Received: 22 April 2013

Revised: 24 June 2013

(wileyonlinelibrary.com) DOI 10.1002/jsfa.6303

Phenolic composition and mouthfeel characteristics resulting from blending Chilean red wines

Alejandro Cáceres-Mella,^a* Álvaro Peña-Neira,^a Pamela Avilés-Gálvez,^a Marcela Medel-Marabolí,^a Rubén del Barrio-Galán,^a Remigio López-Solís^b and Joan Miquel Canals^c

Abstract

BACKGROUND: The blending of wine is a common practice in winemaking to improve certain characteristics that are appreciated by consumers. The use of some cultivars may contribute phenolic compounds that modify certain characteristics in blended wines, particularly those related to mouthfeel. The aim of this work was to study the effect of Carménère, Merlot and Cabernet Franc on the phenolic composition, proanthocyanidin profile and mouthfeel characteristics of Cabernet Sauvignon blends.

RESULTS: Significant differences in chemical composition were observed among the monovarietal wines. Separation using Sep-Pak C₁₈ cartridges revealed differences in the concentration but not in the proportion of various proanthocyanidins. Blending reduced polyphenol concentration differences among the various monovarietal wines. Although no major overall differences were observed after blending the monovarietal wines, this oenological practice produced clear differences in mouthfeel characteristics in such a way that the quality of the perceived astringency was different.

CONCLUSION: This study showed that the use of a particular wine variety (Cabernet Sauvignon) in a higher proportion in wine blending produced blends that were less differentiable from the monovarietal wine, owing to a suppression effect, producing an apparent standardization of the wines regarding chemical composition. © 2013 Society of Chemical Industry

Keywords: wine blending; proanthocyanidins; astringency; Cabernet Sauvignon; Carménère; Merlot; Cabernet Franc

INTRODUCTION

The blending of wine, or coupage, is one of the oldest and most common practices in winemaking. Wines are blended to improve certain characteristics that are appreciated by consumers, such as colour, flavour, alcohol content, body and aromatic composition, and to improve product quality.¹⁻⁵ Although blending wines of different cultivars can provide different aromatic descriptors, it is also true that some cultivars may contribute phenolic compounds that modify certain characteristics in blended wines, particularly those related to mouthfeel.^{6–8} Previous studies on wine blends have shown that polyphenols and colour are affected by the blending process.^{9–11} The different grape cultivars used in blending may result in original wines owing to characteristics acquired in the procedure; individually, these cultivars contribute to different characteristics of the blended wine, thus producing significant organoleptic changes in the final product. Cabernet Sauvignon is one of the most important cultivars in Chile, with approximately 40 800 ha planted. Wines from the Merlot and Cabernet Franc cultivars are widely used in blends throughout the world and in Chile. Carménère, with approximately 10000 ha planted,¹² is the emblematic cultivar of Chile, as it was believed to be extinct in the world after the phylloxera devastation of European grapevines in the mid-19th century and was only rediscovered in Chile in 1994.^{13–15} Although the blending of wines in different proportions can generate significant sensory changes, there are few studies examining the effect of this practice on the phenolic composition of wine blends and, more specifically, on the proportion and concentration of proanthocyanidin fractions and mouthfeel attributes. This study aimed to evaluate the effect of Carménère, Merlot and Cabernet Franc wines on the phenolic content, proanthocyanidin profile and mouthfeel properties of Cabernet Sauvignon blends.

- * Correspondence to: Alejandro Cáceres-Mella, Departamento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile. E-mail: alecaceres@uchile.cl
- a Departamento de Agroindustria y Enología, Facultad de Ciencias Agronómicas, Universidad de Chile, Casilla 1004, Santiago, Chile
- b Programa de Biología Celular y Molecular, Facultad de Medicina, Universidad de Chile, Independencia 1027, 8380453 Santiago, Chile
- c Departamento de Bioquímica y Biotecnología, Facultad de Enología, Universidad Rovira I Virgili, C/ Marcel.lí Domingo s/n, E-43007 Tarragona, Spain

EXPERIMENTAL

Chemical reagents

Methylcellulose (1500 cP viscosity at 20 g L^{-1}) and standards of gallic acid, caffeic acid, p-coumaric acid, caftaric acid, (+)catechin, (-)-epicatechin, guercetin, myricetin, kaempferol and malvidin-3-glucoside were purchased from Sigma Chemical Co. (St Louis, MO, USA). Polyethylene membranes of 0.22 µm pore size were acquired from EMD Millipore (Billerica, MA, USA). Sodium sulfate (anhydrous), potassium metabisulfite, vanillin (990 g L^{-1}), ethyl acetate, diethyl ether, sodium hydroxide, hydrochloric acid, sulfuric acid, high-performance liquid chromatography (HPLC)grade acetonitrile, acetic acid, formic acid and methanol were purchased from Merck (Darmstadt, Germany). All reagents were or analytical grade or higher. Sep-Pak Plus Environmental tC18 cartridges (900 mg) and Sep-Pak Plus Short tC₁₈ cartridges (400 mg) were obtained from Waters (Milford, MA, USA). Phosphate buffer (pH7) was acquired from Mallinckrodt Baker (Phillipsburg, NJ, USA). Nitrogen gas was supplied by Indura SA (Santiago, Chile).

Instrumentation

pH values were measured using an 8417 N pH meter (Hanna Instruments, Smithfield, RI, USA). Phenolic analyses were performed using an 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) consisting of a G1315B photodiode array detector (DAD), a G1311A quaternary pump, a G1379A degasser and a G1329A autosampler. A reverse phase Nova-Pak C₁₈ column (4 μ m, 3.9 mm i.d. \times 300 mm; Waters) was used for the analysis of individual phenolic compounds. Anthocyanin analyses were performed using a D-7000 HPLC system (Merck-Hitachi, Darmstadt, Germany) consisting of an L-7455 DAD, an L-6200 pump and an L-7200 autosampler with a Chromolith C₁₈ column (2 μ m, 4.6 mm i.d. \times 100 mm; Merck). Absorbance values were measured using a PharmaSpec UV-1700 UV-visible spectrophotometer (Shimadzu, Kyoto, Japan).

Wine samples

Four monovarietal red wines (all vintage 2010) were used: Cabernet Sauvignon (CS), Carménère (CR), Merlot (ME) and Cabernet Franc (CF). All monovarietal wines were produced using the same oenological practices at William Fèvre Winery. The grape varieties were cultivated in the same geographical area, the Maipo Valley in central Chile. The wines (150 L of CS and 50 L each of CR, ME and CF) were transported from the private winery to our laboratories in food-grade polyethylene tanks and stored at 10 °C until blending. The wines showed no evidence of malolactic fermentation. The four base wines were all dry (residual sugar: CS, 2.8; CR, 2.2; ME, 2.5; CF, 2.7 g glucose L⁻¹) and presented the following alcohol contents: CS, 14.5; CR, 14.6; ME, 13.7; CF, 14.5% v/v.

Wine blending

The wine blends were prepared in one deposit of 25 L capacity for each blend. Nitrogen flushing was used at all experimental stages to reduce the chance of oxidation. Because the densities of the four wines were essentially the same (CS, 0.987; CR, 0.988; ME, 0.989; CF, 0.989 g cm⁻³), the wines were blended volumetrically. The wine blending was performed 10 days after arrival in our laboratory according to the criteria used by the wine company. A total of ten wines were analysed, including four monovarietal base wines and six two-wine blends with CS as the base wine and CR, ME and CF wines as modifiers in volumetric proportions of 30% (CS-CR (70:30), CS-ME (70:30) and CS-CF (70:30)) and 15% (CS-CR (85:15), CS-ME (85:15) and CS-CF (85:15)). After blending, the wines were adjusted to a free sulfur dioxide level of 30 mg L⁻¹ and immediately bottled in dark green 750 mL glass bottles (CristalChile, Santiago, Chile), closed with roll-on tamper-evident screwcaps (SaranexTM liner) (Amcor, Hawthorn, Australia) and stored at 10 °C until further use.

Wine chemical analyses

The analytical methods recommended by OIV¹⁶ were used to determine the pH, sugar content (g glucose L⁻¹), titratable acidity (g H₂SO₄ L⁻¹) and ethanol content (% v/v). The total phenol content was determined by UV absorptiometry at 280 nm¹⁷ and expressed as mg gallic acid equivalent (GAE) L⁻¹. The total tannin content was measured using methylcellulose as precipitant agent¹⁸ and expressed as mg (+)-catechin equivalent (CE) L⁻¹. The total anthocyanin content was measured using the method described by Ribéreau-Gayon and Stonestreet¹⁹ and expressed as mg malvidin equivalent (ME) L⁻¹. The colour intensity (CI) was estimated using the method described by Glories.¹⁷ The colour coordinates lightness (*L**), chroma (*C**) and hue (*h**) were determined according to Pérez-Magariño and González-Sanjosé.²⁰

Fractionation of proanthocyanidins using Sep-Pak C_{18} cartridges

For fractionation of wine proanthocyanidins according to their polymerization degree, Sep-Pak tC₁₈ cartridges were used following the method described by Sun et al.²¹ Briefly, 7 mL of wine sample was concentrated to dryness in a rotary evaporator at <30 °C and the residue was dissolved in 20 mL of 67 mmol L⁻¹ phosphate buffer (pH7). The resulting solution was adjusted to pH7 under a nitrogen atmosphere. Each sample was passed through two preconditioned neutral Sep-Pak tC₁₈ cartridges (methanol, 10 mL; distilled water, 20 mL; phosphate buffer, 10 mL) connected in series (top, Sep-Pak Plus Environmental tC₁₈ cartridge (900 mg); bottom, Sep-Pak Plus Short tC₁₈ cartridge (400 mg)) and the phenolic acids were eliminated by elution with 10 mL of 67 mmol L^{-1} phosphate buffer (pH 7). After drying the cartridges with nitrogen gas, elution was carried out first with 25 mL of ethyl acetate to elute monomeric and oligomeric proanthocyanidins, and then the polymeric fraction was eluted with 15 mL of methanol. To separate the monomeric fraction from the oligomeric fraction, the ethyl acetate eluate was evaporated to dryness under vacuum at < 30 °C, dissolved in 10 mL of 67 mmol L⁻¹ phosphate buffer (pH 7) and then redeposited onto the same connected cartridges that had been conditioned again as described above. After drying the cartridges with nitrogen gas, monomeric and oligomeric proanthocyanidins were eluted sequentially with 25 mL of diethyl ether (monomeric fraction) followed by 15 mL of methanol (oligomeric fraction). For each fraction obtained previously, flavanols were quantified using the modified vanillin assay described by Sun et al.²² A 2.5 mL aliquot of 1:3 (v/v) H₂SO₄/methanol solution and 2.5 mL of 10 g L^{-1} vanillin in methanol were mixed with 1 mL of the sample. The tubes were incubated at 30 °C for either 15 min (monomeric fraction) or a time sufficient to allow maximal reaction (oligomeric and polymeric fractions). The absorbance at 500 nm was measured. A blank was prepared by replacing the vanillin solution in the reaction mix with methanol.

HPLC-DAD analysis of low-molecular-weight phenolic compounds

Red wines samples (50 mL) were extracted with diethyl ether (3 \times 20 mL) and ethyl acetate (3 \times 20 mL). The resulting extracts

were evaporated to dryness at 30 $^{\circ}$ C, dissolved in 2 mL of 50:50 (v/v) methanol/water and filtered (0.22 µm pore size membrane). Aliquots (25 µL) of the final solution were subjected to reverse phase chromatographic separation at 20°C using a Nova-Pak C_{18} column. The DAD was set from 210 to 360 nm with an acquisition time of 1 s. The two mobile phases used were (A) water/acetic acid (98:2 v/v) and (B) water/acetonitrile/acetic acid (78:20:2 v/v/v). A gradient was applied at a flow rate of 1 mL min⁻¹ from 0 to 55 min and 1.2 mLmin^{-1} from 55 to 90 min as follows: 100-20% A from 0 to 55 min, 20-10% A from 55 to 57 min and 10-0% A from 57 to 90 min. The identification of specific compounds was carried out by comparison of their absorption spectra (from 210 to 360 nm) and retention times with those of standards. Procyanidin dimer, procyanidin gallate, myricetin glycosides, quercetin glycosides and kaempferol glycosides, for which standards were unavailable, were assigned by retention time and spectral parameters. Quantitative determinations were performed using the external standard method with commercial standards. The calibration curves were obtained by injection of standard solutions, under the same conditions as for the samples analysed, over the range of concentrations observed. Compounds for which no standards were available were quantified using standard curves for (+)-catechin (procyanidin dimer and procyanidin gallate), quercetin (quercetin glycosides), myricetin (myricetin glycosides) and kaempferol (kaempferol glycosides). All qualitative and quantitative analyses of the phenolic composition (including extraction) were performed in triplicate.^{23,24}

HPLC-DAD analysis of anthocyanin compounds

A 2 mL sample of wine was filtered through a 0.22 μ m pore size membrane, and 150 μ L aliquots of the sample were subjected to reverse phase chromatographic separation at 20 °C using a Chromolith C₁₈ column. The DAD was set from 210 to 600 nm. The two mobile phases used were (A) water/formic acid (90:10 v/v) and (B) acetonitrile. A gradient was applied at a flow rate of 1.1 mL min⁻¹ from 0 to 22 min and 1.5 mL min⁻¹ from 22 to 35 min as follows: 96–85% A from 0 to 22 min, 85–15% A from 12 to 22 min and 85–70% A from 22 to 35 min. Quantification was performed by peak area measurements at 520 nm. The anthocyanins were quantified and expressed as mg malvidin-3-glucoside L⁻¹. The calibration curves at 520 nm were obtained by injecting different volumes of standard solutions under the same conditions used for the samples.²⁵

Sensory evaluation

A descriptive analysis was conducted on the wines approximately 1 month after bottling. The sensory panel consisted of 12 people (six females and six males aged 24-38 years) who were all students or workers at the Department of Agroindustry and Enology. All judges had previous experience with descriptive analyses. An initial training session was conducted to standardise the criteria among the judges. Subsequently, two sessions of 3 h during 2 days were performed; for each session, each panellist had to assess two flights consisting of five wines each. The wines were evaluated in individual temperature-controlled tasting booths, and water and unsalted crackers were provided for palate cleansing. Aliquots (20 mL) of wine were served at $18-19^{\circ}$ C in dark wine-tasting glasses (RCristal, Mendoza, Argentina) labelled with a threedigit code using a completely randomised order. The use of the dark wine-tasting glasses was to prevent the interaction of visual sensations and to focus the attention of the panellists on mouthfeel sensation. To decrease fatigue, there was a 30 s break between each wine. During each break, the panellists chewed on a cracker and then rinsed the mouth with water. Each wine was evaluated with regard to two sensory attributes, astringency and bitterness, on a 15 cm unstructured linear scale anchored from 'low' to 'high'. The judges also made a descriptive analysis of the astringency of each wine. The astringency terms appropriate for discriminating among wine blends are provided in Table 1. The judges chose a series of mouthfeel terms, and the terms with the highest scores were chosen after the evaluation and expressed as a percentage. These terms were derived from previous studies of mouthfeel properties of red wines.^{7,26,27} All judges rated each wine in duplicate during the 2 day evaluation. The data were collected on a paper ballot.

Statistical analysis

Analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were used for mean separation, with a significance level of 95% (P < 0.05). The descriptive and chemical results were analysed by principal component analysis (PCA). Pearson's correlation was used to understand the relationship between sensory analysis and chemical composition. All statistical analyses were conducted using Statgraphics Centurion Version 15.2 (StatPoint Technologies, Inc., Warrenton, VA, USA) and Excel 2007 Version 12.0 (Microsoft Corp., Redmond, WA, USA).

RESULTS AND DISCUSSION

Wine composition

Table 2 shows the analyses of the global analytical parameters and phenolic analyses of the wine samples. The total phenol content ranged from 794.99 to 1006.78 mg GAE L⁻¹, with the highest value for the CF (100) wine and the lowest value for the ME (100) wine. For the analyses of total tannins, the content of the wine blends varied from 1700.66 to 1992.77 mg CE L⁻¹; the highest content was observed in CF (100) and in the blends with this monovarietal wine. With regard to the total anthocyanins, ME (100) showed the lowest content, whereas CR (100) showed the highest content; in the case of the wine blends, the anthocyanin content ranged from 426.68 to 533.27 mg ME L⁻¹. A comparison of the anthocyanin content among the monovarietal wines showed that CS presented 45% less anthocyanins than CR and 19 and 30% more anthocyanins than ME and CF respectively. Such differences in the content of tannins and anthocyanins could affect the chromatic characteristics of the wine. There were significant differences among the wine samples

Table 1. G	rouping of astring	ency terms rated by panel and their
Grouping	Term	Definition
Harsh	Abrasiveness	Excessive astringency of a strongly rough nature
	Hardness	Combined effect of astringency and bitterness
Dynamic	Adhesiveness	A sensation that the mouth surfaces are adhering to one another
Drying	Dryness	Feeling of a lack of lubrication in the mouth
Complex	Mouthcoating	A sensation of a coating film that adheres to the mouth surfaces, which decreases with time

www.soci.org

Table 2.	Chemical com	position of wi	ne samples						
Wine	рН	TA (g H ₂ SO ₄ L ⁻¹)	Phenols (mg GAE L ⁻¹)	Tannins (mg CE L ⁻¹)	Anthocyanins (mg ME L^{-1})	CI (a.u.)	L*	С*	h*
CS (100)	$3.53\pm0.01b$	$3.53\pm0.10ab$	$893.69\pm4.3d$	$1704.12 \pm 168.9b$	$486.04\pm2.0c$	$17.17\pm0.04e$	$37.21\pm0.01f$	$58.06\pm0.02f$	$17.62\pm0.08e$
CR (100)	$3.52\pm0.01 bc$	$3.59\pm0.06a$	$922.85\pm4.9b$	$1866.05\pm286.9b$	$707.80\pm3.8a$	$23.88\pm0.02a$	$28.60\pm0.01i$	$60.29\pm0.03a$	$20.30\pm0.07a$
ME (100)	$3.58\pm0.01a$	$3.27\pm0.06c$	794.99 \pm 3.6f	$1790.84\pm155.7b$	$393.03\pm3.7f$	$14.79\pm0.03h$	$41.65\pm0.05a$	$55.69\pm0.05i$	$15.47\pm0.11f$
CF (100)	$3.52\pm0.01 bc$	$3.50\pm0.06ab$	$1006.78 \pm 7.1a$	$2254.66\pm54.1a$	$342.71\pm2.9g$	$17.49\pm0.02c$	$36.70\pm0.00g$	$58.38\pm0.08e$	$18.66\pm0.05c$
CS-CR (70:30)	$3.51\pm0.01 bc$	$3.63\pm0.10a$	$924.54\pm2.3b$	$1710.53\pm21.9b$	$533.27\pm3.4b$	$19.75\pm0.01b$	$33.61\pm0.02h$	$60.17\pm0.01b$	$19.36\pm0.02b$
CS-ME (70:30)	$3.53\pm0.01b$	$3.46 \pm 0.06abc$	$857.87\pm6.7e$	$1700.76\pm3.0b$	$436.22\pm4.9e$	$17.21\pm0.04e$	$37.60\pm0.10e$	$59.05\pm0.01c$	18.23 \pm 0.01d
CS-CF (70:30)	$3.50\pm0.01c$	$3.53\pm0.10ab$	$907.91\pm0.4c$	1992.77 \pm 22.8ab	$429.67\pm3.7e$	$16.40\pm0.05f$	$38.85\pm0.05d$	$57.77\pm0.05g$	$18.16\pm0.14d$
CS-CR (85:15)	$3.50\pm0.01c$	$3.56\pm0.01ab$	$882.24\pm2.9d$	$1722.65\pm97.0b$	$494.64\pm4.5c$	$17.34\pm0.02d$	$37.31\pm0.01f$	$58.52\pm0.04d$	$18.81\pm0.09c$
CS-ME (85:15)	$3.52\pm0.01 bc$	$3.36\pm0.01 bc$	$863.09\pm3.1e$	$1735.88\pm100.5b$	447.35 \pm 2.2d	$15.57\pm0.01g$	$40.55\pm0.05b$	$57.38\pm0.06h$	$17.53\pm0.07e$
CS-CF (85:15)	$3.50\pm0.01c$	$3.59\pm0.01a$	894.99 \pm 6.0 cd	$1860.05 \pm 32.3b$	$426.68\pm5.3e$	$16.35\pm0.01f$	$39.05\pm0.05c$	$57.71 \pm 0.00 g$	$18.61\pm0.07c$

Values are expressed as mean \pm standard deviation (n = 3). Different letters in a column denote significant differences among samples (P < 0.05, Tukey's HSD test). CS, Cabernet Sauvignon; CR, Carménère; ME, Merlot; CF, Cabernet Franc; TA, titratable acidity; GAE, gallic acid equivalent; CE, (+)-catechin equivalent; ME, malvidin equivalent; CI, colour intensity; a.u., absorbance units.

in terms of colour intensity. The greater colour intensity was found for the CR (100) and CS-CR (70:30) wines, which had the highest anthocyanin content. The same pattern was observed for the colour coordinates L^* , chroma (C^*) and hue (h^*), demonstrating that wine blending produced significant changes in the colour of the wine.

In general terms, the higher concentrations of total phenols, tannins and anthocyanins in the wines blended with CF and CR were due to the higher content of these compounds in these monovarietal wines (Table 2). The blending of wines produced significant changes in their phenolic composition, corroborating the results of other authors.^{2,10,11} However, the changes in the blended wines depended on the contribution of the monovarietal wines added to the mixture, so the initial amount of each cultivar influenced the wine produced. The relative content of phenolic compounds observed in this study is in agreement with those reported by other authors.^{25,28–30}

The PCA illustrated the relationship between the ten wines and the compositional analysis (Fig. 1). PC1 and PC2 accounted for

80.6% of the total variation (60.1 and 20.5% respectively). PC1 was characterised by colour attributes, specifically, *L**, colour intensity, chroma (*C**), hue (*h**) and titratable acidity. PC2 was characterised by pH and phenolic compounds such as total phenols, tannins and anthocyanins. The monovarietal wines CR (100), ME (100) and CF (100) were located outside the central region where the blends, CS (100) and most analytical parameters were located. As expected, all four base wines were significantly different from each other, corroborating the results shown in Table 2. The monovarietal wines each had distinct characteristics that distanced them from the blends, though the respective monovarietal wine and corresponding blend were located in the same quadrant. The overall results indicate that the wine blends including CR were correlated with a high amount of anthocyanins, whereas a blend including CF was correlated with a high amount of tannins.

