Quantification of organic acids in wine and grape by direct infusion electrospray ionization mass spectrometry

Flamys Lena do Nascimento Silva,[a] Eduardo Morgado Schmidt,[a] Cláudio Luiz Messias,[b] Marcos Nogueira Eberlin*[a] and Alexandra Christine Helena Frankland Sawaya[a,c*]

5

[a] *ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas– UNICAMP, CEP 13084-971, Campinas, SP, Brazil. Fax: (19)3521-3073, Tel: (19)3521-3426, flsilva@iqm.unicamp.br*

[b] *School of Agriculture Engineering-UNICAMP, CEP 13083-875, Campinas, SP, Brazil. Tel: (19)99847-4003, cmessias@feagri.unicamp.br*

[c] *Department of Plant Biology, Pharmacy Course and BTPB Program, Institute of Biology, University of Campinas – UNICAMP, CEP 13084-971,*
10 *Campinas, SP, Brazil. Tel: (19)3521-6211,*

*Corresponding authors Tel. +55 019 35213073, fax. +55 019 35213073, eberlin@iqm.unicamp.br and achfsawa@unicamp.br

Abstract

Several organic acids such as malic, tartaric and citric acids are key grape and wine constituents. They greatly contribute to the
15 organoleptic properties of grapes and wines and are responsible for their acidity, a crucial wine property that is known to considerably vary as a function of grape variety, environmental conditions and viticulture, as well as during wine maturation. A rapid and simple method, requiring minimum sample pre-treatment and no chromatographic separation, based on direct infusion electrospray ionization mass spectrometry (ESI-MS) was developed and validated to quantitate organic acids in wine and grapes. The method was also demonstrated to be able to monitor a common wine adulteration procedure, that is, addition of citric acid for pH adjustment. ESI-MS data
20 when treated via principal component analysis (PCA) was also found to group wine samples based on the profiles and concentrations of organic acids.

Keywords: wine, grape, organic acids, direct infusion ESI-MS quantification

1. Introduction

Organic acids greatly contribute to the organoleptic quality of wine and grapes and their preservative properties increase physical and
25 chemical stability of this highly appreciated and widely consumed beverage. Being produced from grapes, the organic acids found in wine are mainly those found in grapes such as malic and tartaric acids, which are the main components responsible for the acidity (ca. pH 3) and the balance of the gustatory characteristics of wine (Andrade Lima, Schuler, Guerra, Pereira, Andrade de Lima, & Rocha, 2010). Grapes are one of the few fruits where the L isomer (+) of tartaric acid is found in high amounts, being that most of the acid taste of wines is due to this acid. The L (-) isomer of malic acid is found in leaves and fruits and is very common in nature but has little

resistance to oxidation. Although present in small amounts in grapes and wine, citric acid plays an important role in the Krebs cycle, inhibits the growth of yeasts, and is therefore frequently used as an acidifying agent in food and beverages. Therefore, an excessive amount of citric acid in wine indicates adulteration. The analysis of organic acids in wines is necessary for quality control as well as to monitor the evolution of acidity during the different stages of winemaking since important changes in wine may be detected by the alterations in the acid content (Mato, Suárez-Luque, & Huidobro, 2005).

The level of tartaric acid is also a critical control parameter in the stabilization of wine. Malic and tartaric acids are dominant in grapes and their levels are often used to determine the date of harvest, since each acid behaves differently during the maturation process. The content of malic acid declines continuously during the ripening process whereas the content of tartaric acid remains nearly constant resulting in different ratios between these acids during ripening. The ideal harvest date of grapes may be established based on the malic/tartaric acid ratio (Palma, & Barroso, 2002) and their profile and concentration have also been found to correlate with the grape variety, region of winemaking, processing techniques (alcoholic and malolactic fermentation) and maturation (Zheng, Duan, Zhang, Pan, Li, & Huang, 2009).

Various techniques have been used to identify and quantify organic acids in grapes, juice and wine, such as chromatography, electrophoresis and spectrophotometry (Villiers, Alberts, Tredoux, & Nieuwoudt, 2012; Ehling, & Cole, 2011; Frayne, 1986). Although capillary electrophoresis has been shown to be faster and may be used in a broader analytical range (Peres, Moraes, Micke, Tonin, Tavares, e Rodriguez-Amaya, 2009; Mato, Suárez-Luque, e Huidobro, 2007), liquid chromatography seems to be more sensitive and precise (Zotou,Loukou, e Karava, 2004; Zheng, Duan, Zhang, Pan, Li, e Huang, 2009).

Direct infusion electrospray ionization mass spectrometry (ESI-MS) has been offered as an attractive alternative to characterize complex chemical mixtures. The main advantages of ESI-MS are that neither chromatographic separation nor derivatization are needed, having minimal or no sample pretreatment at all, being also relatively simple and rapid. Our group and others have been successfully applying this method for the fingerprinting characterization of several complex mixtures such as those found in propolis (Cottica, Sawaya, Eberlin, Franco, Zeoula, & Visentainer, 2011), plant extracts (Schiozer, Cabral, Godoy, Chaves, Poppi, Riveros, Eberlin, & Barata, 2012), wine (Sawaya, Catharino, Facco, Fogaça, Godoy, Daudt, & Eberlin, 2011; Cooper & Marshall, 2001; Villagra et al., 2012; Zeng, Duan, Zhang, Pan, Li & Huang, 2009), coffee (Amorin, Hovell, Pinto, Eberlin, Arruda, Pereira, Bizzo, Catharino, Moraes Filho, & Rezende, 2009), biodiesel (Catharino, Milagre, Saraiva, Garcia, Schuchardt, & Eberlin, 2007) fruit juices (Roesler, Catharino, Malta, Eberlin, & Pastore, 2008; Flores, Hellin, & Fenoll, 2012) and essential oils (Møller, Catharino, & Eberlin, 2007).

We have already applied direct infusion ESI-MS fingerprinting for wine analysis, more specifically to characterize samples of must from six grape varieties during the fermentation process and samples of their respective wines after malolactic fermentation (Catharino, Cunha, Fogaça, Facco, Godoy, Daudt, Eberlin & Sawaya, 2006). Chemometric analysis of wine ESI-MS fingerprints was shown to group the samples and define diagnostic ions. The ESI-MS fingerprints also reflected the changes in composition due to the fermentation process. The method was also used to monitor the aging process of wines produced from diverse varieties grapes (Sawaya, Catharino,

2

Facco, Fogaça, Godoy, Daudt, & Eberlin, 2011) where PCA of the ESI-MS data was found to group the samples pinpointing markers for each group. But in these studies the quantitation abilities of the method was not tested since it was applied mainly for wine typification and quality control.

The aim of the present study was therefore to test the ability of this rapid and simple direct infusion ESI-MS fingerprinting method a step further in wine analysis. An analytical method was therefore developed and validated, not only to typify wines by their organic acid contents but also to quantitate these organic acids both in wines and grapes.

2. Materials and methods

2.1. Reagents

The standards used had a purity exceeding 99.0%: L-(-) malic acid, tartaric acid and citric acid (Sigma Aldrich). Stock solutions of these acids were prepared in a de-acidified red wine (DARW) at a concentration of 1000 μg / mL. Calibration curves were prepared from these stock solutions.

DARW was prepared from red wine and the acids were washed out using SPE cartridges. These C18 SPE cartridges (Supelco) were initially activated with 1 mL of methanol (analytical grade, JTBaker, 99.97%), then rinsed with 1 mL of ultrapure water, (Milli-Q, Millipore). Subsequently, 3 ml of red wine was loaded on the cartridge and washed with 3 mL of ultrapure water to remove the acids. The matrix retained in the cartridges was then extracted with 3 ml methanol and stored at 6 ^{0}C in a refrigerator.

2.2. Wine samples

As Table 1 summarizes, samples of wines from different countries produced from the Syrah variety of *Vitis vinifera*, as well as samples of wine from Paulista hybrid cultivar (Maximum IAC 138-22) and Brazilian wines from other grape varieties such as Carmen, Merlot and Bordô, were used. Bordô is a common denomination for *Vitis labrusca* grapes cultivated in the south of Brazil (Lago-Vanzela, Silva, Gomes, García-Romero & Hermosín-Gutiérrez, 2011).

2.3. Grape samples

Samples of Carmen BRS-hybrid grapes, derived from Muscat Belly and BRS-Rúbea developed by the Brazilian Agricultural Research (Embrapa) were donated by the Corol Cooperative juice industry, located in Rolândia, Parana State, Brazil. Samples of Bordô grapes (*Vitis labrusca*) were purchased from farmers in Santa Catarina State, Brazil. The other grape samples: a hybrid (Maximum IAC 138-22), an American (Niagara) and *Vitis vinifera* (Syrah) were donated by the College of Agricultural Engineering of Campinas (FEAGRI), located in Campinas, São Paulo State, Brazil. The grape samples were frozen at -20 ° C until analysis.

2.4. Preparation, extraction and recovery of samples of grapes

An ultrasound assisted extraction method was used (UAE) to extract and isolate organic acids from the grapes. Samples of approximately 2.10 g of skins, pulp and seeds of grapes were separately weighed, and lyophilized. Aliquots of 100 mg of the frozen lyophilized samples were ground in the presence of liquid N_2 and subsequently extracted with 1 ml of a methanol: water (1:1) solution,

stirred in sorbex (BenchMixer-Benchmark) mixer for 1 min and then placed in an ultrasonic bath (UNIQUE computed 1400) for 30 min. This material was then centrifuged for 2 min and the supernatant was separated. Extraction was repeated two more times on the remaining precipitate and the supernatants pooled. These extracts were filtered through a 22 μm membrane and diluted again in methanol: water (1:1) before ESI-MS analysis in different proportions, because the dilution was optimized for each part of the grapes. The validation of the recovery of tartaric and malic acids from this material was performed using extracts of skins and Syrah grapes at three concentration levels: low (1 mg / mL), medium (2 mg / mL) and high (3 mg / mL).

2.5. ESI-MS

Samples were analyzed by direct infusion ESI-MS with an Acquity TQD mass spectrometer (Waters, Manchester, UK) in the negative ion mode under the following conditions: capillary voltage -3.5 kV, cone -30 V, source temperature 150 °C, desolvation temperature 350 °C, mass range 100-620. An aliquot of 10 μL of wine was dissolved in 990 μL of methanol containing 0.1% ammonium hydroxide solution (Hexis). The extracts of skins, pulp and seed were diluted in methanol: water in the proportions of 1:25, 1:100 and 1:40 respectively. All samples were analyzed in triplicate.

2.6. Statistical Analysis

Principal component Analysis (PCA) was performed using the Pirouette software version 2.60 of Infometrix, