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Influence of chips, lees and micro-oxygenation during aging on the phenolic composition of a red Sangiovese wine

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Abstract

A red *Sangiovese* wine was aged using separate or combined different technological treatments and techniques (chips, chips and micro-oxygenation, chips and lees and micro-oxygenation), to evaluate their influence on phenolic compounds.

The use of chips induced slight modifications on wine phenolic composition, while micro-oxygenation, jointly with chips, increased wine colour even if a lower level of monomeric pigments was determined. Aging with lees (jointly with chips and micro-oxygenation treatments) slightly affected phenolic composition but increased colour and red polymeric pigments more than other treatments. The application of different technologies during bottle storage affected wine composition and micro-oxygenated wines were more stable.
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Keywords: Red wine; Micro-oxygenation; Yeast lees; Chips; Phenolic compounds

1. Introduction

Oxygen is very important for the phenolic evolution of red wine particularly during aging. Aging in a wood barrel affects wine because of the permeation of oxygen (Vivas, 2000); a mild oxygenation during storage in stainless steel tanks was proposed to improve wine quality (Moutounet, Ducournau, Chassin, & Lemaire, 1996; Pontallier & Ribereau-Gayon, 1983). Whereas a strong oxygenation increases wine oxidation, controlled oxygenation with the same amount of oxygen (3–9 ml/l/month) (Ferrarini, Girardi, De Conti, & Castellari, 2001) could stabilise wine colour (Atanasova, Fulcrand, Cheynier, & Moutounet, 2002; Malien-Aubert, Dangles, & Amiot, 2002) inducing the formation of blue-red polymeric pigments from the direct condensation of anthocyanins with other flavonoids or from a combination of pigments and flavonoids with ethyl bridges (Atanasova et al., 2002; Timberlake & Bridle, 1976). These chemical reactions may induce some phenolic modifications producing highly coloured compounds (Timberlake & Bridle, 1976) reducing astringency and affecting both wine colour and taste (Moutounet et al., 1996).

Chips have been tested to reduce costs and to improve the technological process of wine aging (Spillman, 1999). French wood extracts seemed to be richer in phenols and poorer in aromatic compounds than American ones (Singleton, 1974). Oak chips may influence a wine's sensorial and chemical profile, because many compounds, such as gallic, ferulic, vanillic, syringic, ellagic acids, ellagitannins (Viriot, Scalbert, Herve du Penhoat, & Moutounet, 1994) and tannins (Fernández de Simon, Cadahía, Conde, & García- Vallejo, 1996) can be extracted from oak wood.

At the end of fermentation wine could be aged using the technique of *bâtonnage* for 3–8 months; during this time, yeast cells may release some components that could contribute to wine aging (Escot, Feuillat, Dulau, & Charpetier,

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2001). The application of this technique could improve wine stabilisation in terms of colour and proteins (Escot et al., 2001; Moine-Ledoux & Dubourdieu, 1997). Oxygen consumption in wine increases with yeast lees contact (Fornairon-Bonnefond & Salmon, 2003; Salmon, Fornairon-Bonnefond, Mazauric, & Moutounet, 2000).

Castel, Morland, Pujol, Peyron, and Naudin (2001) Salmon, Fornairon-Bonnefond, and Mazauric (2002) studied the effects of micro-oxygenation and lees on phenolic composition. McCord (2003) examined the combined effect of micro-oxygenation and aging in toasted oak barrels. Finally, Bosso, Panero, and Follis (2004) looked at the combined use of chips and lees on wine sensorial profile.

No scientific work was carried out to put in evidence the effects of the combination of these different technological practices (chips, yeast lees and micro-oxygenation) on wine phenolic composition. The aim of this work was to assess the influence of chips, yeast lees and micro-oxygenation techniques at the end of aging and during bottle storage on colour and phenolic composition of a red Sangiovese wine.