Table 3 displays the concentrations of low-molecular-weight phenolic compounds quantified in the wine samples. The hydroxybenzoic acid quantified was gallic acid. The hydroxycinnamic acids quantified were caftaric, caffeic and *p*-coumaric acids. The flavanols



Figure 1. PCA comparing general analytical parameters of wine samples. CS, Cabernet Sauvignon; CR, Carménère; ME, Merlot; CF, Cabernet Franc.

lable 3. Low-molect	llar-weight phe	nolic compound	as quantined in	wine samples						
Compound (mg L $^{-1}$)	CS (100)	CR (100)	ME (100)	CF (100)	CS-CR (70:30)	CS-ME (70:30)	CS-CF (70:30)	CS-CR (85:15)	CS-ME (85:15)	CS-CF (85:15)
Non-flavonoid phenolics										
Gallic acid	$25.00\pm0.5de$	$22.40\pm0.2f$	$26.43\pm0.8d$	$50.73 \pm 0.7a$	23.30 ± 1.4ef	$26.43\pm0.6d$	$33.03\pm0.8b$	$22.83\pm0.6ef$	$24.03\ \pm\ 0.9ef$	29.47 ± 1.3c
Hydroxybenzoic acids	$25.00\pm0.5de$	$22.40 \pm 0.2f$	$26.43\pm0.8d$	$50.73 \pm 0.7a$	23.30 ± 1.4ef	$26.43\pm0.6d$	$33.03\pm0.8b$	$22.83 \pm 0.6ef$	$24.03\ \pm\ 0.9ef$	$29.47 \pm 1.3c$
Caftaric acid	$3.80 \pm 0.1cd$	3.57 ± 0.1 cd	$3.30 \pm 0.1d$	$7.30 \pm 0.1a$	$4.00 \pm 0.4 \mathrm{bc}$	$4.00 \pm 0.1 \text{bc}$	$4.53\pm0.3b$	$3.70 \pm 0.1cd$	$3.50\pm0.2cd$	$4.50\pm0.2b$
Caffeic acid	$4.30\pm0.2e$	$3.80 \pm 0.1f$	$3.10\pm0.1\mathrm{g}$	$7.30 \pm 0.1a$	$5.43 \pm 0.1c$	$4.40 \pm 0.1e$	$6.30\pm0.1b$	$4.87\pm0.1d$	$4.10 \pm 0.1ef$	$4.87\pm0.2d$
<i>p</i> -Coumaric acid	1.40 ± 1.1d	$4.33\pm0.2a$	$3.40 \pm 0.1abc$	$3.10 \pm 0.1 \text{bc}$	$3.67\pm0.2ab$	$2.50\pm0.1c$	$2.70 \pm 0.0bc$	$3.60 \pm 0.0ab$	$2.40\pm0.1cd$	$2.67 \pm 0.1 \text{bc}$
Hydroxycinnamic acids	9.50 ± 1.4e	$11.70 \pm 0.2cd$	$9.80\pm0.2e$	$17.70 \pm 0.2a$	$13.10 \pm 0.6bc$	$10.90 \pm 0.3 de$	$13.53 \pm 0.4b$	$12.17 \pm 0.2bcd$	$10.00\pm0.4e$	$12.03 \pm 0.4 bcd$
Total non-flavonoid phenolic	:s 34.50 ± 1.9d	$34.10\pm0.4d$	$36.23 \pm 0.9d$	68.43 ± 0.9a	$36.40 \pm 2.0d$	37.33 ± 0.9d	$46.57 \pm 1.1b$	$35.00 \pm 0.7d$	34.03 ± 1.3d	$41.50 \pm 1.7c$
Flavonoid phenolics										
(+)-Catechin	$32.03 \pm 1.2b$	68.83 ± 0.4a	19.73 ± 0.9e	$70.10 \pm 2.3a$	$20.20 \pm 0.2e$	$24.13 \pm 1.4 cd$	$20.63\pm0.6de$	$19.70 \pm 0.2e$	$21.60\pm0.7cde$	24.67 土 2.4c
(–)-Epicatechin	$34.40\pm0.4b$	$55.00 \pm 1.8a$	$28.00\pm1.1f$	$32.73 \pm 1.0bcd$	33.40 ± 1.0bc	$29.10 \pm 0.5ef$	31.13 ± 0.8 cde	31.73 ± 1.8 bcde	30.73 ± 1.0 cdef	29.93 \pm 0.2def
Procyanidin dimer	$10.90\pm3.5cd$	$17.67 \pm 0.8b$	24.20 ± 0.9a	$11.00 \pm 0.6c$	$7.20 \pm 0.7e$	$7.47 \pm 0.2 de$	$6.50 \pm 0.3e$	8.40 ± 0.2 cde	$7.17 \pm 0.2e$	$6.87 \pm 0.1e$
Procyanidin gallate	$10.50 \pm 2.6a$	$6.27 \pm 1.0 bc$	$4.57 \pm 0.7c$	$10.70 \pm 1.1a$	$8.10\pm0.2ab$	$5.80 \pm 0.1 \text{bc}$	$6.73 \pm 0.3 bc$	$7.50 \pm 0.1b$	$6.00 \pm 0.1 \text{bc}$	$6.90 \pm 0.1 \text{bc}$
Flavanols	87.83 ± 7.7c	147.77 ± 3.4a	$\textbf{76.50}\pm\textbf{3.5d}$	124.53 ± 4.9b	$68.90 \pm 2.1 de$	$66.50 \pm 2.1 de$	65.00 ± 1.9e	67.33 ± 2.3 de	$65.50 \pm 1.9e$	68.37 ± 2.7de
Myricetin-3-glucoside	$12.40 \pm 0.3a$	$7.50 \pm 0.2d$	$7.70 \pm 0.5d$	$8.00 \pm 0.3d$	$10.97 \pm 1.0bc$	$10.47 \pm 0.1c$	$10.33 \pm 0.1c$	$11.87 \pm 0.1ab$	$10.30\pm0.0c$	$10.23 \pm 0.1c$
Myricetin-3-galactoside	$3.60 \pm 0.3 bc$	$3.03 \pm 0.1c$	$4.00 \pm 0.3b$	$4.93 \pm 0.2a$	$3.47 \pm 0.6bc$	$3.40 \pm 0.0 bc$	$3.30 \pm 0.2 bc$	$3.27 \pm 0.4bc$	$3.33 \pm 0.1bc$	$3.20\pm0.1c$
Myricetin-3-rutinoside	$1.93 \pm 0.4abc$	1.67 ± 0.1 abc	$1.03 \pm 0.1c$	$1.40 \pm 0.1 \text{ bc}$	$2.23 \pm 1.0ab$	$1.00 \pm 0.0c$	$1.63 \pm 0.1abc$	$2.40\pm0.2a$	$1.03 \pm 0.1c$	$1.43 \pm 0.1 bc$
Quercetin-3-glucoside	$1.90 \pm 0.2b$	$2.03 \pm 0.1b$	$0.43 \pm 0.1e$	$2.03 \pm 0.1b$	$1.57 \pm 0.1c$	$0.67 \pm 0.1e$	$1.83 \pm 0.2 bc$	$2.40\pm0.2a$	$1.00 \pm 0.0d$	$1.73 \pm 0.1 bc$
Quercetin-3-galactoside	$0.37 \pm 0.2d$	$3.90\pm0.1a$	$0.43\pm0.1cd$	$1.60 \pm 0.1b$	$1.93 \pm 0.7b$	$1.63 \pm 0.1b$	$1.33 \pm 0.5 bc$	$1.63 \pm 0.7b$	$1.70 \pm 0.0b$	$1.87 \pm 0.1b$
Kaempferol-3-glucoside	$1.33 \pm 0.1a$	$1.03 \pm 0.1ab$	$1.07 \pm 0.1ab$	$1.53 \pm 0.1a$	$1.30 \pm 0.1ab$	$1.13 \pm 0.2ab$	$1.33 \pm 0.1a$	$0.77 \pm 0.6b$	$1.17 \pm 0.1ab$	$1.30 \pm 0.0ab$
Kaempferol-3-galactoside	$1.33 \pm 0.2ab$	$0.47 \pm 0.1d$	$1.03 \pm 0.1 bc$	$1.70 \pm 0.4a$	$1.07 \pm 0.1bc$	$1.30 \pm 0.1b$	$0.90\pm0.1c$	$1.13 \pm 0.1bc$	$1.20 \pm 0.0bc$	$1.00 \pm 0.0 bc$
Quercetin	$4.77 \pm 0.2bc$	$6.50\pm0.4a$	$4.97 \pm 0.2 bc$	$6.67 \pm 0.4a$	$5.40 \pm 0.7b$	$4.67 \pm 0.2 bc$	$5.00 \pm 0.1 \text{bc}$	$4.37 \pm 0.2c$	$4.30\pm0.2c$	$4.73 \pm 0.1bc$
Flavonols	$27.63 \pm 1.7a$	$26.13 \pm 0.8a$	$20.67 \pm 1.1b$	$27.87 \pm 1.3a$	$27.93\pm4.0a$	$24.27 \pm 0.6ab$	$25.67 \pm 1.2a$	$27.83 \pm 2.2a$	$24.03~\pm~0.4ab$	$25.50 \pm 0.4 ab$
Total flavonoid phenolics	$115.47 \pm 9.3c$	$173.90 \pm 4.1a$	$97.17 \pm 4.5d$	$152.40\pm6.3b$	$96.83\pm6.1d$	$90.77 \pm 2.7d$	$90.67 \pm 3.0d$	$95.17 \pm 4.5d$	$89.53\pm2.3d$	93.87 ± 3.0d
Total non-anthocyanin										
phenolics	149.97 ± 11.2b	208.00 ± 4.4a	133.40 ± 5.4bc	220.83 ± 7.1a	133.23 ± 8.1bc	128.10 ± 3.5c	137.23 ± 4.1bc	$130.17 \pm 5.2c$	123.57 ± 3.5c	135.37 ± 4.7bc
Values are expressed as mea Franc.	in, \pm standard devia	tion ($n = 3$). Differer	nt letters in a row de	enote significant diff	erences among sam	ples ($P < 0.05$, Tuke	y's HSD test). CS, Cal	oernet Sauvignon; C	R, Carménère; ME, I	Merlot; CF, Cabernet

www.soci.org

quantified were (+)-catechin, (-)-epicatechin, procyanidin dimer and procyanidin gallate. The flavonols identified and quantified were myricetin-3-glucoside, myricetin-3-galactoside, myricetin-3-rutinoside, quercetin-3-glucoside, quercetin-3-galactoside, kaempferol-3-glucoside, kaempferol-3-galactoside and guercetin. All identified compounds were detected in all wines used in this study. Flavonoids were the most abundant fraction (from 66.1 to 83.6%) compared with non-flavonoids (from 16.4 to 33.9%), as also reported by other authors.^{25,30,31} The monovarietal wines differed in the concentration of phenolic compounds, observing that CF had the highest concentration of gallic and hydroxycinnamic acids. In addition, CF and its respective blends had the highest concentration of non-flavonoid phenolics. With regarding to (+)-catechin, CF and CR had the highest concentration; however, ME had the lowest concentration. In addition, flavanols were the major class of flavonoid compounds quantified in all wines evaluated. These compounds are important qualitative factors in red wine owing to their role in astringency and bitterness.^{6,8,32,33}

In the case of anthocyanins (Table 4), the glucosylated anthocyanins found included delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside and malvidin-3-glucoside. Peonidin-3-(6'-acetyl) glucoside and malvidin-3-(6'-acetyl) glucoside were the only acetylated anthocyanins found in this study, while peonidin-3-(6'-p-coumaroyl) glucoside and malvidin-3-(6'-p-coumaroyl) glucoside were the only coumaroylated anthocyanins found. All identified anthocyanins were detected in all wines examined. Among the monovarietal wines, CF had the highest concentration of glucosylated and coumaroylated anthocyanins. The group of monoglucosides represented the highest proportion of all anthocyanins, with values ranging from 81.2 to 84.3%. With respect to acylated and coumaroylated derivatives, the wine samples presented a similar proportion of both anthocyanins. The relative content of anthocyanins quantified in the present study is higher than those published by other authors.^{9,25,30} According to the results of the detailed polyphenolic analyses (Tables 3 and 4), there was a decrease in the differences in concentrations of the wine blends compared with the single base wines. This pattern can be observed in a better way through the PCA, which illustrates the relationship between the ten wines and the HPLC analysis of lowmolecular-weight phenolic compounds and anthocyanins (Fig. 2). PC1 and PC2 accounted for 80.3% of the total variation (61.6 and 18.7% respectively). PC1 was characterised by hydroxybenzoic and hydroxycinnamic acids and glucosylated and coumaroylated anthocyanins. PC2 was characterised by flavanols, flavonols and acetylated anthocyanins. As expected, all four base wines were significantly different from each other, corroborating the results provided by the analytical parameters (Table 2, Fig. 1).

