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Flavour characterization of red wines by descriptive analysis and ESI mass spectrometry

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ABSTRACT

Direct infusion electrospray ionization mass spectrometry (ESI-MS) is a new technique that allows for the identification of volatile and non-volatile compounds in foods and beverages, without the need for extraction, isolation and prior chromatographic separation of the sample constituents. The objective of this research was to explore the potential of ESI-MS to identify the acid and phenolic compounds in red wines important for the beverage flavour profile. Thus, descriptive flavour data from red wines elaborated with *Vitis labrusca*, hybrid and *Vitis vinifera* grapes was initially generated by a trained panel ($n_1 = 12$ panellists; $n_2 = 6$ repetitions) and subsequently correlated with ESI-MS data collected in the negative ion mode. Considerable variation concerning several acid and polyphenolic compounds identified in the samples was observed amongst the wines. The Principal Component Analysis (PCA) and correlation analyses of the data showed that the succinic acid contents were good predictors of sourness ($r = 0.63$, $p = 0.05$). A negative correlation was also observed between grape flavour and the carbonic acid contents ($r = -0.76$, $p = 0.02$), amongst other findings. Overall, the results indicated ESI-MS fingerprinting as a fast and reliable technique with potential use in wineries for quality control.

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1. Introduction

Knowledge of the volatile composition of a wine is of great interest since these compounds are highly related to beverage flavour. Thus, several studies associating the wine volatiles with the grape variety (Chisholm, Guiher, Vonah, & Beaumont, 1994; Lee, Lee, Kim, Kim, & Koh, 2006; Lee & Noble, 2006), climatic conditions (Falcão, de Revel, Perello, Moutsiu, & Zanus, et al., 2007; Louw et al., 2009) and various winemaking practices (Aznar, López, Cacho, & Ferreira, 2003; Chatonnet, 1998) have been carried out. On the other hand the non-volatile compounds also promote considerable impact on wine flavour, notably the organic acids and phenolic compounds.

Organic acids produce a pleasant and refreshing sourness in wines (Ebeler, 2001) but in excess, they promote an unpleasant acidity, suppressing the perception of other desirable flavour and mouth-feel attributes, especially the perception of sweetness (Jackson, 2000). This is the case of tartaric acid, the most abundant acid in wines (Margalit, 2004), which, in high concentrations, gives a sharp, unpleasant taste to the beverage (Sass-Kiss, Kiss, Havadi, &

Adányi, 2008). Malic acid promotes an additional taste described as harsh that can be reduced via the malolactic fermentation, which transforms malic into lactic acid (Jackson, 2000). Succinic acid promotes a bitter note in the wine, causing salivation and accentuating the overall flavour of the beverage (Ribéreau-Gayon, Glories, Maujean, & Dubourdieu, 2006). Citric acid is occasionally added to wines as an acidifying agent, but since this acid is not dominant in grapes, large additions may result in what many would regard as a citrus-like flavour (Zoecklein, Fugelsang, Gump, & Nury, 1999). Other fixed acids, such as fumaric and pyruvic acids, are generally found in minor amounts in wines, and do not usually cause any sensory impact on the beverage. A possible exception is α -ketoglutaric acid, which can bind with free sulphur dioxide, reducing its active level (Jackson, 2000) and the unpleasant odour and pungency that this sulphur compound imparts to the wine (Garde-Cerdan & Ancín-Azpilicueta, 2007). Thus α -ketoglutaric acid may improve the wine flavour, especially when free sulphur dioxide is present at high levels (Garde-Cerdan & Ancín-Azpilicueta, 2007). Acetic acid, which usually occurs in wines as a by-product of yeast and bacterial metabolism, may impart a vinegar-like aroma/flavour note, which is detectable only in wines spoiled by microbes. However, according to Jackson (2000), at levels below 300 mg/l, acetic acid adds a desirable complexity to the wine aroma and flavour.

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To a large extent the wine flavour also depends on its phenolic composition, since polyphenolic compounds affect beverage bitterness, sourness, astringency, body and balance (Jackson, 2000). In red wines, the astringency is strongly related to the presence of tannins, which are polymers of flavonoids. Monomeric flavonoids, such as myricetin, quercetin and catechin, mostly influence beverage bitterness (Preys et al., 2006; Robichaud & Noble, 1990), while anthocyanins, mainly present in red wines, increase the mouth perception of beverage fullness (Vidal et al., 2004). Cheynier, Fulcrand, Broussaud, Asselin, and Moutounet (1998, chap. 10) showed that for Cabernet Franc wines, higher anthocyanin to tannin ratios implied in samples with better sensory balance and mellowness. Finally, since phenolic acids such as caffeic, p -coumaric, ferulic and other acids are potential H⁺ donors, they may also contribute to wine sourness (Fischer & Noble, 1994).