2. Materials and methods

2.1. Samples

Sangiovese red grapes were destemmed, crushed and combined with 50 mg/l of SO_2 and 150 mg/l of dry selected yeasts (*Saccharomyces cerevisiae* 404 IMIA, DI.PRO.VAL strain collection). Alcoholic fermentation was carried out on skins for 7 days at 28 °C in stainless steel tanks. At the end of malolactic fermentation wine was racked, filtered and adjusted to 50 mg/l of total sulfites. Wine samples' chemical parameters were alcohol 12.64% (v/v), pH 3.24, titratable acidity 6.81 g/l, volatile acidity 0.35 g/l, SO_2 50 mg/l, total phenols 2160 mg/l.

2.2. Experimental conditions

At the end of malolactic fermentation, four aging processes were carried out using 50 l stainless steel containers.

The first sample (Control) (C) was carried out without any additional treatments; in the second sample (Control + Chips) (C + Chips) 2 g/l of chips were added; in the third sample (Control + Chips + Micro-oxygenation) (C + Chips + μO_2) 2 g/l of chips were added and the wine was aged using 3 ml/l/month of oxygen. Finally, in the fourth sample (Control + Chips + Lees + Micro-oxygenation) (C + Chips + Lees + μO_2) 2 g/l of chips were added, with 90 mg/l of yeast lees and aged using 9 ml/l/month of oxygen.

French chips toasted at medium degree (Vason, Verona, Italy) and *Saccharomyces cerevisiae* yeast lees were used.

At the end of aging (90 days), wines were filtered using a cartridge line (cut-off 8, 5, 3 μm) (Sartorious S.p.A, Florence, Italy) and bottled. The experiments were carried out in triplicate.

Sample analysis was carried out at the end of aging (0 days) and 50, 100 and 150 days after bottling.

2.3. Chemical analysis

Total phenols were evaluated as stated by Singleton and Rossi (1965) using Folin–Ciocalteau reagent. The quantification of total phenols was carried out using a calibration curve prepared with known amounts of gallic acid.

Optical density at 620 nm was carried out according to Glories (1984), colour intensity and hue were evaluated as stated by Sudraud (1958), measuring the optical density (OD) at 420 nm and at 520 nm. The colour intensity is the sum of the two OD values and the colour hue is their ratio (OD 420 nm/OD 520 nm). Red polymerised phenols were evaluated according to Somers and Evans (1977); phenolic acid, flavanols, flavonols and anthocyanins were quantified using HPLC (Castellari, Arfelli, Riponi, & Amati, 1998; Castellari, Sartini, Fabiani, Arfelli, & Amati, 2002).

2.4. Statistical procedures

Regression curves (concentration *vs* time) were prepared using the data taken during bottle storage.

Statistical treatments of data were carried out using Statistica 5.0 for Windows (StatSoft, Inc., Tulsa, OK). ANOVA and LSD tests were used to show the effects and interactions of the independent variables on chemical data. Principal component analysis (PCA) was carried out to prove the relationship between the chemical data and the different conditions used in wine aging. Only two principal components (PC) were extracted and resulted significant, according to the Kaiser criterion (eigenvalue > 1).

3. Results and discussion

Table 1 shows wine properties at the end of aging ($t = 0$). Colour intensity is statistically higher in micro-oxygenated wines. Oxygen consumption during aging induced colour stabilisation because of red polymers formation. Red and violet polymeric compounds increase colour intensity and improve pigment stabilisation (Escribano-Bailón, Alvarez-Garcia, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001; Llaudy et al., 2006; Timberlake & Bridle, 1976); their formation is supported by micro-oxygenation and by yeast lees releasing activity. Also micro-oxygenated wines and those aged using micro-oxygenation and yeast lees were different, confirming that yeast lees stabilise colour, as confirmed by other authors (Escot et al., 2001; Rosi, Gheri, & Ferrari, 1998). Wines with added chips showed the lowest colour intensity probably because of the absorbing capacity of chips (Amati & Arfelli, 2001).

Colour hue was statistically lower in micro-oxygenated wines. This trend is against the findings of Alcade-Eon, Escribano-Bailón, Santos-Buelga, and Rivas-Gonzalo (2006), who showed an increase of the orange compounds during wood aging. This lower value could be explained by

Table 1
Composition of wine at the end of aging ($t = 0$)

	C	C + Chips	C + Chips + μO_2	C + Chips + Lees + μO_2
Total phenolics (mg/l)	1973 ± 12	1931 ± 16	1987 ± 25	1948 ± 15
Colour intensity	7.7 ± 0.010^{b}	7.645 ± 0.006^{a}	7.788 ± 0.009^{c}	7.84 ± 0.003^{d}
Colour hue	0.54 ± 0.002^{b}	0.541 ± 0.003^{b}	0.506 ± 0.002^{a}	0.507 ± 0.004^{a}
Red polymeric color	2.51 ± 0.005^{a}	2.548 ± 0.007^{a}	2.613 ± 0.007^{b}	2.665 ± 0.013^{c}
Optical density (620 nm)	0.68 ± 0.010^{ab}	0.663 ± 0.006^{a}	0.704 ± 0.009^{b}	0.69 ± 0.003^{ab}
Malvidin-3-glucoside (mg/l)	194 ± 0.1^{b}	191 ± 0.8^{b}	185 ± 1.7^{a}	184 ± 0.6^{a}
Total anthocyanins (mg/l)	315 ± 0.8^{b}	316 ± 0.2^{b}	305 ± 1.8^{a}	301 ± 1.6^{a}
Gallic acid (mg/l)	59.1 ± 0.56	59.3 ± 0.24	58.8 ± 0.31	58.9 ± 0.17
(+)-Catechin (mg/l)	45.3 ± 0.36^{b}	44.5 ± 0.16^{b}	39.5 ± 0.18^{a}	38.9 ± 0.13^{a}
(−)-Epicatechin (mg/l)	40.7 ± 0.15^{b}	39.4 ± 0.10^{b}	37.3 ± 0.23^{a}	37.2 ± 0.36^{a}
Caftaric acid (mg/l)	45.4 ± 0.82^{b}	45.7 ± 0.07^{b}	43.3 ± 0.32^{a}	45.6 ± 0.05^{b}
Quercetin (mg/l)	8.36 ± 0.173^{c}	5.61 ± 0.074^{b}	4.16 ± 0.293^{a}	5.1 ± 0.030^{ab}
Miricetin (mg/l)	3.43 ± 0.185^{c}	2.81 ± 0.023^{b}	2.26 ± 0.049^{a}	2.89 ± 0.027^{b}

[a–d] Different letters on the same line indicate a difference at $p = 0.05$. Each value is the mean of three replicates.

the formation of red polymeric pigments. During micro-oxygenation some ethyl bridge compounds and condensation products that decrease the colour hue could be formed (Cano-López, Pardo-Minguez, López-Roca, & Gómez-Plaza, 2006).

Red polymeric colour (PPC) confirmed what was observed above regarding colour intensity and hue. Micro-oxygenated wines and those with added yeast lees were richer in PPC.

Optical density at 620 nm, indicating the blue colour of wine (Glories, 1984), had higher values in micro-oxygenated wines, probably due to co-pigmented complexes and to polymerisation phenomena with ethyl bridges, which generated red-violet compounds (Atanasova et al., 2002).

Malvidin-3-glucoside and monomeric anthocyanins had similar values in micro-oxygenated wines, but these values were statistically lower than those of the control wine and of wines with only chips added. Micro-oxygenation affected the concentration of these compounds more than chips addition, so the stabilising effect of oxygen on pigments (Atanasova et al., 2002) was more important than the adsorbing effect of chips.

According to other authors (Ferrarini et al., 2001), (+)-catechin and (−)-epicatechin concentration is lower in micro-oxygenated wines because of the high reactivity of these molecules in an oxidant environment.