Clearly, the oenological practice of blending produced a modification in the proportion and concentration of phenolic compounds, while the differences among the monovarietal wines decreased. Although the highest polyphenolic content in previous studies^{2,11} was found for wine blends rather than monovarietal wines, the reasons for the differing results in our study are not clear. Only the wine blends with a modifier wine having a high content of polyphenols, such as CF or CR, showed additive effects in increasing the polyphenol concentration in the final wine; however, blending, in general, produced a decrease in the concentration of phenolic compounds. These results regarding an apparent standardization of the wines with blending could be due to the proportion of CS used in the blend caused a decrease in the phenolic compounds.

-	
www.soci.org	

S (100)	CR (100)	ME (100)	CF (100)	CS-CR (70:30)	CS-ME (70:30)	CS-CF (70:30)	CS-CR (85:15)	CS-ME (85:15)	CS-CF (85:15)	
土 3.2b	39.32 ± 3.4cd	31.48 ± 2.9de	75.22 ± 5.8a	42.37 ± 2.9c	36.80 ± 1.4cd	26.36 土 1.3e	$37.10 \pm 1.3cd$	39.22 ± 3.9cd	33.47 ± 3.3cde	
土 2.5e	12.39 ± 1.4de	13.32 ± 2.4 de	$19.87 \pm 1.0cd$	$3.97 \pm 0.3f$	46.31 ± 4.5a	$21.54 \pm 3.1c$	$7.59 \pm 0.3ef$	14.68 ± 3.5 cde	29.42 ± 3.4b	_
土 4.5cd	$51.55 \pm 6.0bc$	$36.42 \pm 1.9d$	82.51 ± 1.0a	$56.61 \pm 9.8b$	38.37 ± 2.4 cd	33.93 ± 3.0d	$59.36~\pm~8.4b$	$36.74 \pm 2.4d$	38.88 ± 3.7cd	_
土 1.6a	$17.11 \pm 1.4bcd$	9.62 ± 3.1e	$20.05 \pm 1.2b$	$14.03 \pm 3.3bcde$	12.47 ± 1.8 de	9.03 ± 1.7e	19.15 ± 3.5bc	$12.92 \pm 0.8cde$	$10.64 \pm 0.9e$	_
土 3.2de	224.96 ± 0.9h	163.24 土 1.8i	443.76 ± 1.6a	$316.25 \pm 10.6b$	243.93 ± 3.6 fg	$227.78 \pm 2.6gh$	$290.77 \pm 6.9c$	$269.09 \pm 6.4d$	248.52 ± 11.0ef	_
土 14.9bcd 🔅	345.33 土 13.1ef	$254.08 \pm 12.2g$	641.41 ± 10.6a	$433.23 \pm 26.8b$	$377.88 \pm 13.6cde$	318.64 ± 11.6f	$413.97\ \pm\ 20.4bc$	$372.65 \pm 16.9cde$	360.93 ± 22.3def	
土 1.7b	7.40 ± 1.4ab	6.73 ± 0.3b	$8.10 \pm 2.6ab$	12.85 ± 3.2a	$8.66 \pm 2.1ab$	7.99 ± 1.1ab	8.06 ± 3.3ab	4.12 ± 0.3b	5.00 ± 1.0b	
土 1.9a	39.53 ± 3.3c	38.82 ± 3.4c	63.02 ± 2.9a	$57.96 \pm 4.9ab$	54.19 土 3.4ab	48.61 ± 2.1bc	$56.48 \pm 4.2ab$	$55.18 \pm 4.9ab$	50.15 ± 1.0b	
土 3.5ab	46.93 ± 4.7c	45.55 ± 3.7c	$71.12 \pm 5.5a$	$70.81 \pm 8.1a$	$62.85 \pm 5.5ab$	$56.60 \pm 3.2abc$	$64.54 \pm 7.5ab$	$59.30 \pm 5.2abc$	$55.15 \pm 2.0bc$	
± 0.0bc	$2.65 \pm 0.0bc$	$2.02\pm0.3cd$	3.86 ± 0.0a	4.14 ± 0.4a	$0.87 \pm 0.1ef$	2.90 ± 0.1b	1.31 ± 0.3de	$1.25 \pm 0.7 def$	$0.46 \pm 0.0f$	
土 0.8e	$14.65 \pm 0.2 \text{cd}$	11.16 ± 1.1de	44.37 ± 2.1a	19.05 ± 1.2b	11.67 ± 0.1de	11.38 ± 1.0de	16.13 ± 1.0bc	13.29 ± 1.9cde	12.18 ± 1.5de	
± 0.8 cd	17.30 ± 0.2c	13.18 ± 1.4 cd	48.23 ± 2.1a	23.19 ± 1.6b	$12.54 \pm 0.2d$	$14.28 \pm 1.1 cd$	17.44 ± 1.4c	$14.54 \pm 2.6 cd$	$12.64 \pm 1.5d$	
± 19.3bc	409.56 ± 18.1d	312.81 ± 17.3e	760.76 ± 18.2a	527.23 ± 36.5b	$453.27 \pm 19.3 cd$	389.52 ± 15.9d	495.95 ± 29.3bc	$446.49 \pm 24.7 cd$	$428.72 \pm 25.8 cd$	
ndard deviati	ion ($n = 3$). Differen	it letters in a row de	enote significant dil	fferences among sam	ples (<i>P</i> < 0.05, Tuke	y's HSD test). CS, Ca	abernet Sauvignon; (CR, Carménère; ME, I	Aerlot; CF, Cabernet	
	5(100) = 3.2b = 3.2b = 4.5cd = 4.5cd = 1.6a = 1.6a = 1.7b = 1.7b = 1.7b = 1.7b = 1.9a = 1.9a = 0.0bc = 0.0	$5(100)$ $CR(100)$ $\pm 3.2b$ $39.32 \pm 3.4cd$ $\pm 2.5e$ $12.39 \pm 1.4de$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $\pm 1.6a$ $17.11 \pm 1.4bcd$ $\pm 3.2de$ $224.96 \pm 0.9h$ $\pm 1.4.9bcd$ $345.33 \pm 13.1ef$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $\pm 1.9a$ $39.53 \pm 3.3c$ $\pm 1.9a$ $39.53 \pm 3.3c$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $\pm 0.8e$ $14.65 \pm 0.2cd$ $\pm 0.8cd$ $17.30 \pm 0.2cd$ $\pm 0.8cd$ $17.30 \pm 0.2c$ $\pm 19.3bc$ $409.56 \pm 18.1d$ $\pm 19.3bc$ $409.56 \pm 18.1d$ $\pm 19.3bc$ $409.56 \pm 18.1d$	$S(100)$ CR (100)ME (100) $\pm 3.2b$ $39.32 \pm 3.4cd$ $31.48 \pm 2.9de$ $\pm 2.5e$ $12.39 \pm 1.4de$ $31.32 \pm 2.4de$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $\pm 1.6a$ $17.11 \pm 1.4bcd$ $9.62 \pm 3.1e$ $\pm 1.2de$ $224.96 \pm 0.9h$ $163.24 \pm 1.8i$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $\pm 1.9a$ $39.53 \pm 4.7c$ $45.55 \pm 3.7c$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $2.02 \pm 0.3cd$ $\pm 0.0bc$ $2.65 \pm 0.2cd$ $11.16 \pm 1.1de$ $\pm 0.8e$ $14.65 \pm 0.2cd$ $11.16 \pm 1.4cd$ $\pm 0.8cd$ $17.30 \pm 0.2cd$ $13.18 \pm 1.4cd$ $\pm 0.8cd$ $17.30 \pm 0.2cd$ $13.18 \pm 1.4cd$ $\pm 19.3bc$ $409.56 \pm 18.1d$ $312.81 \pm 17.3e$ $\pm 19.3bc$ $409.56 \pm 18.1d$ $312.81 \pm 17.3e$	$5(100)$ CR (100)ME (100)CF (100) $\pm 3.2b$ $39.32 \pm 3.4cd$ $31.48 \pm 2.9de$ $75.22 \pm 5.8a$ $\pm 2.5e$ $12.39 \pm 1.4de$ $13.32 \pm 2.4de$ $19.87 \pm 1.0cd$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $82.51 \pm 1.0a$ $\pm 1.6a$ $17.11 \pm 1.4bcd$ $9.62 \pm 3.1e$ $20.05 \pm 1.2b$ $\pm 1.4.9bcd$ $31.31ef$ $254.08 \pm 12.2g$ $641.41 \pm 10.6a$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $8.10 \pm 2.6ab$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $8.10 \pm 2.6ab$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $2.02 \pm 0.3cd$ $3.86 \pm 0.0a$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $2.02 \pm 0.3cd$ $3.86 \pm 0.0a$ $\pm 0.8e$ $14.65 \pm 0.2cd$ $11.16 \pm 1.1de$ $44.37 \pm 2.1a$ $\pm 0.8e$ $17.30 \pm 0.2c$ $13.18 \pm 1.4cd$ $48.23 \pm 2.1a$ $\pm 0.8cd$ $17.30 \pm 0.2c$ $13.18 \pm 1.4cd$ $48.23 \pm 2.1a$ $\pm 19.3bc$ $409.56 \pm 18.1d$ <td>$5(100)$CR (100)ME (100)CF (100)CS-CR (70:30)$\pm 3.2b$$39.32 \pm 3.4cd$$31.48 \pm 2.9de$$75.22 \pm 5.8a$$42.37 \pm 2.9c$$\pm 4.5cd$$51.55 \pm 6.0bc$$36.42 \pm 1.9d$$82.51 \pm 1.0cd$$3.97 \pm 0.3f$$\pm 4.5cd$$51.55 \pm 6.0bc$$36.42 \pm 1.9d$$82.51 \pm 1.0a$$56.61 \pm 9.8b$$\pm 1.6a$$17.11 \pm 1.4bcd$$9.62 \pm 3.1e$$20.05 \pm 1.2b$$14.03 \pm 3.3bcde$$\pm 1.3ade$$23.33 \pm 13.1ef$$254.08 \pm 12.2g$$641.41 \pm 10.6a$$316.25 \pm 10.6b$$\pm 1.7b$$7.40 \pm 1.4ab$$6.73 \pm 0.3b$$8.10 \pm 2.6ab$$12.85 \pm 3.2a$$\pm 1.7b$$7.40 \pm 1.4ab$$6.73 \pm 0.3b$$8.10 \pm 2.6ab$$12.85 \pm 3.2a$$\pm 1.9bcd$$39.53 \pm 3.3c$$8.10 \pm 2.6ab$$12.85 \pm 3.2a$$\pm 1.9a$$39.53 \pm 3.3c$$8.322 \pm 