Thus determining the acid and polyphenolic contents of wines can help to predict important sensory attributes of the beverage such as sourness, bitterness and astringency, amongst others. However, these determinations are very time-consuming, usually requiring prior extraction, purification and separation of the individual sample compounds. As a consequence, undesirable sample modifications may occur during the above mentioned analyses (Cooper & Marshall, 2001).

Direct infusion electrospray ionization mass spectrometry (ESI-MS) is a new, fast and robust technique that in recent years has been employed for the chemical characterization of alcoholic beverages such as beer (Araújo et al., 2005), whisky (Moller, Catharino, & Eberlin, 2005), wine (Catharino et al., 2006), rum and cachaça (de Souza, Augusti, Catharino, Siebald, Eberlin, & Augusti, 2007). The main advantage of ESI-MS is that the sample can be analyzed with no preparation such as extraction, isolation and/or concentration of its chemical compounds. Thus, the use of ESI-MS in the chemical characterization of wines may be advantageous, since it eliminates the need for extraction, isolation and prior chromatographic separation of the sample components, minimizing, as a consequence, the time of analysis and the formation of artefacts (Cooper & Marshall, 2001).

Cooper and Marshall (2001) were amongst the first researchers to use ESI-MS for the direct analysis of wines. The technique was used in both the positive and negative ion mode, and allowed for the identification of several anthocyanins, tannins and other phenolic compounds. The authors reported better performance in the negative ion mode ESI-MS in order to differentiate the samples. Catharino et al. (2006) used negative ion mode ESI-MS for a fast characterization of the musts of six varieties of grape and their corresponding wines at the end of the malolactic fermentation. The

authors showed that negative ion mode ESI-MS easily ionised the organic acids and polyphenolic compounds originally present in the samples, allowing for rapid identification of the differences amongst them. More recently, Gollücke, Catharino, Souza, Eberlin, and Tavares (2009) successfully used ESI-MS in the negative ion mode to analyse the impact of pasteurization, concentration and storage on the antioxidant phenolic compounds of grape juice.

Although ESI-MS shows a good potential to generate useful information for a better understanding of the acid and phenolic compound contents of wines and their relation with the beverage flavour profile, this technique has not yet been fully explored with this objective. Thus in the present research, descriptive flavour data from red wines elaborated with *Vitis labrusca*, hybrid and *Vitis vinifera* grapes was correlated with ESI-MS data, in order to explore the potential of this novel analytical technique to characterize the chemical and sensory profiles of red wines.

2. Materials and method

2.1. Wines

Using information provided by the Association of Brazilian wine producers, the authors invited all the relevant *V. labrusca* and hybrid wineries that produce and/or bottle their beverages in the State of Sao Paulo, Brazil, to participate in the current study. This is the wealthiest and one of the largest wine markets in the country. Of the 20 wineries invited, 10 agreed to participate in the present research. Thus the samples consisted of nine red table wines produced from *V. labrusca* and/or hybrid grapes, and one sample produced from *V. vinifera* (Barbera) grapes. All the wines were collected directly from the wineries, whose owners/managers voluntarily agreed to participate in this study. Table 1 specifies the grape species and varieties employed to produce each wine, as well as their vintage and the location of each winery.

2.2. Descriptive analysis

The flavour profiles were generated by a panel of 12 trained judges between 18 and 35 years of age, undergraduate or postgraduate students from the University of Campinas, Brazil, and experienced in food and beverage sensory evaluation using quantitative descriptive analysis (Meilgaard, Civillie, & Carr, 1999; Stone & Sidel, 2004; Stone, Sidel, Oliver, Woosley, & Singleton, 1974). The panellists were initially screened based on: (i) sensitivity to recognize the basic tastes and to perceive astringency in standard

Table 1
Characterization of the red table wines analyzed in the current study.

Wine codes	Varieties	Species	Vintage	Origin	Productivity (litres/year)	US\$/bottle 750 ml
1	Unknown	Not specified	2006	Rio Grande do Sul State, Brazil	25 millions	2.9
2	Ives and Isabella	<i>Vitis labrusca</i> and <i>Vitis labrusca</i>	2006	Rio Grande do Sul State, Brazil	10 millions	4
3	Ives	<i>Vitis labrusca</i>	2006	Rio Grande do Sul State, Brazil	4 millions	4.5
4	Máximo	Hybrid (Syrah × Seibel 11342)	2006	Sao Paulo State, Brazil	15,000	5.8
5	Ives, Máximo and Sanches	<i>Vitis labrusca</i> , hybrid (Syrah × Seibel 11342), hybrid (Máximo × IAC 577-8)	2006	Sao Paulo State, Brazil	3500	5
6	Máximo	Hybrid (Syrah × Seibel 11342)	2006	Sao Paulo State, Brazil	6500	6
7	Seibel 2	Hybrid (<i>Vitis lincecumii</i> × Alicante Bouschet)	2006	Sao Paulo State, Brazil	12,000	4.6
8	Isabella, Máximo, Ives and Seibel 2	<i>Vitis labrusca</i> , hybrid (Syrah × Seibel 11342), <i>Vitis labrusca</i> and hybrid (<i>Vitis lincecumii</i> × Alicante Bouschet)	2006	Rio Grande do Sul State, Brazil	118,750	4.5
9	Barbera	<i>Vitis vinifera</i>	2006	Sao Paulo State, Brazil	5000	7
10	Ives	<i>Vitis labrusca</i>	2006	Sao Paulo State, Brazil	6000	5.1

solutions containing slightly above-threshold concentrations of pure compounds such as sucrose, citric acid, caffeine, sodium chloride and tartaric acid (ASTM, 1981; ISO 22935, 2006; Meilgaard et al., 1999); (ii) subject discrimination capability to determine differences in the flavour of red wine samples in the difference-from-control test, (Meilgaard et al., 1999); and (iii) the ability of the individual to identify 32 odours present in the Wine Aroma Wheel® and usually perceived in red wines, such as: rose, black pepper, cloves, lemon, blackberry, strawberry and peach, amongst others (Noble, Arnold, Buechsenstein, Leach, Schmidt, & Stekron, 1987).

2.2.1. Lexicon and panel training

Using Kelly's Repertory Grid Method described in Moskowitz (1983), the above-mentioned panellists evaluated the 10 wines listed in Table 1 during five distinct sessions. In each session, the subjects received three wine samples and individually described their similarities and differences with respect to their flavour. As a group, the panellists then discussed the terms generated by each individual and, with the supervision of a panel leader, they consensually defined the terms that adequately described the flavour similarities and differences amongst the 10 wines, writing down their definitions and suggesting references for training purposes. In subsequent sessions, the suggested references were presented, discussed and approved or modified by the group. During this process, 13 flavour descriptors were consensually generated, as well as the written definitions and references for each one (Table 2).

In consensus the panellists also elaborated a flavour descriptive ballot for the wines, associating each descriptor with a 9-cm unstructured scale, anchored at its left and right extremes by the terms "none/weak" and "strong", respectively.

The descriptive ballot, the 10 samples of red wine and the reference standards listed in Table 2 were then used for panel training. After a training period, a final selection of the panellists was carried out, where each judge evaluated four red wines with four replications using the descriptive ballot, and an ANOVA (Source of variation: wine and replications) was carried out for each panellist and each attribute. The level of significance (p) of the F value calculated by the above mentioned ANOVA for the source of variation "wine" (pF_{wine}), was used as the criterion to estimate the discriminative power of each judge, and the level of significance (p) of the F value calculated for the source of variation "replication" ($pF_{\text{replication}}$), was used as the criterion to estimate the reproducibility of each judge. Only individuals showing adequate discriminative power ($pF_{\text{wine}} \leq 0.30$), reproducibility ($pF_{\text{replication}} \geq 0.05$) and consensus with the rest of the panel for at least 80% of the descriptors present in the ballot, were selected to take part in the descriptive panel (Damásio & Costell, 1991).

Table 2
Attributes and reference standards generated by the sensory panel.

Flavours/tastes	Reference standard
Grape juice	100% lves processed grape juice produced by a Brazilian cooperative (Aecia – Rio Grande do Sul State, Brazil)
Grape	Niagara grapes
Sweetness	Aqueous solution containing 1.6% sugar cane
Sourness	Aqueous solution containing 0.07% citric acid
Bitterness	Aqueous solution containing 0.06% caffeine
Alcoholic	Aqueous solution containing 30% ethanol (food grade)
Blackberry	Frozen blackberries
Dried fruit (raisin/fig)	Mixture of raisins and dried figs
Wood	Oak chips (5 g) soaked in water
Seed	Niagara grape seeds
Yeast	Aqueous solution containing 1% snapshot dried biological yeast (Dr. Oetker – Sao Paulo State, Brazil)
Astringency body	Aqueous solution containing 0.3% tannic acid Red dessert wine produced by Góes winery (Sao Paulo State, Brazil)

2.2.2. Flavour profile

Samples of red wine (30 ml) were presented in clear tulip-shaped glasses covered with watch glasses, and coded with random three-digit numbers. The temperature of the wines was maintained at 20 ± 2 °C, and the evaluations were made in individual booths under white light. The data was collected using the descriptive ballot consensually generated by the panel.

In order to control the contrast effect amongst the samples (Amerine, Pangborn, & Roessler, 1965), a Cochran and Cox (1957) incomplete balanced block design was used (design plan 11.6) in which four wines were tested in each session (block). Overall, each judge evaluated each wine with six repetitions, giving a total of 15 sessions. For each repetition, a different bottle of wine was tested. In each session, the order of presentation of the samples was balanced for the first-order effect (MacFIE, Bratchell, Greenhoff, & Vallis, 1989).

2.3. ESI-MS analyses

For the fingerprinting ESI-MS analysis, a hybrid high-resolution and high-accuracy (5 mg/l) Micromass Q-TOF mass spectrometer (Micromass, Manchester, UK) was used. The general conditions were: source temperature of 100 °C, capillary voltage of 3.0 KV and cone voltage of 40 V. The wines were diluted in a solution containing 50% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA), 50% (v/v) deionized water and 0.5% of ammonium hydroxide (Merck, Darmstadt, Germany). ESI-MS was performed by direct infusion with a flow rate of $10 \mu\text{l min}^{-1}$ using a syringe pump (Harvard Apparatus). Mass spectra were acquired and accumulated over 60 s, and the spectra were scanned in the range between 50 and 1000 m/z . A structural analysis of single ions in the mass spectra of the wine samples was performed by ESI-MS/MS. Ions with m/z values of interest were selected and submitted to 15–45 eV collisions with argon in the collision quadrupole. The collision gas pressure was optimised to produce extensive fragmentation of the ions under investigation.

The mass-spectra were expressed as the intensities of individual [M–H]⁺ ions. The compounds were identified by comparing their ESI-MS/MS fragmentation spectra with those in the literature whenever possible. The compounds whose fragmentation spectra were not found in the literature were tentatively identified based on their high-resolution mass.

2.4. Data analyses

All statistical analyses were performed using the SAS version 9.1.3 (SAS® Institute, Cary, NC). The sensory descriptive data was evaluated by ANOVA (sources of variation: wines, judges, wine * judge) followed by the Tukey test for multiple mean comparisons ($p \leq 0.05$). The sensory and analytical data were also analyzed by the Principal Component Analysis (PCA) and correlated with the analytical data using the Pearson correlation coefficient.

3. Results and discussion

3.1. Descriptive analyses

Significant differences ($p \leq 0.05$) were found amongst the 10 red wines listed in Table 1, for all the 13 descriptors generated by the trained panel (Table 3). No significant wine * judge interaction ($p \leq 0.05$) was detected, confirming the adequate training of the descriptive panel. In the wine literature, non-significant judges * sample interactions for all the sensory descriptors evaluated is not common, but similar results are reported once in a

Table 3
Means of descriptive attributes for flavour rated by the sensory trained panel for each red table wine ($n_1 = 12$ judges, $n_2 = 6$ repetitions/sample).

Attributes	Wines/variety ^a									
	1	2	3	4	5	6	7	8	9	10
Alcoholic	5.3 ^{ab}	5.1 ^{ab}	4.6 ^b	5.5 ^a	5.1 ^{ab}	5.4 ^a	5.6 ^a	5.3 ^{ab}	5.2 ^{ab}	5.1 ^{ab}
Yeast	2.2 ^{cd}	1.9 ^d	2.1 ^{cd}	3.2 ^a	2.3 ^{cd}	3.2 ^{ab}	2.5 ^{bc}	2.3 ^{cd}	3.2 ^{ab}	2.1 ^{cd}
Bitterness	2.4 ^{bcd}	2.3 ^{cd}	2.1 ^d	3.5 ^a	2.7 ^{bcd}	3.6 ^a	2.0 ^{ab}	2.9 ^{abc}	3.0 ^{ab}	2.4 ^{bcd}
Sweetness	2.6 ^e	3.3 ^{abcd}	3.5 ^{ab}	2.8 ^{cde}	3.6 ^a	2.8 ^{de}	2.9 ^{cde}	2.9 ^{bcd}	2.6 ^e	3.4 ^{abc}
Sourness	4.3 ^{bc}	4.4 ^{abc}	3.9 ^c	4.3 ^{bc}	4.0 ^{bc}	4.4 ^{abc}	4.6 ^{abc}	4.8 ^{ab}	5.2 ^a	4.1 ^{bc}
Wood	2.8 ^{ab}	2.4 ^b	2.4 ^b	3.2 ^a	2.6 ^b	3.2 ^a	3.2 ^a	2.9 ^{ab}	3.1 ^a	2.4 ^b
Grape juice	3.3 ^{de}	4.5 ^{abc}	5.2 ^a	4.0 ^{cd}	4.9 ^{ab}	4.1 ^{bc}	4.0 ^{cd}	4.0 ^{cd}	2.9 ^e	4.9 ^{ab}
Grape	3.1 ^{abc}	3.4 ^{abc}	3.5 ^{ab}	2.7 ^c	3.5 ^{ab}	2.9 ^{bc}	3.1 ^{abc}	3.0 ^{abc}	2.8 ^{bc}	3.7 ^a
Seed	2.4 ^{abcde}	2.0 ^e	2.1 ^{de}	2.9 ^a	2.1 ^{cde}	2.7 ^{abcd}	2.6 ^{abcde}	2.8 ^{abc}	2.8 ^{abc}	2.2 ^{bcd}
Dried fruit (raisin/fig)	2.5 ^{ab}	1.9 ^{bc}	1.9 ^c	2.4 ^{bc}	2.2 ^{bc}	2.4 ^{bc}	2.3 ^{bc}	2.1 ^{bc}	3.1 ^a	2.2 ^{bc}
Blackberry	2.6 ^d	3.9 ^{ab}	4.0 ^a	3.0 ^{cd}	4.2 ^a	3.2 ^{bcd}	3.6 ^{abc}	3.8 ^{ab}	3.0 ^{cd}	4.2 ^a
Body	2.7 ^d	3.1 ^{cd}	3.7 ^{bc}	4.8 ^a	3.7 ^{bc}	4.2 ^{ab}	3.3 ^{cd}	3.7 ^{bc}	2.8 ^d	3.2 ^{cd}
Astringency	4.3 ^{bcd}	4.0 ^d	4.1 ^{cd}	5.2 ^a	3.9 ^d	4.7 ^{abcd}	4.9 ^{abc}	5.0 ^{ab}	4.3 ^{abcd}	4.1 ^{cd}