Caftaric acid showed a lower concentration in micro-oxygenated wines without lees. Lees prevented the oxidation of caftaric acid (Fornairon-Bonnefond & Salmon, 2003; Salmon et al., 2000). Rutin absorption on chips (Amati & Arfelli, 2001) influenced the concentration of this compound (data not shown). Also quercetin was higher in the control. Wines obtained using only chips showed a lower quantity because of the adsorbing power of this co-adjuvant (Amati & Arfelli, 2001). Quercetin was reduced in micro-oxygenated wines, probably due to the high reactivity of this molecule with oxygen (Rice-Evans, Miller, & Paganga, 1996). Yeast lees may protect miricetin from oxidation because they adsorb oxygen (Fornairon-Bonnefond & Salmon, 2003; Salmon et al., 2000).

Wine evolution was evaluated five months after bottling (Table 2). Total phenols decrease in the bottle. The decrease of these substances is moderate because of the formation of polymeric compounds during aging. Colour intensity and PPC, show no variation during bottle conservation, confirming the stabilization of the phenolic substances in the aging process.

Colour hue increases during bottle conservation because of the increase of the optical density at 420 nm. This is an expected trend in bottled wine, in which the formation of brown pigment due to oxidation and polymerization phenomena can occur (Alcade-Eon et al., 2006; Baranowski & Nagel, 1983; Bonaga, Pallotta, & Syrghi, 1990; Jurd, 1969; Liao, Cai, & Haslam, 1992; Santos-Buelga, Bravo-Haro, & Rivas-Gonzalo, 1995; Somers, 1971). During bottle conservation, optical density at 620 nm increases, and is probably related to the formation of blue anthocyanin pigments (Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & de Freitas, 2003).

All the measured monomeric phenolic compounds decrease during bottle storage, confirming that polymerisation and oxidation reactions continue in the bottle. Also the monomeric anthocyanins decrease during bottle storage. The evolution of anthocyanins could be affected by the aging technologies used and by the natural decrease of these substances due to polymerisation during bottle storage (Singleton & Trousdale, 1992; Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002).

Table 3 shows the gradient of the regression curves relating to each parameter evolution during bottle storage. In all tests, total phenolic compounds decreased during bottle storage. A statistically decrease was induced by lees addition, probably because of some compounds, released by lees during aging, that remained in the wine after filtration. These substances could have interacted with wine phenolic compounds during bottle storage because they had more time for reaction. In all tests, colour intensity (Table 3) increases but samples with added chips showed a higher increase than the control. Lees also had their own positive effect on colour intensity. Micro-oxygenation did not affect

Table 2
Evolution of wine composition during bottle storage

	Days			
	0[a]	50[a]	100[a]	150[a]
Total phenols (mg/l)	2111 ± 13[B]	2089 ± 15[B]	1959 ± 11[A]	1942 ± 25[A]
Colour intensity	7.743 ± 0.022	7.775 ± 0.027	7.804 ± 0.031	7.828 ± 0.032
Color hue	0.523 ± 0.005[A]	0.560 ± 0.002[B]	0.574 ± 0.003[C]	0.657 ± 0.003[D]
Red polymeric colour	2.584 ± 0.018	2.593 ± 0.018	2.601 ± 0.017	2.607 ± 0.032
Optical density (620 nm)	0.683 ± 0.006[A]	0.745 ± 0.002[B]	0.756 ± 0.005[BC]	0.771 ± 0.007[C]
Malvidin-3-glucoside (mg/l)	188 ± 2.0[D]	161 ± 1.9[C]	108 ± 1.7[B]	70.6 ± 2.60[A]
Total anthocyanins (mg/l)	309 ± 2.7[D]	261 ± 4.2[C]	174 ± 3.5[B]	115 ± 4.20[A]
Gallic acid (mg/l)	59.0 ± 0.18[D]	56.1 ± 0.43[C]	53.8 ± 0.37[B]	40.3 ± 0.32[A]
(+)-Catechin (mg/l)	42.0 ± 0.83[D]	35.7 ± 0.84[C]	29.5 ± 0.32[B]	20.0 ± 0.54[A]
(−)-Epicatechin (mg/l)	38.6 ± 0.44[C]	30.9 ± 1.14[B]	21.3 ± 0.68[A]	18.4 ± 0.55[A]
Caftaric acid (mg/l)	45.2 ± 0.42[D]	42.7 ± 0.69[C]	39.1 ± 0.15[B]	27.1 ± 0.67[A]
Quercetin (mg/l)	5.81 ± 0.460[C]	4.08 ± 0.230[B]	3.02 ± 0.320[AB]	2.46 ± 0.300[A]
Miricetin (mg/l)	2.85 ± 0.130[C]	2.05 ± 0.180[B]	1.70 ± 0.030[AB]	1.55 ± 0.120[A]