3.4c$$63.02 \pm 2.9a$$41.926 \pm 4.9ab$$\pm 1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.4c$$63.02 \pm 2.9a$$7.96 \pm 4.9ab$$\pm 1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.4c$$63.02 \pm 2.9a$$7.96 \pm 4.9ab$$\pm 1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.4c$$63.02 \pm 2.9a$$70.81 \pm 8.1a$$\pm 2.0bcc$$2.65 \pm 0.0bc$$2.02 \pm 0.3cd$$3.16 \pm 2.5a$$70.81 \pm 8.1a$$\pm 0.0bc$$2.65 \pm 0.0bc$$2.02 \pm 0.3cd$$3.16 \pm 2.5a$$70.81 \pm 8.1a$$\pm 0.0bc$$14.65 \pm 0.2cd$$11.16 \pm 1.1de$$44.37 \pm 2.1a$$19.05 \pm 1.2b$$\pm 0.0bc$$10.65 \pm 1.31b \pm 1.4cd$$48.23 \pm 2.1a$$23.19 \pm 1.6b$$\pm 0.8cd$$17.30 \pm 0.2cd$$13.18 \pm 1$</td> <td>5(100)CR(100)ME (100)CF (100)CS-CR (70:30)CS-ME (70:30)$\pm 3.2b$$39.32 \pm 3.4cd$$31.48 \pm 2.9de$$75.22 \pm 5.8a$$42.37 \pm 2.9c$$36.80 \pm 1.4cd$$\pm 2.5e$$12.39 \pm 1.4de$$31.32 \pm 2.4de$$19.87 \pm 1.0cd$$3.97 \pm 0.3f$$46.31 \pm 4.5a$$\pm 4.5cd$$51.55 \pm 6.0bc$$36.42 \pm 1.9d$$82.51 \pm 1.0a$$56.61 \pm 9.8b$$38.37 \pm 2.4cd$$\pm 1.6a$$17.11 \pm 1.4bcd$$9.62 \pm 3.1e$$20.05 \pm 1.2b$$14.03 \pm 3.3bcde$$12.47 \pm 1.8de$$\pm 1.6a$$17.11 \pm 1.4bcd$$9.62 \pm 3.1e$$20.05 \pm 1.2b$$14.03 \pm 3.3bcde$$12.47 \pm 1.8de$$\pm 1.7b$$7.40 \pm 1.4abcd$$9.62 \pm 3.1e$$20.05 \pm 1.2b$$14.03 \pm 3.3bcde$$23.788 \pm 13.6cde$$\pm 1.7b$$7.40 \pm 1.4abcd$$9.62 \pm 3.1c$$20.05 \pm 1.2b$$14.03 \pm 3.3bcde$$23.738 \pm 13.6cde$$\pm 1.7b$$7.40 \pm 1.4ab$$6.73 \pm 0.3b$$8.10 \pm 2.6ab$$37.52 \pm 3.2a$$8.66 \pm 2.1ab$$\pm 1.9bccd$$39.53 \pm 3.3c$$33.52 \pm 3.7c$$71.12 \pm 5.5a$$70.81 \pm 8.1a$$62.85 \pm 5.5ab$$\pm 1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.7c$$71.12 \pm 5.5a$$70.81 \pm 8.1a$$62.85 \pm 5.5ab$$\pm 0.0bc$$2.65 \pm 0.0bc$$2.02 \pm 0.3cd$$4.337 \pm 2.1a$$12.85 \pm 3.2a$$8.66 \pm 2.1ab$$\pm 1.9a$$39.53 \pm 3.3cd$$11.6a$$12.87 \pm 3.2a$$8.66 \pm 2.1ab$$46.93 \pm 4.7c$$45.55 \pm 3.7c$$71.12 \pm 5.5a$$\pm 1.9a$$20.56 \pm 4.03 b$$45.55 \pm 3.7c$$71.12 \pm 5.5a$$70.81 \pm 8.1a$$62.85 \pm 5.2ab$</td> <td>5 (100)CF (100)CF (100)CF (100)C5-CF (70:30)C5-CF (70:30)C5-CF (70:30)C5-CF (70:30)$\pm 3.2b$$39.32 \pm 3.4cd$$31.48 \pm 2.9de$$75.22 \pm 5.8a$$42.37 \pm 2.9c$$36.80 \pm 1.4cd$$26.36 \pm 1.3e$$\pm 4.5cd$$51.55 \pm 6.0bc$$36.42 \pm 1.9d$$13.32 \pm 2.4de$$19.87 \pm 1.0cd$$3.97 \pm 0.3f$$46.31 \pm 4.5a$$21.54 \pm 3.1c$$\pm 4.5cd$$51.55 \pm 6.0bc$$36.42 \pm 1.9d$$82.51 \pm 1.0a$$56.61 \pm 9.8b$$38.37 \pm 2.4cd$$33.93 \pm 3.0d$$\pm 1.1da$$17.11 \pm 1.4bcd$$9.62 \pm 3.1e$$20.05 \pm 1.2b$$14.03 \pm 3.3bcde$$12.47 \pm 1.8de$$9.03 \pm 1.7e$$\pm 3.2de$$21.49bcd$$36.42 \pm 1.3b$$43.76 \pm 1.6a$$316.52 \pm 10.6b$$237.38 \pm 3.36fg$$21.79 \pm 2.4cd$$\pm 1.1.7b$$7.40 \pm 1.4abcd$$65.3 \pm 0.3bb$$8.10 \pm 2.6bb$$12.47 \pm 1.8de$$9.03 \pm 1.7e$$\pm 1.1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.2d$$316.24 \pm 1.8d$$2.926 \pm 2.1ab$$7.99 \pm 1.1ab$$\pm 1.1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.2d$$31.72 \pm 5.5a$$7.96 \pm 4.9ab$$7.92 \pm 2.4dc$$32.92 \pm 2.6db$$\pm 1.9a$$39.53 \pm 3.3c$$38.82 \pm 3.7c$$71.12 \pm 5.5a$$70.81 \pm 8.1a$$62.85 \pm 5.5ab$$7.96 \pm 2.90 \pm 0.1b$$\pm 1.9a$$46.93 \pm 4.7c$$31.84 \pm 1.1db$$43.75 \pm 1.2b$$7.94 \pm 0.2d$$11.6f \pm 2.1bc$$\pm 1.9a$$39.53 \pm 3.3cd$$46.93 \pm 4.7c$$38.84 \pm 0.2db$$11.64 \pm 0.1db$$11.65 \pm 0.2db$$11.66 \pm 2.2db$$\pm 1.9a$$46.93 \pm 4.7c$$38.82 \pm 3.7c$$71$</td> <td>5 (100)CR (100)CF (100)CF (100)CS-CR (70:30)CS-CF (70:30)CS-CF (70:30)CS-CR (85:15)$\pm 3.22 \pm 3.4cd$$31.48 \pm 2.04e$$75.22 \pm 5.8a$$42.37 \pm 2.9c$$36.30 \pm 1.4cd$$26.36 \pm 1.3e$$37.10 \pm 1.3cd$$\pm 2.5ce$$12.39 \pm 1.44e$$13.48 \pm 2.04e$$75.22 \pm 5.8a$$42.37 \pm 2.9c$$36.30 \pm 1.4cd$$26.36 \pm 1.3e$$37.10 \pm 1.3cd$$\pm 4.5cd$$31.32 \pm 2.4de$$31.48 \pm 2.04e$$19.37 \pm 1.1cd$$3.55.4 \pm 1.9d$$33.37 \pm 2.4dcd$$33.33 \pm 3.3dd$$53.56 \pm 3.3dc$$\pm 1.5bc$$35.33 \pm 13.1ef$$23.34 \pm 1.8i$$43.35 \pm 11.06a$$33.33 \pm 2.6dc$$23.33 \pm 1.7e$$13.99 \pm 3.3dc$$\pm 1.7bc$$34.33 \pm 13.1ef$$23.40 \pm 1.20$$43.37 \pm 3.2dcd$$243.33 \pm 1.7e$$13.92 \pm 2.6db$$290.77 \pm 5.9c$$\pm 1.7bc$$34.53 \pm 1.31ef$$23.40 \pm 1.20$$43.37 \pm 2.6db$$33.33 \pm 2.6dc$$23.33 \pm 1.7e$$13.97 \pm 2.04bc$$\pm 1.7bc$$34.33 \pm 1.31ef$$23.40 \pm 1.20$$43.37 \pm 3.2dcd$$21.32 \pm 3.2dcd$$21.36 \pm 2.14bc$$30.77 \pm 5.9c$$\pm 1.7bc$$34.33 \pm 1.31ef$$23.40 \pm 1.22$$43.37 \pm 2.64b$$33.32 \pm 2.64b$$290.77 \pm 5.9c$$\pm 1.7bc$$34.33 \pm 1.31ef$$23.40 \pm 1.22$$43.37 \pm 2.64b$$33.32 \pm 2.64b$$30.77 \pm 5.9c$$\pm 1.7bc$$34.33 \pm 1.31ef$$23.40 \pm 1.23$$43.37 \pm 2.64b$$33.32 \pm 2.64b$$43.64 \pm 7.54b$$\pm 1.9bc$$39.53 \pm 3.32c$$38.52 \pm 3.76c$$7.99 \pm 1.1ab$$43.64 \pm 7.54b$$\pm 1.9bc$$46.31 \pm 4.72a$$3.32 \pm 0.2$</td> <td>5 (100) CR (100) ME (100) CF (100) CF (70:30) C5 CF (70:30) C5 CF (70:30) C5 CR (85:15) C5 ME (85:15) 2 3 2 2 3 3 2 3 3 4 2 3 4 2 3 2 1 3 4 8 2 9 0 6 7 3 2 2 4 3 6 8 1 4 4 6 7 3 2 4 4 6 3 1 4 4 5 7 2 3 1 3 4 3 3 3 3 3 3 3 3 3 6 3 4 3 5 4 2 4 4 3 3 3 3 3 3 3 3 3 6 3 4 3 5 4 6 4 4 3 5 6 6 6 7 3 4 2 4 4 3 3 3 3 3 3 3 3 3 3 3 3 3 6 7 4 3 3 3 4 2 4 6 7 1 1 4 1 4 b c 4 9 6 2 4 3 3 3 7 4 5 4 7 4 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 6 7 4 5 2 4 6 6 4 4 4 3 5 6 6 6 7 3 4 4 3 7 6 4 1 6 0 3 4 4 3 7 6 4 1 6 8 3 5 1 4 2 4 6 3 1 4 4 3 5 6 6 7 3 4 4 3 7 6 4 1 8 6 3 3 1 6 5 2 4 1 8 0 4 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 6 4 4 1 6 6 4 4 4 3 7 6 4 6 4 4 4 3 7 6 4 6 4 1 4 1 6 0 6 4 4 4 3 7 6 4 6 4 4 1 4 6 6 6 4 4 4 3 7 6 8 6 6 4 4 4 3 6 6 4 4 3 6 6 4 4 4 3 6 6 4 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 4 3 6 6 4 4 3 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 6</td> <td>(100)$(R(100)$$(R(100)$$(F(10)$$(F(100)$<t< td=""></t<></td>	$5(100)$ CR (100)ME (100)CF (100)CS-CR (70:30) $\pm 3.2b$ $39.32 \pm 3.4cd$ $31.48 \pm 2.9de$ $75.22 \pm 5.8a$ $42.37 \pm 2.9c$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $82.51 \pm 1.0cd$ $3.97 \pm 0.3f$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $82.51 \pm 1.0a$ $56.61 \pm 9.8b$ $\pm 1.6a$ $17.11 \pm 1.4bcd$ $9.62 \pm 3.1e$ $20.05 \pm 1.2b$ $14.03 \pm 3.3bcde$ $\pm 1.3ade$ $23.33 \pm 13.1ef$ $254.08 \pm 12.2g$ $641.41 \pm 10.6a$ $316.25 \pm 10.6b$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $8.10 \pm 2.6ab$ $12.85 \pm 3.2a$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $8.10 \pm 2.6ab$ $12.85 \pm 3.2a$ $\pm 1.9bcd$ $39.53 \pm 3.3c$ $8.10 \pm 2.6ab$ $12.85 \pm 3.2a$ $\pm 1.9a$ $39.53 \pm 3.3c$ $8.322 \pm 3.4c$ $63.02 \pm 2.9a$ $41.926 \pm 4.9ab$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $7.96 \pm 4.9ab$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $7.96 \pm 4.9ab$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.4c$ $63.02 \pm 2.9a$ $70.81 \pm 8.1a$ $\pm 2.0bcc$ $2.65 \pm 0.0bc$ $2.02 \pm 0.3cd$ $3.16 \pm 2.5a$ $70.81 \pm 8.1a$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $2.02 \pm 0.3cd$ $3.16 \pm 2.5a$ $70.81 \pm 8.1a$ $\pm 0.0bc$ $14.65 \pm 0.2cd$ $11.16 \pm 1.1de$ $44.37 \pm 2.1a$ $19.05 \pm 1.2b$ $\pm 0.0bc$ $10.65 \pm 1.31b \pm 1.4cd$ $48.23 \pm 2.1a$ $23.19 \pm 1.6b$ $\pm 0.8cd$ $17.30 \pm 0.2cd$ 13.18 ± 1	5(100)CR(100)ME (100)CF (100)CS-CR (70:30)CS-ME (70:30) $\pm 3.2b$ $39.32 \pm 3.4cd$ $31.48 \pm 2.9de$ $75.22 \pm 5.8a$ $42.37 \pm 2.9c$ $36.80 \pm 1.4cd$ $\pm 2.5e$ $12.39 \pm 1.4de$ $31.32 \pm 2.4de$ $19.87 \pm 1.0cd$ $3.97 \pm 0.3f$ $46.31 \pm 4.5a$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $82.51 \pm 1.0a$ $56.61 \pm 9.8b$ $38.37 \pm 2.4cd$ $\pm 1.6a$ $17.11 \pm 1.4bcd$ $9.62 \pm 3.1e$ $20.05 \pm 1.2b$ $14.03 \pm 3.3bcde$ $12.47 \pm 1.8de$ $\pm 1.6a$ $17.11 \pm 1.4bcd$ $9.62 \pm 3.1e$ $20.05 \pm 1.2b$ $14.03 \pm 3.3bcde$ $12.47 \pm 1.8de$ $\pm 1.7b$ $7.40 \pm 1.4abcd$ $9.62 \pm 3.1e$ $20.05 \pm 1.2b$ $14.03 \pm 3.3bcde$ $23.788 \pm 13.6cde$ $\pm 1.7b$ $7.40 \pm 1.4abcd$ $9.62 \pm 3.1c$ $20.05 \pm 1.2b$ $14.03 \pm 3.3bcde$ $23.738 \pm 13.6cde$ $\pm 1.7b$ $7.40 \pm 1.4ab$ $6.73 \pm 0.3b$ $8.10 \pm 2.6ab$ $37.52 \pm 3.2a$ $8.66 \pm 2.1ab$ $\pm 1.9bccd$ $39.53 \pm 3.3c$ $33.52 \pm 3.7c$ $71.12 \pm 5.5a$ $70.81 \pm 8.1a$ $62.85 \pm 5.5ab$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.7c$ $71.12 \pm 5.5a$ $70.81 \pm 8.1a$ $62.85 \pm 5.5ab$ $\pm 0.0bc$ $2.65 \pm 0.0bc$ $2.02 \pm 0.3cd$ $4.337 \pm 2.1a$ $12.85 \pm 3.2a$ $8.66 \pm 2.1ab$ $\pm 1.9a$ $39.53 \pm 3.3cd$ $11.6a$ $12.87 \pm 3.2a$ $8.66 \pm 2.1ab$ $46.93 \pm 4.7c$ $45.55 \pm 3.7c$ $71.12 \pm 5.5a$ $\pm 1.9a$ $20.56 \pm 4.03 b$ $45.55 \pm 3.7c$ $71.12 \pm 5.5a$ $70.81 \pm 8.1a$ $62.85 \pm 5.2ab$	5 (100)CF (100)CF (100)CF (100)C5-CF (70:30)C5-CF (70:30)C5-CF (70:30)C5-CF (70:30) $\pm 3.2b$ $39.32 \pm 3.4cd$ $31.48 \pm 2.9de$ $75.22 \pm 5.8a$ $42.37 \pm 2.9c$ $36.80 \pm 1.4cd$ $26.36 \pm 1.3e$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $13.32 \pm 2.4de$ $19.87 \pm 1.0cd$ $3.97 \pm 0.3f$ $46.31 \pm 4.5a$ $21.54 \pm 3.1c$ $\pm 4.5cd$ $51.55 \pm 6.0bc$ $36.42 \pm 1.9d$ $82.51 \pm 1.0a$ $56.61 \pm 9.8b$ $38.37 \pm 2.4cd$ $33.93 \pm 3.0d$ $\pm 1.1da$ $17.11 \pm 1.4bcd$ $9.62 \pm 3.1e$ $20.05 \pm 1.2b$ $14.03 \pm 3.3bcde$ $12.47 \pm 1.8de$ $9.03 \pm 1.7e$ $\pm 3.2de$ $21.49bcd$ $36.42 \pm 1.3b$ $43.76 \pm 1.6a$ $316.52 \pm 10.6b$ $237.38 \pm 3.36fg$ $21.79 \pm 2.4cd$ $\pm 1.1.7b$ $7.40 \pm 1.4abcd$ $65.3 \pm 0.3bb$ $8.10 \pm 2.6bb$ $12.47 \pm 1.8de$ $9.03 \pm 1.7e$ $\pm 1.1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.2d$ $316.24 \pm 1.8d$ $2.926 \pm 2.1ab$ $7.99 \pm 1.1ab$ $\pm 1.1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.2d$ $31.72 \pm 5.5a$ $7.96 \pm 4.9ab$ $7.92 \pm 2.4dc$ $32.92 \pm 2.6db$ $\pm 1.9a$ $39.53 \pm 3.3c$ $38.82 \pm 3.7c$ $71.12 \pm 5.5a$ $70.81 \pm 8.1a$ $62.85 \pm 5.5ab$ $7.96 \pm 2.90 \pm 0.1b$ $\pm 1.9a$ $46.93 \pm 4.7c$ $31.84 \pm 1.1db$ $43.75 \pm 1.2b$ $7.94 \pm 0.2d$ $11.6f \pm 2.1bc$ $\pm 1.9a$ $39.53 \pm 3.3cd$ $46.93 \pm 4.7c$ $38.84 \pm 0.2db$ $11.64 \pm 0.1db$ $11.65 \pm 0.2db$ $11.66 \pm 2.2db$ $\pm 1.9a$ $46.93 \pm 4.7c$ $38.82 \pm 3.7c$ 71	5 (100)CR (100)CF (100)CF (100)CS-CR (70:30)CS-CF (70:30)CS-CF (70:30)CS-CR (85:15) $\pm 3.22 \pm 3.4cd$ $31.48 \pm 2.04e$ $75.22 \pm 5.8a$ $42.37 \pm 2.9c$ $36.30 \pm 1.4cd$ $26.36 \pm 1.3e$ $37.10 \pm 1.3cd$ $\pm 2.5ce$ $12.39 \pm 1.44e$ $13.48 \pm 2.04e$ $75.22 \pm 5.8a$ $42.37 \pm 2.9c$ $36.30 \pm 1.4cd$ $26.36 \pm 1.3e$ $37.10 \pm 1.3cd$ $\pm 4.5cd$ $31.32 \pm 2.4de$ $31.48 \pm 2.04e$ $19.37 \pm 1.1cd$ $3.55.4 \pm 1.9d$ $33.37 \pm 2.4dcd$ $33.33 \pm 3.3dd$ $53.56 \pm 3.3dc$ $\pm 1.5bc$ $35.33 \pm 13.1ef$ $23.34 \pm 1.8i$ $43.35 \pm 11.06a$ $33.33 \pm 2.6dc$ $23.33 \pm 1.7e$ $13.99 \pm 3.3dc$ $\pm 1.7bc$ $34.33 \pm 13.1ef$ 23.40 ± 1.20 $43.37 \pm 3.2dcd$ $243.33 \pm 1.7e$ $13.92 \pm 2.6db$ $290.77 \pm 5.9c$ $\pm 1.7bc$ $34.53 \pm 1.31ef$ 23.40 ± 1.20 $43.37 \pm 2.6db$ $33.33 \pm 2.6dc$ $23.33 \pm 1.7e$ $13.97 \pm 2.04bc$ $\pm 1.7bc$ $34.33 \pm 1.31ef$ 23.40 ± 1.20 $43.37 \pm 3.2dcd$ $21.32 \pm 3.2dcd$ $21.36 \pm 2.14bc$ $30.77 \pm 5.9c$ $\pm 1.7bc$ $34.33 \pm 1.31ef$ 23.40 ± 1.22 $43.37 \pm 2.64b$ $33.32 \pm 2.64b$ $290.77 \pm 5.9c$ $\pm 1.7bc$ $34.33 \pm 1.31ef$ 23.40 ± 1.22 $43.37 \pm 2.64b$ $33.32 \pm 2.64b$ $30.77 \pm 5.9c$ $\pm 1.7bc$ $34.33 \pm 1.31ef$ 23.40 ± 1.23 $43.37 \pm 2.64b$ $33.32 \pm 2.64b$ $43.64 \pm 7.54b$ $\pm 1.9bc$ $39.53 \pm 3.32c$ $38.52 \pm 3.76c$ $7.99 \pm 1.1ab$ $43.64 \pm 7.54b$ $\pm 1.9bc$ $46.31 \pm 4.72a$ 3.32 ± 0.2	5 (100) CR (100) ME (100) CF (100) CF (70:30) C5 CF (70:30) C5 CF (70:30) C5 CR (85:15) C5 ME (85:15) 2 3 2 2 3 3 2 3 3 4 2 3 4 2 3 2 1 3 4 8 2 9 0 6 7 3 2 2 4 3 6 8 1 4 4 6 7 3 2 4 4 6 3 1 4 4 5 7 2 3 1 3 4 3 3 3 3 3 3 3 3 3 6 3 4 3 5 4 2 4 4 3 3 3 3 3 3 3 3 3 6 3 4 3 5 4 6 4 4 3 5 6 6 6 7 3 4 2 4 4 3 3 3 3 3 3 3 3 3 3 3 3 3 6 7 4 3 3 3 4 2 4 6 7 1 1 4 1 4 b c 4 9 6 2 4 3 3 3 7 4 5 4 7 4 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 6 7 4 5 2 4 6 6 4 4 4 3 5 6 6 6 7 3 4 4 3 7 6 4 1 6 0 3 4 4 3 7 6 4 1 6 8 3 5 1 4 2 4 6 3 1 4 4 3 5 6 6 7 3 4 4 3 7 6 4 1 8 6 3 3 1 6 5 2 4 1 8 0 4 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 6 4 4 1 6 6 4 4 4 3 7 6 4 6 4 4 4 3 7 6 4 6 4 1 4 1 6 0 6 4 4 4 3 7 6 4 6 4 4 1 4 6 6 6 4 4 4 3 7 6 8 6 6 4 4 4 3 6 6 4 4 3 6 6 4 4 4 3 6 6 4 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 3 6 6 4 4 4 3 6 6 4 4 3 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 4 4 4 3 6 6 6 6	(100) $(R(100)$ $(R(100)$ $(F(100)$ $(F(10)$ $(F(100)$ <t< td=""></t<>

ni hodin



Figure 2. PCA comparing HPLC anthocyanin and phenolic compounds from wine samples. CS, Cabernet Sauvignon; CR, Carménère; ME, Merlot; CF, Cabernet Franc.

in the resulting blends, even if the blend used was done with CF or CR, which had a high concentration of phenolic compounds. This trend suggests that the smaller differences among wine blends with regard to certain parameters are due to a suppression or masking effect, producing blends that are less different from the monovarietal base wine.¹¹

Figure 3 displays the monomeric, oligomeric and polymeric flavan-3-ol proportions in the wine samples. Sun et al.²¹ indicated that the monomeric fraction consists only of (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-O-gallate, whereas the oligomeric fraction is formed by proanthocyanidins of degree of polymerization ranging from 2 to 12-15, and the polymeric fraction is composed of polymeric proanthocyanidins (more than 12-15 units). The relative percentages of the proanthocyanidin fraction in the wine blends were as follows: flavan-3-ol polymers (86.1-91.4%), followed by flavan-3-ol oligomers (8.7-11.7%) and a lower percentage of flavan-3-ol monomers (1.1-2.3%). These results are in agreement with those reported by other authors for red wines.^{25,34,35} The wines blended with ME showed a higher concentration of monomeric and oligomeric flavan-3-ol fractions. Although ME had a lower concentration of monomers of flavanols such as (+)-catechin and (-)-epicatechin (Table 3), the highest concentration of monomeric proanthocyanidins in blended wines could be due to an additive effect of CS. Moreover, the higher content of oligomeric proanthocyanidins may be due to the higher content of procyanidin dimer in ME that caused this trend (Table 3). This pattern was also observed for the CF wine, which contained a high concentration of oligomers and polymers of flavan-3ol, though blending with this monovarietal wine produced a decrease in the concentration of the oligomeric and polymeric fractions. This decrease could be due to the higher proportion of CS used, which had a lower concentration of oligomeric and polymeric proanthocyanidins, causing a suppression effect in the respective blend. Also, it could produce condensation reactions between proanthocyanidins and other compounds, but this research was done only in one stage and not over the wine evolution, so further studies should take into account the time factor and the implications of the condensation reaction of these compounds in blended wine. Although the four monovarietal



Figure 3. (A) Monomeric, (B) oligomeric and (C) polymeric fractions of proanthocyanidins in wine samples. Different letters denote significant differences among wine samples (P < 0.05, Tukey's HSD test). CS, Cabernet Sauvignon; CR, Carménère; ME, Merlot; CF, Cabernet Franc.

wines showed differences in the contents of the three fractions, blending produced a decrease in concentration differences among the wine varietals, which is particularly important in the case of the oligomeric and polymeric fractions. Moreover, the pooled data showed that blending produced only a change in the



Figure 4. PCA showing descriptive analysis scores for wine samples (n = 12 judges $\times 2$ replicates). CS, Cabernet Sauvignon; CR, Carménère; ME, Merlot; CF, Cabernet Franc.

concentration but not in the proportions of the different fractions. Other interesting results showed that, when blended with such wines as ME or CF, with a higher content of oligomers and polymers of flavan-3-ols, the CS base wine could produce an additive effect, increasing the concentration of these fractions in the final wine. The various fractions of flavan-3-ols could produce differences in sensory properties such as astringency and bitterness, thus influencing the wine quality,^{7,8} a result that suggests that blending would impact the mouthfeel characteristics of wines by modifying these parameters.

Sensory and mouthfeel characteristics of wines

A sensory evaluation was conducted to complement the chemical analysis and determine whether the blending of wines produced changes in their organoleptic characteristics. Table 5 shows the parameters evaluated by the judges with their respective scores. There were no differences in perceived astringency and

bitterness. Regarding the chemical results of overall phenolic composition (Table 2), the differences among the blends were lower than among the monovarietals, which could be a reason why no significant differences in astringency and bitterness were found, although there was no difference among monovarietal wines that differed clearly in the concentration of total tannins (Table 2). Based on the various flavan-3-ol fractions obtained by separation through Sep-Pak C₁₈ cartridges (Fig. 3), differences were observed among the wine blends, with an increase in the polymeric and oligomeric fractions in some cases, which may cause an increase in such mouthfeel properties as astringency and bitterness. According to Vidal et al.,³⁶ astringency is augmented when proanthocyanidin polymerisation increases. In this case, the increase in the concentration of flavan-3-ol polymers must have caused an increase in the sensory perception of astringency; the same should occur with the case of oligomers, which should be more bitter than astringent,^{8,36} but the sensory panel did not perceive these changes. Wine tasting depends on other

Table 5. Mou	thfeel attributes of wi	ine samples					
Wine	Abrasiveness (%)	Hardness (%)	Adhesiveness (%)	Dryness (%)	Mouthcoating (%)	Astringency	Bitterness
CS (100)	12.40b	18.24bc	24.34b	38.56b	6.46f	10.62a	7.61a
CR (100)	6.81c	19.71bc	19.82c	40.33a	13.34e	10.81a	7.77a
ME (100)	6.19c	6.05e	5.87e	40.92a	40.97a	10.69a	7.43a
CF (100)	5.32c	5.27e	15.74c	31.69b	41.98a	11.98a	8.26a
CS-CR (70:30)	19.97a	13.44d	13.39d	20.12d	33.06b	11.36a	7.62a
CS-ME (70:30)	5.99c	17.88b	23.11b	29.39c	23.63d	11.37a	7.98a
CS-CF (70:30)	9.52b	4.72e	33.34a	19.08d	33.34b	11.11a	7.76a
CS-CR (85:15)	17.67a	11.77d	17.86c	29.25c	23.44d	11.77a	7.72a
CS-ME (85:15)	11.32b	22.15a	11.14d	33.42b	21.97d	11.31a	7.31a
CS-CF (85:15)	10.61b	15.72c	15.64c	26.06c	31.98c	11.55a	7.80a

Values are expressed as mean score of 12 judges (*n* = 2). Different letters in a column denote significant differences among samples (*P* < 0.05, Tukey's HSD test). CS, Cabernet Sauvignon, CR, Carménère; ME, Merlot; CF, Cabernet Franc.

Table 6. Cor	relation ma	itrix of com	positional	measurement	s with descrip	tive analysis	of mouthfee	l attributes in m	onovarietal a	nd blended wind	SS			
	ТР	Ц	TA	Monomers	Oligomers	Polymers	Flavanols	Abrasiveness	Hardness	Adhesiveness	Dryness	Mouthcoating	Astringency	Bitterness
TP	1.000			I	I	I	I	I	I	Ι	I	Ι	Ι	Ι
Ħ	0.5858	1.000	I		I	I	Ι							
TA	0.0644	-0.3028	1.000		Ι	Ι	Ι				Ι			I
Monomers	-0.5584^{*}	-0.3881^{*}	-0.5794^{*}	1.000	Ι	Ι	Ι			I	Ι			
Oligomers	-0.2446	0.1391	-0.7652*	0.5982*	1.000	Ι	Ι			I	Ι			
Polymers	0.3117	0.5761*	-0.6429^{*}	0.0020	0.5316*	1.000				Ι	Ι	I	I	
Flavanols	0.5445*	0.4216*	0.4515*	-0.8419^{*}	-0.4545^{*}	0.2601	1.000							
Abrasiveness	0.0211	-0.3545	0.1196	0.2656	-0.1826	-0.4447*	-0.5477^{*}	1.000			Ι			I
Hardness	-0.2005	-0.4735^{*}	0.3857*	0.1755	-0.2614	-0.3059	-0.0750	0.0812	1.000		Ι	I		I
Adhesiveness	0.2740	0.2373	-0.0487	0.0293	-0.1484	-0.3117	-0.1022	0.0277	-0.1966	1.000				
Dryness	-0.2768	0.1059	-0.1198	-0.0699	0.0515	0.4535*	0.4206^{*}	-0.6429^{*}	0.0992	-0.3975*	1.000			
Mouthcoating	1 0.1463	0.5432*	-0.7004^{*}	0.0795	0.6089*	0.5994*	-0.1723	-0.1388	-0.6924^{*}	0.0348	-0.1134	1.000		I
Astringency	0.2275	-0.0769	-0.1596	0.0058	0.0684	0.2429	-0.0041	0.1263	-0.0512	-0.0096	-0.1260	0.2021	1.000	
Bitterness	0.1357	0.2652	-0.0478	-0.1641	0.1072	0.0818	0.0566	-0.0681	-0.0611	0.0759	-0.0292	0.0599	-0.3579	1.000
*Significance	at <i>P</i> < 0.05.	TP, total pł	henols; TT, t	total tannins; T	A, total anthc	ocyanins.								

factors such as alcohol level, acidity, aromatic intensity and other parameters, which can influence these sensations.^{37,38} However, these chemical parameters were similar for all wines studied (Table 2), which shows that wine blending did not produce changes in astringency and bitterness, and even the perceived difference in the amount of wine modifier would not impact these characteristics.

Although no differences were observed in bitterness and astringency, a different result was seen when analysing the mouthfeel descriptors. For the term 'abrasiveness', the CS (100) wine presented a higher value than ME (100) and CR (100), with CF (100) presenting the lowest value. Blending with CF and CR produced an additive effect for this attribute. In the case of 'hardness', the CS and CR wines showed an additive effect with regard to increasing this sensation in the wine blends. For the term 'dryness', all monovarietal wines presented high values, and blending produced a decrease in this sensation. No clear relation was observed for 'adhesiveness'.

In general terms, the monovarietal wines presented differences in the values of the mouthfeel properties, with the CS wines showing higher values of 'hardness', 'dryness' and 'adhesiveness' and lower values of 'mouthcoating'. CR was less abrasive and more dry; in contrast, ME and CF were less abrasive, less harsh, more dry and had more mouthcoating sensation.

A PCA was performed to examine the interrelationships between the mouthfeel variables (Fig. 4). PC1 and PC2, with eigenvalues > 1, explained 62.0% of the total variation (36.4 and 25.6% respectively). PC3 and PC4 explained another 13.9 and 10.8% of the variation respectively (data not shown). PC1 was most heavily loaded in the positive direction with 'dryness' and 'bitterness' and in the negative direction with 'adhesiveness' and 'astringency'. PC2 was positively loaded with 'mouthcoating' and negatively loaded with 'abrasiveness' and 'hardness'.

Predictably, all four base wines were significantly different from each other and were located outside the central region. The distribution of wines on the PCA illustrates that CS (100) and CR (100) were located in the right and lower part of the plot, which was dominated by the descriptor 'hardness'. The wines blended with ME and the CS-CF (85:15) wine were located near the descriptors 'hardness', 'dryness' and 'bitterness'. The wines blended with CR presented a higher relationship with the descriptors 'adhesiveness' and 'abrasiveness'. Lastly, the wine blended with CF in a proportion of 30% and the monovarietal CF (100) wine were located in the left and upper part of the plot, which was dominated by the descriptors 'mouthcoating' and 'astringency', particularly the CS-CF (70:30) wine, which was interestingly different compared with base wine CS. The high relationship between astringency and the CF (100) and CS-CF (70:30) wines could be due to the higher concentration of polymeric proanthocyanidins and total tannins in these wines (Table 2, Fig. 3).

