the seedless treatment, and should therefore be used cautiously for grape phenolics analyses. The machine-crushed method and microwaved phenolic extraction method, which mimics winemaking conditions, generally showed less significant differences between the treatments for phenolics compared to the other two extraction methods. Further research is required to assess the extraction efficiency and suitability of the machine-crushed method to indicate phenolics extracted from seeds.

The levels of phenolic compounds were affected by the cultivar, as well as by the presence of seeds. In general, the

TABLE 7
HPLC-phloroglucinolysis individual tannin monomeric flavanols of Shiraz grape extracts and wines.

	Extension subunits			Terminal subunits		
	C-P (nmol)	**EC-P (nmol)**	**ECG-P (nmol)**	**C (nmol)**	**EC (nmol)**	**ECG (nmol)**
Glories SL	0.04 ± 0.001[a]	0.04 ± 0.002	-	0.037 ± 0.0004[a]	0.045 ± 0.001[a]	0.013 ± 0.001[a]
Glories 1xS	0.045 ± 0.002[b]	0.05 ± 0.01	0.014 ± 0.001[a]	0.046 ± 0.003[b]	0.051 ± 0.003[b]	0.012 ± 0.0003[b]
Glories 2xS	0.049 ± 0.001[c]	0.06 ± 0.01	0.017 ± 0.001[b]	0.057 ± 0.003[c]	0.081 ± 0.003[c]	0.014 ± 0.001[c]
Iland SL	-	-	-	-	-	-
Iland 1xS	0.038 ± 0.001[a]	0.05 ± 0.001[a]	0.011 ± 0.0003[a]	0.038 ± 0.0004[a]	0.039 ± 0.001[a]	-
Iland 2xS	0.042 ± 0.002[b]	0.071 ± 0.002[b]	0.013 ± 0.0002[b]	0.043 ± 0.0003[b]	0.045 ± 0.001[b]	-
Machine SL	0.04 ± 0.001[a]	0.047 ± 0.01[a]	-	0.039 ± 0.001[a]	-	0.012 ± 0.001
Machine 1xS	0.04 ± 0.001[a]	0.056 ± 0.01[ab]	0.011 ± 0.0004[a]	0.042 ± 0.001[b]	0.043 ± 0.002	0.012 ± 0.0004
Machine 2xS	0.045 ± 0.001[b]	0.079 ± 0.01[b]	0.013 ± 0.0002[b]	0.049 ± 0.001[c]	0.069 ± 0.003	0.012 ± 0.001
Wine SL	0.045 ± 0.0004	0.042 ± 0.002	-	0.039 ± 0.001[a]	-	0.012 ± 0.0002
Wine 1xS	0.048 ± 0.001	0.043 ± 0.001	0.014 ± 0.004	0.048 ± 0.002[b]	0.06 ± 0.004[a]	0.012 ± 0.0004
Wine 2xS	0.052 ± 0.01	0.046 ± 0.004	0.012 ± 0.0001	0.054 ± 0.002[c]	0.08 ± 0.01[b]	0.012 ± 0.0002

Means and standard deviations with different letters are significantly different ($p < 0.05$). C-P – catechin-phloroglucinol, EC-P – epicatechin-phloroglucinol, ECG-P – Epicatechin gallate-phloroglucinol, C – catechin, EC – epicatechin, ECG – epicatechin-3-O-gallate. SL – seedless, 1xS – 1 x seeds, 2xS – 2 x seeds.

TABLE 8
HPLC-phloroglucinolysis individual tannin monomeric flavanols of Merlot grape extracts and wines.

	Extension subunits			Terminal subunits		
	C-P (nmol)	**EC-P (nmol)**	**ECG-P (nmol)**	**C (nmol)**	**EC (nmol)**	**ECG (nmol)**
Gl SL	0.036 ± 0.001	0.044 ± 0.001[a]	0.027 ± 0.001	0.038 ± 0.001[a]	0.038 ± 0.001[a]	0.012 ± 0.0001
Gl 1xS	0.037 ± 0.001	0.061 ± 0.002[b]	0.025 ± 0.002	0.083 ± 0.01[b]	0.092 ± 0.01[b]	0.012 ± 0.001
Gl 2xS	0.038 ± 0.001	0.079 ± 0.001[c]	0.023 ± 0.001	0.76 ± 0.01[c]	0.09 ± 0.05[b]	0.012 ± 0.0001
Iland SL	0.034 ± 0.0001	0.034 ± 0.0001[a]	0.013 ± 0.0003	0.036 ± 0.001[a]	0.098 ± 0.002[a]	-
Iland 1xS	0.034 ± 0.001	0.037 ± 0.001[b]	0.013 ± 0.001	0.047 ± 0.002[b]	0.045 ± 0.003[b]	-
Iland 2xS	0.034 ± 0.0004	0.039 ± 0.001[b]	0.013 ± 0.001	0.049 ± 0.001[b]	0.047 ± 0.001[c]	-
Mach SL	0.036 ± 0.0002[a]	0.045 ± 0.001	0.035 ± 0.0003	0.037 ± 0.001[a]	0.039 ± 0.002[a]	0.014 ± 0.004
Mach 1xS	0.039 ± 0.001[b]	0.049 ± 0.005	0.035 ± 0.004	0.051 ± 0.002[b]	0.063 ± 0.002[b]	0.012 ± 0.001
Mach 2xS	0.036 ± 0.001[ab]	0.048 ± 0.001	0.036 ± 0.0004	0.058 ± 0.001[c]	0.072 ± 0.002[c]	0.012 ± 0.0002
Wine 7 SL	0.048 ± 0.01	0.039 ± 0.003	0.027 ± 0.001	0.048 ± 0.01[a]	0.047 ± 0.003[a]	0.022 ± 0.001[a]
Wine 7 1xS	0.051 ± 0.004	0.042 ± 0.01	0.027 ± 0.0002	0.084 ± 0.002[b]	0.087 ± 0.003[b]	0.019 ± 0.001[b]
Wine 7 2xS	0.049 ± 0.01	0.04 ± 0.01	0.026 ± 0.001	0.13 ± 0.003[c]	0.145 ± 0.002[c]	0.022 ± 0.0003[a]
Wine 14 SL	0.048 ± 0.004[a]	0.038 ± 0.004	0.026 ± 0.001	0.044 ± 0.002[a]	0.05 ± 0.01[a]	0.02 ± 0.004[a]
Wine 14 1xS	0.057 ± 0.005[b]	0.041 ± 0.003	0.028 ± 0.003	0.10 ± 0.001[b]	0.1 ± 0.002[b]	0.02 ± 0.0004[ab]
Wine 14 2x S	0.044 ± 0.002[a]	0.035 ± 0.0003	0.026 ± 0.0003	0.16 ± 0.002[c]	0.17 ± 0.01[c]	0.026 ± 0.001[b]

Means and standard deviations with different letters are significantly different ($p < 0.05$). C-P – catechin-phloroglucinol, EC-P – epicatechin-phloroglucinol, ECG-P – epicatechin gallate-phloroglucinol, C – catechin, EC – epicatechin, ECG – epicatechin-3-O-gallate. SL – seedless, 1xS – 1 x seeds, 2xS – 2 x seeds.

2 x seeds treatment led to higher tannin and phenolic content than the other treatments. Extended maceration time led to increased extraction of phenolic compounds from seeds, as observed in the Merlot wines. The presence or absence of seeds during fermentation also affected the mDP, catechin derivatives and percentage gallolylation. Knowledge obtained from the results may assist winemakers to decide on winemaking practices to favour or prevent seed tannin extraction for a specific wine style.

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