[A–D] Different letters on the same line indicate a difference at $p = 0.01$. Each value is the mean of three replicates.
[a] $n = 12$.

Table 3
Interaction between time of bottle storage and technological treatments: slopes of regression lines

	r^2	Control	C + Chips	C + Chips + μO_2	C + Chips + Lees + μO_2
Total phenolics	0.773	−0.85887 ± 0.207619[a]	−1.36667 ± 0.074955[a]	−1.2889 ± 0.096975[a]	−2.0498 ± 0.092482[b]
Colour intensity	0.881	0.00009 ± 0.000043[a]	0.00059 ± 0.000051[b]	0.00063 ± 0.000069[b]	0.00097 ± 0.000013[c]
Colour hue	0.850	0.00105 ± 0.000021	0.00099 ± 0.000021	0.0009 ± 0.000059	0.00091 ± 0.000046
Red polymeric colour	0.636	0.0002 ± 0.000026	0.0001 ± 0.000044	0.00018 ± 0.000079	0.00015 ± 0.000085
Optical density (620 nm)	0.788	0.00066 ± 0.000060	0.00041 ± 0.000036	0.00062 ± 0.000040	0.00052 ± 0.000017
Malvidin-3-glucoside	0.980	−0.78643 ± 0.000971	−0.82823 ± 0.024489	−0.78057 ± 0.034482	−0.85873 ± 0.028138
Total anthocyanins	0.982	−1.27637 ± 0.005223	−1.38017 ± 0.028606	−1.28813 ± 0.042305	−1.40413 ± 0.042585
Gallic acid	0.826	−0.11707 ± 0.003739[ab]	−0.1265 ± 0.000732[b]	−0.11453 ± 0.002850[ab]	−0.10927 ± 0.000720[a]
(+)-Catechin	0.972	−0.15757 ± 0.007426[b]	−0.17297 ± 0.001506[b]	−0.1269 ± 0.000939[a]	−0.12167 ± 0.001419[a]
(−)-Epicatechin	0.927	−0.14923 ± 0.002926[b]	−0.16653 ± 0.000196[c]	−0.12557 ± 0.001149[a]	−0.1217 ± 0.001370[a]
Caftaric acid	0.850	−0.11402 ± 0.004746[b]	−0.1488 ± 0.003169[c]	−0.08833 ± 0.003616[a]	−0.11207 ± 0.003142[ab]
Quercetin	0.907	−0.0282 ± 0.001509[b]	−0.02243 ± 0.000671[ab]	−0.02197 ± 0.001793[ab]	−0.01613 ± 0.000191[a]
Miricetin	0.763	−0.0111 ± 0.000432[b]	−0.00737 ± 0.000144[a]	−0.00717 ± 0.000260[a]	−0.00837 ± 0.000144[a]

[a–c] Different letters on the same line indicate a difference at $p = 0.05$. Each value is the mean of three replicates. The r^2 value is the mean of 12 values.

total phenolic compounds and colour intensity because C + Chips and C + Chips + μO_2 were not statistically different. Colour hue, PPC, optical density at 620 nm, malvidin 3-glucoside and total anthocyanins were not affected by the different treatments during bottled storage. A different evolution of total phenolic compounds and colour intensity was expected because their values were influenced by several variables. Table 3 shows that micro-oxygenation had a positive influence on the stabilisation of the considered flavans ((+)-catechin and (−)-epicatechin) and of caftaric acid. The stabilising effect on these easily oxidisable flavans could be also due to their decrease during aging (Table 1). Chips (Table 3) had a positive influence on quercetin evolution but this effect became statistically significant only with lees addition. This is probably due to the components released by lees, that may stabilise quercetin.