^a In the same line means showing common letter are not significantly different ($p = 5\%$).

while, such as Chapman, Roby, Ebeler, Guinard, and Matthews (2005).

In spite of the sensory differences mentioned above, Table 3 revealed some similarities amongst a number of the wines. For instance, all the wines containing *V. labrusca* grapes and their hybrids, notably the two Ives wines (samples 3 and 10), showed greater ($p \leq 0.05$) fruity flavour notes described as blackberry and grape juice, as compared to the *V. vinifera* Barbera wine (sample 9). This finding is in agreement with several authors who reported that *V. labrusca* varieties, such as Ives, Concord, Isabella and Niagara, amongst others, contained methyl anthranilate, a phenol-derived ester that imparts fruity and/or artificial grape aroma/flavour notes to the wine (Amerine & Singleton, 1984; Jackson, 2002; Reynolds, Lowrey, & Savigny, 2005) On the other hand, the *V. vinifera* wine (Barbera) was the sourest sample, significantly differing ($p \leq 0.05$) from several of the other wines tested, notably the two Ives (samples 3 and 10). The *V. vinifera* wine was also highest ($p \leq 0.05$) in dried fruit flavour, and lowest in sweet taste, as compared to all the others, except sample 1.

The two wines elaborated exclusively with Máximo grapes, a hybrid from Syrah with Seibel (samples 4 and 6), were the most bitter, woody and seedy samples, significantly differing ($p \leq 0.05$) from those elaborated exclusively with Ives grapes (samples 3 and 10), which showed low intensities of the above mentioned attributes. The two Máximo wines (samples 4 and 6) were also the beverages imparting the greatest mouth feel for body, as compared to the other samples.

Fig. 1 shows the results for the Principal Component Analysis (PCA), which was used to illustrate the similarities and differences amongst the wines with respect to their sourness, bitterness, sweetness, astringency and body.