Although there were no differences in general astringency and bitterness (Table 5), these data confirm that the blending of wine produces changes in astringency quality descriptors, which, in some cases, depend on the amount of wine modifier used in the blend. Clearly, the wines blended with ME and CR showed descriptors grouped in harsh and drying terms, which are negative hedonic groups that suggest aspects of excessive unbalanced astringency, excessive roughness and/or bitterness and associated green flavour notes (Table 1). In contrast, the wines blended with CF showed descriptors grouped in complex terms, which are a positive hedonic group consisting of a pleasing astringent sensation, flavour and balanced acidity.²⁶

Correlations were determined for the analytical data and mouthfeel attributes of the wines to understand the relationships among the wine components (Table 6). Total phenols were positively correlated with total tannins and flavanols and negatively correlated with flavan-3-ol monomers. There was a moderately strong correlation between tannins and flavan-3-ol polymers. There were moderately strong correlations between total anthocyanins and proanthocyanidin fractions. In that case, the total anthocyanins were negatively correlated with the monomeric, oligomeric and polymeric proanthocyanidins. Relating to the mouthfeel attributes, specifically, the 'mouthcoating' descriptor presented a moderately strong correlation in a positive way with total tannins, flavan-3-ol oligomers and flavan-3-ol polymers and in a negative way with total anthocyanins and 'hardness'. The blending with CF produced an increase in the mouthcoating sensation that was mainly due to the highest content of tannins and flavan-3-ol polymers, which was confirmed with this analysis.

Astringency is one of the most important sensory characteristics of red wine,^{39,40} so understanding its presence is important. Indeed, balanced astringency is related to high-quality wines; therefore, if the astringency is too low, the wine could be considered flat and dull.⁶ All evaluated wines were astringent, but the differentiation in the type of perceived astringency may be important for wine consumers and the acceptance of these types of wines. Although it is difficult to note which monovarietal wine offers best attributes for blending with CS, clearly the blending produced differences in the type of astringency. Notably, the largest proportion of CS in the blend caused standardization in respective blends. This aspect could be in agreement with the technical decision to blend a wine in a winery, which helps the winery maintain a consistent product from bottle to bottle and from vintage to vintage in some categories of wines.

CONCLUSIONS

In general terms, blending resulted in a decrease in the differences in concentration among the various wines blended, producing an apparent standardization of the blends, principally due to the higher proportion of Cabernet Sauvignon used in the blending. The data from the analysis of the different fractions of proanthocyanidins after their separation by Sep-Pak C₁₈ cartridges showed differences in the concentration but not in the proportion of fractions. Although there was no difference in perceived astringency and bitterness, wine blending produced changes in mouthfeel descriptors. Further studies that take into account the time and the wine evolution and the use of other proportions of wine blends are necessary to determine whether the apparent standardization of the wines changes and also the variety that produces the best characteristics in wine blends for improving wine quality and consumer acceptance.

ACKNOWLEDGEMENTS

This study was supported by Conicyt-Chile (grants Fondecyt 1080559 and 1110832). The authors are also grateful to Mr Gonzalo Pino from William Fèvre Winery (Maipo Valley, Chile) for providing the wines used in the study.

REFERENCES

1 Iacono F, Nicolini G and Alonzo E, Wine blending techniques and examples of qualitative result definition (sensory properties). *Vignevini* **26**:81–86 (1999).

- 2 Monagas M, Bartolomé B and Gómez-Cordovés C, Effect of the modifier (Graciano vs. Cabernet Sauvignon) on blends of Tempranillo wine during ageing in the bottle. I. Anthocyanins, pyranoanthocyanins and non-anthocyanin phenolics. LWT – Food Sci Technol **39**:1133–1142 (2006).
- 3 Monagas M, Martín-Álvarez P, Gómez-Cordovés C and Bartolomé B, Effect of the modifier (Graciano vs. Cabernet Sauvignon) on blends of Tempranillo wine during ageing in the bottle. II. Colour and overall appreciation. LWT – Food Sci Technol 40:107–115 (2007).
- 4 Dooley L, Threlfall R and Meullenet J, Optimization of blended wine quality through maximization of consumer liking. *Food Qual Prefer* **24**:40–47 (2011).
- 5 Ferrier J and Block D, Neural-network-assisted optimization of wine blending based on sensory analysis. *Am J Enol Vitic* **52**:386–395 (2001).
- 6 Gawel R, Red wine astringency: a review. Aust J Grape Wine Res 4:74–95 (1998).
- 7 Gawel R, lland P and Francis L, Characterizing the astringency of red wine: a case study. *Food Qual Prefer* **12**:83–94 (2001).
- 8 Brossaud F, Cheynier V and Noble A, Bitterness and astringency of grape and wine polyphenols. Aust J Grape Wine Res 7:33-39 (2001).
- 9 Escudero-Gilete M, Gonzalez-Miret M and Heredia F, Implications of blending wines on the relationships between the color and the anthocyanic composition. *Food Res Int* **43**:745–752 (2010).
- 10 Dooley L, Threlfall R, Meullenet J and Howard L, Compositional and sensory impacts from blending red wine varietals. *Am J Enol Vitic* 63:241–250 (2012).
- 11 Hopfer H, Ebeler S and Heymann H, How blending affects the sensory and chemical properties of red wine. *Am J Enol Vitic* **63**:312–324 (2012).
- 12 SAG (Servicio Agrícola y Ganadero), Catastro Nacional de Vides de Vinificación 2013 [Online]. Available: http://www.sag.gob.cl [11 March 2013].
- 13 Granett J, Rooting out the wine plague. Nature 428:20 (2004).
- 14 Mattivi F, Vrhovsek U, Masuero D and Trainotti D, Differences in the amount and structure of extractable skin and seed tannins amongst red grape varieties. Aust J Grape Wine Res 15:27–35 (2009).
- 15 Obreque-Slier E, Peña-Neira A, López-Solís R, Zamora-Marín F, Ricardo-Da Silva J and Laureano O, Comparative study of the phenolic composition of seeds and skins from Carménère and Cabernet Sauvignon grape varieties (*Vitis vinifera* L.) during ripening. J Agric Food Chem **58**:3591–3599 (2010).
- 16 OIV, Compendium of International Methods of Wine and Must Analysis, Vol. I–II, Sect. 3. OIV, Paris (2012).
- 17 Glories Y, La coleur des vins rouges. 2eme Partier. Mesure, origine et interpretation. Conaiss Vigne Vin 18:253-271 (1984).
- 18 Sarneckis C, Dambergs R, Jones P, Mercurio M, Herderich M and Smith P, Quantification of condensed tannins by precipitation with methylcellulose: development and validation of an optimized tool for grape and wine analysis. Aust J Grape Wine Res 12:39–49 (2006).
- 19 Ribéreau-Gayon J and Stonestreet E, Le dosage des anthocyanes dans le vin rouge. *Bull Soc Chim* **9**:2649–2652 (1965).
- 20 Pérez-Magariño S and González-Sanjosé M, Application of absorbance values used in wineries for estimating CIELAB parameter in red wines. *Food Chem* 81:301–306 (2003).
- 21 Sun B, Leandro C, Ricardo da Silva J and Spranger I, Separation of grape and wine proanthocyanidins according to their degree of polymerization. J Agric Food Chem 46:1390–1396 (1998).
- 22 Sun B, Ricardo da Silva J and Spranger I, Critical factors of the vanillin assay from catechins and proanthocyanidins. *J Agric Food Chem* **46**:4267–4274 (1998).
- 23 Peña-Neira A, Hernández T, García-Vallejo C, Estrella I and Suarez J, A survey of phenolic compounds in Spanish wines from different geographical origins. *Eur Food Res Technol* **210**:445–448 (2000).
- 24 Peña-Neira A, Cáceres A and Pastenes C, Low molecular weight phenolic and anthocyanins composition of grape skins from cv. Syrah (*Vitis vinifera* L.) in the Maipo Valley (Chile): effect of cluster thinning and vineyard yield. *Food Sci Technol Int* **13**:153–158 (2007).
- 25 Fanzone M, Peña-Neira A, Gil M, Jofré V, Assof M and Zamora F, Impact of phenolic and polysaccharidic composition on commercial value of Argentinean Malbec and Cabernet Sauvignon wines. *Food Res Int* 45:402–414 (2012).
- 26 Gawel R, Oberholster A and Francis L, A 'mouth-feel wheel': terminology for communicating the mouth-feel characteristics of red wine. *Aust J Grape Wine Res* **6**:203–207 (2000).

- 27 Vidal S, Francis L, Noble A, Kwiatkowski M, Cheynier V and Waters E, Taste and mouth-feel properties of different types of tannin-like polyphenolic compounds and anthocyanins in wine. *Anal Chim Acta* 513:57–65 (2004).
- 28 Ginjom I, D'Arcy B, Caffin N and Gidley M, Phenolic contents and antioxidants activities of major Australian red wines throughout the winemaking process. J Agric Food Chem 58:10133–10142 (2010).
- 29 Rezaei J and Reynolds A, Characterization of Niagara peninsula Cabernet Franc wines by sensory analysis. *Am J Enol Vitic* **61**:1–14 (2010).
- 30 Di Profio F, Reynolds A and Kasimos A, Canopy management and enzyme impacts on Merlot, Cabernet Franc, and Cabernet Sauvignon. II. Wine composition and quality. *Am J Enol Vitic* **62**:152–168 (2011).
- 31 Granato D, Katayama F and de Castro I, Phenolic composition of South American red wines classified according to their antioxidant activity, retail price and sensory quality. *Food Chem* **129**:366–373 (2011).
- 32 Kennedy J, Ferrier J, Harbertson J and Peyrot des Gachons C, Analysis of tannins in red wine using multiple methods: correlation with perceived astringency. *Am J Enol Vitic* **57**:481–485 (2006).
- 33 Landon J, Weller K, Harbertson J and Ross C, Chemical and sensory evaluation of astringency in Washington state red wines. Am J Enol Vitic 59:153–158 (2008).

- 34 Monagas M, Gómez-Cordovés C, Bartolomé B, Laureano O and Ricardo da Silva J, Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J Agric Food Chem* 51:6475–6481 (2003).
- 35 Cosme F, Ricardo-Da-Silva J and Laureano O, Tannin profiles of *Vitis vinifera* L. cv. red grapes growing in Lisbon and from their monovarietal wines. *Food Chem* **112**:197–204 (2009).
- 36 Vidal S, Francis L, Guyot S, Marnet N, Kwiatkowski M, Gawel R, *et al.*, The mouth-feel properties of grape and apple proanthocyanidins in a wine-like medium. *J Sci Food Agric* **83**:564–573 (2003).
- 37 Fontoin H, Saucier C, Teissedre P and Glories Y, Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution. *Food Qual Prefer* **19**:286–291 (2008).
- 38 Obreque-Slier E, Peña-Neira A and López-Solís R, Enhancement of both salivary protein–enological tannin interactions and astringency perception by ethanol. J Agric Food Chem 58:3729–3735 (2010).
- 39 Llaudy M, Canals R, Canals JM, Rozés N, Arola L and Zamora F, New method for evaluating astringency in red wine. J Agric Food Chem 52:742-746 (2004).
- 40 Lesschaeve I and Noble A, Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *Am J Clin Nutr* **81**:330S-335S (2005).