Miricetin decrease was statistically lower in all of the samples with added chips, compared to the control, and this could be due to some compounds, such as ellagitannins, flavans and flavones released by chips (Fernández de Simon et al., 1996; Viriot et al., 1994).

Gallic acid was different from the other compounds. Chips addition and micro-oxygenation did not affect evolution relative to the control. The highest differences were observed between C + Chips and C + Chips + Lees + μO_2. Therefore, a synergic effect of lees and micro-oxygenation was assumed.

Principal component analysis (Fig. 1) showed that wines with added chips (C + Chips) were not different from the control wine; C + Chips + μO_2 were different from control both in principal component (PC) 1 and 2 and from C + Chips only in PC 1. Wines micro-oxygenated with added chips and lees (C + Chips + Lees + μO_2) were different from the control only in PC 1 and from C + Chips both in PC 1 and 2.

C + Chips + μO_2 and C + Chips + Lees + μO_2 were discriminated by delphinidin-3-glucoside, cyanindin-3-glucoside, and petunidin-3-glucoside, on PC 2 while, in PC 1, were not discriminated. PC 1 discriminated micro-oxygenated wines from those not micro-oxygenated for gallic acid, peonidin-3-glucoside, colour intensity and colour hue.

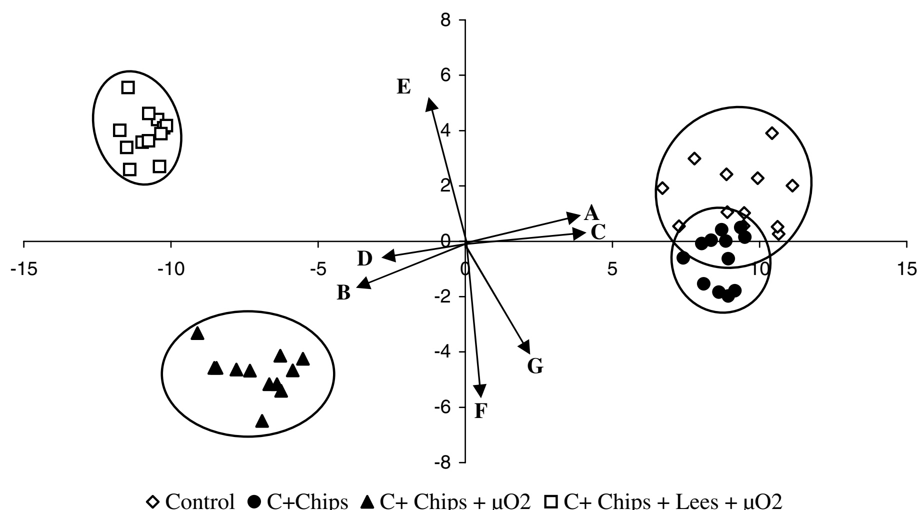

Fig. 1. Principal component analysis (PCA) of red Sangiovese wines (PC 1 – 86.79%, PC 2 – 10.63%). Factorial weights higher than 3 on PC 1 and higher than 4 on PC 2 are represented as vectors. A, Gallic acid; B, peonidin-3-glucoside; C, colour hue; D, colour intensity; E, delphinidin-3-glucoside; F, cyanidin-3-glucoside; G, petunidin-3-glucoside.

Finally, all data showed that wines produced using chips, lees and micro-oxygenation have a more stable colour because of red-blue polymeric pigments. Wines' chemical profiles and their stability in bottle were affected by the application of these three techniques. Different effects induced by a combined application of chips, lees and micro-oxygenation may be used by the winemaker to obtain particular products.

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