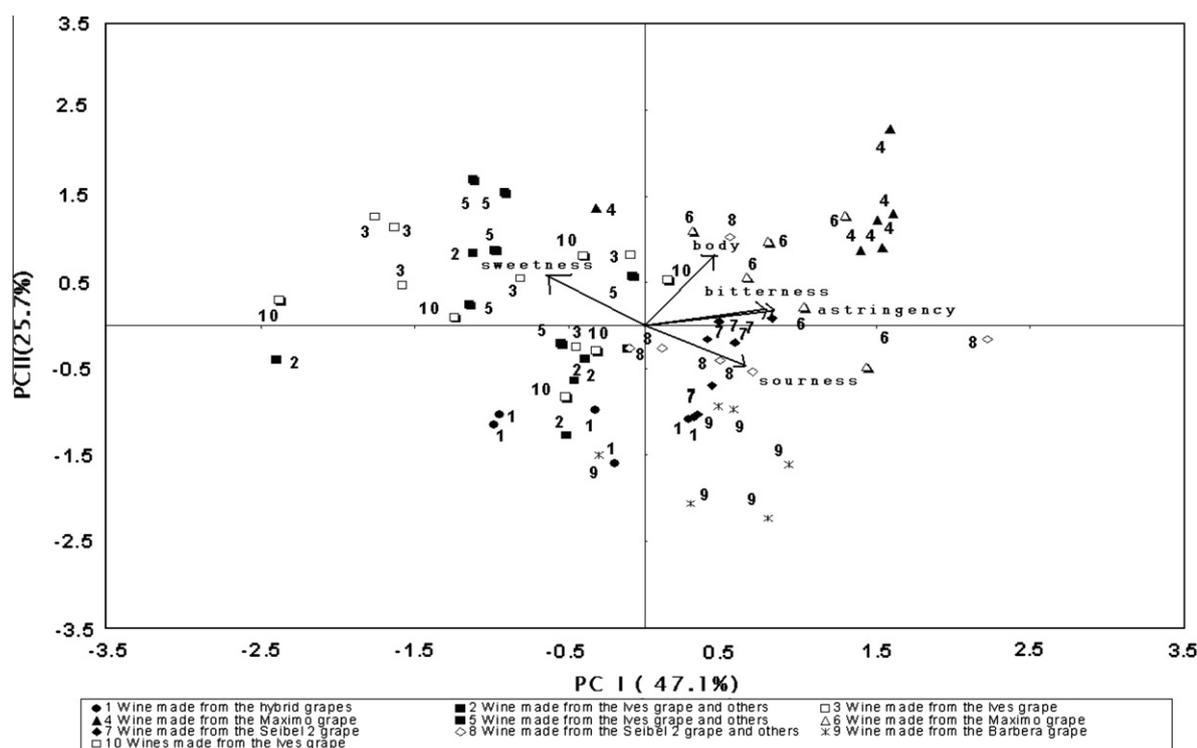


Fig. 1. PCA generated with the sensory data for sourness, bitterness, sweetness, astringency and body.

sweetness, astringency and body; all sensory perceptions known to be related to the sample contents of organic acids and phenolic compounds. In Fig. 1 the sensory descriptors are represented as vectors and the wines as numbers from 1 to 10. Each wine is represented six times; corresponding to the six repetitions performed by the descriptive panel.

Fig. 1 explains 72.8% of the total sensory variation amongst the wines. As shown, the axis I of the PCA is strongly related to the astringency and bitterness of the wines and negatively related to their sweetness; while axis II is positively related to the body of the wines. Thus, in Fig. 1, the position of the two wines elaborated exclusively with Máximo grapes, notably sample 4, suggests that these sample were the most bitter and astringent ones as compared to the others. On the other hand, the wines elaborated exclusively with Ives grapes (samples 3 and 10) or containing this grape in their composition (samples 2 and 5), were the sweetest and least bitter and astringent of the wines. Finally, Fig. 1 also suggests that the *V. vinifera* Barbera wine (sample 9) was very sour as compared to the remaining samples, which is to be expected for this variety (Jackson, 2000; Amerine & Singleton, 1984). Overall, all the information generated by the PCA (Fig. 1) was confirmed by the Tukey test (Table 3).

3.2. Chemical analysis

The ESI-MS analysis allowed for a rapid identification of 28 compounds, three inorganic acids, 11 organic acids and 14 phenolic compounds (Table 4).

3.2.1. Organic and inorganic acids

Carbonic (m/z 61), sulphurous (m/z 81) and phosphoric (m/z 97) acids were the inorganic acids identified in some of the wines (Table 4). Carbonic and sulphurous acids are inorganic acids frequently present in wines, usually dissolved in their gaseous form, which do not affect the beverage pH or sour taste (Jackson, 2000).

Of the 11 organic acids identified in the wines, tartaric was the major acid present in the samples, followed by succinic, malic and acetic acids (Table 4). All these organic acids are frequently present in red wines (Ough & Amerine, 1988). Tartaric and malic acids come from the grape and are usually found at levels ranging from 0.2% to 1.0% (wt/vol) and from 0.1% to 0.8% (wt/vol), respectively, in wines. According to Jackson (2000), tartaric and malic acids are the dominant acids in grapes and wines. On the other hand, succinic and acetic acids are produced at the beginning of the alcoholic fermentation of the must (Zoecklein et al., 1999).

In this research, tartaric acid was indeed the dominant organic acid present in all the wines except sample 6, a wine produced exclusively with Máximo grapes. Overall, malic and succinic acids were the second most abundant organic acids present in the wines, with the exception of samples 4 and 9, respectively Máximo and Barbera wines, which did not contain malic acid. The absence of malic acid in these two wines might be attributed to the fact that during ripening of the grapes, especially when this occurred at the end of the season and under hot harvesting conditions, the concentration of malic acid would decrease sharply (Rice, 1974). In fact, musts produced with ripe grapes from countries in the southern hemisphere, such as Brazil, contain from two to three times less malic acid than musts produced in countries in the northern hemisphere (Ribéreau-Gayon et al., 2006). Unlike malic acid, the concentration of tartaric acid does not decline significantly during grape ripening (Jackson, 2000). Thus, the absence of malic acid in two of the 10 wines tested (samples 4 and 9) might be attributed both to the hot Brazilian weather, especially during the summer, and to the inadequate harvesting practice often used by small wineries, who harvest their grapes after the coolest morning hours of the day. In fact, Table 1 shows that the two wines lacking malic

acid (samples 4 and 9) came from small family wineries. One important organic acid generally present in *V. vinifera* wines, but not identified in the 10 samples evaluated in the current study, is lactic acid (Ough & Amerine, 1988), possibly because, as reported by the wineries, the malolactic fermentation was not carried out during the production of the wines tested.

Finally, as shown in Table 4, the palmitic and stearic fatty acids were found in several of the wines evaluated. Their presence probably indicates the use of excessive pressure during grape crushing, which fractures the grape seeds releasing their oils. These fatty acids may subsequently suffer oxidation, generating an unpleasant rancid odour in the wines. Modern crushers have almost eliminated this source of wine contamination (Jackson, 2000).

Thus, while the malic acid content may be a good indicator to evaluate the adequacy of the harvest practiced by the Brazilian wineries, the palmitic and stearic fatty acid contents of the wines indicate the adequacy of the crushing step, and both these analyses can easily be carried out by ESI-MS in the negative ion mode, thus helping to monitor the quality of Brazilian wines during and after their production.

3.2.2. Polyphenolic compounds

The non-flavonoid phenolic compounds such as the phenolic acids, are structurally simple, and in wines mainly originate during juice extraction and post-fermentation treatments, including aging of the beverage in oak barrels. In wines, non-flavonoid phenolic compounds commonly occur etherified with sugars, alcohols or organic acids, particularly tartaric acid (Jackson, 2000; Zoecklein et al., 1999). In the current research, hydrolysis products of anthocyanins, such as the phenolic acids p -coumaric acid (m/z 163), p -coumaric acid-glucoside (m/z 325), caffeic acid (m/z 179), caffeoyltartaric acid (m/z 311), and ferulic acid (m/z 193), were identified. Ferulic acid was identified in all the 10 wines, while caffeic and p -coumaric acids in only a few. These results are in agreement with several studies reported in the wine literature. For instance, while p -coumaric acid was present in all five Merlot wines analysed by Makris, Kallithraka, and Mamilos (2006), it was absent in 3 of the 7 Cabernet Sauvignon beverages investigated by the same authors. In the study by Goldberg, Tsang, Karumanchiri, Diamandis, and Soleas (1996), ferulic acid was found in just one of the Italian red wines analysed, being absent in the samples elaborated in Australia, France and USA. Several additional phenolic acids usually found in *V. vinifera* wines, were not identified in the 10 wines investigated in the current study, such as gallic (Kallithraka et al., 2001; Preys et al., 2006), caftaric (Makris et al., 2006; Preys et al., 2006) and coumaric acids (Makris et al., 2006).

Tyrosol (m/z 137), a non-flavonoid phenolic compound produced by yeasts from tyrosine during wine fermentation, was found in one of the Ives wines (sample 3). Previous studies by Pour Nikfardjam and Pickering (2008) and Monagas, Gómez-Cordovés and Begona (2007), found tyrosol in all the *V. vinifera* wines analyzed, including samples of Kadarka, Lemberger, Cabernet Sauvignon and Tempranillo wines, and also in hybrid wines such as Maréchal Foch and Baco Noir. Thus, the presence of tyrosol was expected in all the wines analyzed in the current study. However, in wines this polyphenolic compound is derived from the alcoholic fermentation by the yeast, so its formation is highly dependent on factors that affect the growth and metabolism of the yeast cells, such as the must composition and the pH (Monagas et al., 2007). Several flavonoids usually present in grape skin, seed and pulp (Zoecklein et al., 1999) were identified in the wines evaluated. The anthocyanin peonidin (m/z 299) was identified in all 10 wines evaluated. Nonetheless, four other anthocyanins usually found in red grapes, such as malvidin, delphinidin, cyanidin and petunidin-3- D -glucoside (Zoecklein et al., 1999) were not identified in the wines analyzed in the present study. However, this is not un-

Table 5
Correlation coefficients and significance values between the sensory and chemical data.

Attributes	Positive correlation	Negative correlation
Sourness	Carbonic acid (<i>m/z</i> 61) $r = 0.58$, $p = 0.08$; succinic acid (<i>m/z</i> 135) $r = 0.63$, $p = 0.05$	–
Bitterness	Ascorbic acid (<i>m/z</i> 175) $r = 0.73$, $p = 0.02$	–
Sweetness	–	Carbonic acid (<i>m/z</i> 61) $r = -0.64$, $p = 0.05$; succinic acid (<i>m/z</i> 117) $r = -0.58$, $p = 0.08$
Astringency	Succinic acid (<i>m/z</i> 117) $r = 0.58$, $p = 0.08$	–
Grape juice	–	Succinic acid (<i>m/z</i> 135) $r = -0.59$, $p = 0.07$
Grape	–	Carbonic acid (<i>m/z</i> 61) $r = -0.76$, $p = 0.02$
Blackberry	–	Carbonic acid (<i>m/z</i> 61) $r = -0.60$, $p = 0.06$; propionic acid (<i>m/z</i> 73) $r = -0.64$, $p = 0.05$
Yeast	Ascorbic acid (<i>m/z</i> 175) $r = 0.76$, $p = 0.01$; succinic acid (<i>m/z</i> 117) $r = 0.62$, $p = 0.06$	Phosphoric acid (<i>m/z</i> 97) $r = -0.57$, $p = 0.08$
Wood	Succinic acid (<i>m/z</i> 117) $r = 0.72$, $p = 0.02$	–
Seed	Succinic acid (<i>m/z</i> 117) $r = 0.59$, $p = 0.07$; Succinic acid (<i>m/z</i> 135) $r = 0.63$, $p = 0.05$	Phosphoric acid (<i>m/z</i> 97) $r = -0.57$, $p = 0.08$
Dried fruit	Carbonic acid (<i>m/z</i> 61) $r = 0.72$, $p = 0.02$	–

sual since, for instance, Kallithraka et al. (2001) only found malvidin in the 33 red wines from different varieties that they analyzed, and Makris et al. (2006) did not find cyanidin in any of the wines they studied. The flavonols isoquercetin (*m/z* 463), quercetin 3-O-glucuronide (*m/z* 477) and myricetin-3-O- β -D-glucuronopyranose (*m/z* 493) were identified in one of the Seibel wines (sample 7) and the flavonol myricetin-3-hexose (*m/z* 479) was identified in one of the Máximo wines (sample 6). Finally, the flavonols resveratrol (*m/z* 227) and resveratrol glucoside (*m/z* 389) were identified in just a few of the 10 wines analysed. According to Zoeklein et al. (1999), the concentration of resveratrol declines substantially with grape maturation. In addition, since resveratrol is found in the grape skins, the winemaking practices also affect the contents of this and other polyphenols in the wines. In fact, comparing the resveratrol contents of wines from different countries and varieties, Stervbo, Vang, and Bonnesen (2007) did not find any resveratrol in Australian Merlot wines, Pinot Noir samples from Switzerland, Shiraz and Cabernet Sauvignon wines from Greece or in the Italian Zinfandel wine.

No flavan-3-ols such as catechin and epicatechin, or flavan-3,4-diols such as leucoanthocyanidins and leucoanthocyanins, or condensed tannins (procyandins), usually formed from the polymerization of monomeric flavan-3-ol or flavan-3,4-diols, were identified in any of the 10 wine samples. However the absence of the above mentioned compounds in wines is also not unusual. Makris et al. (2006) evaluated nine Syrah wines from several regions of Greece and also failed to find any catechin, epicatechin or procyanidin. Similarly, the absence of catechin and epicatechin compounds may have occurred due to the polymerization of these compounds into condensed tannins. In the study by Preys et al. (2006), catechins and epicatechins were not detected in the free monomeric form in red wines, but were found as sub-units of the tannins.

3.3. Correlations between the sensory attributes and chemical compounds

Table 5 shows the results of the correlation analysis between the sensory and chemical data. As can be seen, a positive correlation occurred between sourness in the wines and their contents of succinic acid ($r = 0.63$, $p = 0.05$). It is interesting to note that no significant correlation was found between the sourness perceived in the wines and their content of tartaric and malic acids, both major organic acids usually found in wines.

In wine, sourness is a perception elicited by several parameters: the hydrogen ion concentration present in the beverage at equilibrium (pH), the potential hydrogen ions from the non-dissociated

acids (titratable acidity) and also the anion part of the acid molecules present in the beverage. According to Noble, Philbrick, and Boulton (1986), at equal pH and equal titratable acidity, the sourness of succinic acid is significantly greater than that of tartaric and malic acids. Thus, the current research, suggests that succinic acid is a potential predictor of the sourness of Brazilian red wines. The suppression effect explains the negative correlation between the perception of fruity flavour notes in the wines (Table 5), such as grape juice, grape or blackberry flavours, and their contents of carbonic ($r = -0.76$, $p = 0.02$) and propionic ($r = -0.64$, $p = 0.05$) acids. Previous studies also reported negative correlation ($p \leq 0.05$) between apple ($r = -0.89$) and lemon ($r = -0.72$,) aroma notes in white wines and the beverage volatile acidity (Zamora & Guirao, 2002), as well as between the lactic acid content in red wines and their intensity of ripe-fruit aromas ($r = -0.98$) (Lee et al., 2006).

On the other hand, organic acids, notably succinic acid, also promote bitterness in wines (Ribéreau-Gayon et al., 2006). In the current study, bitterness was positively correlated ($r = 0.73$, $p = 0.02$) with the ascorbic acid content of the wines (Table 5).

In general, since this was not a causal study, all the above-mentioned correlations should be interpreted with caution, since they do not necessarily imply in the cause.

No correlation was found between astringency and the content of polyphenolic compounds, possibly because few flavonoids and no tannins were identified by the ESI-MS method used.

4. Conclusion

In red wines with no sample pre-treatment, ESI-MS in the negative ion mode, scanning in the *m/z* range between 50 and 1000, was able to quickly and easily identify the organic and phenolic acids present in the red wines. Some of these compounds were shown to be good predictors of sensory attributes related to the sensory quality of the wines, such as sourness (succinic acid) and bitterness (ascorbic acid). The method is reliable and reproducible, and may offer a fast and simple technique for the quality control and identification of acid and phenolic compounds in the wines. The authors envisage that this powerful method could be used to establish a correlation between other non-volatile and volatile components and the sensory profiles of wines.

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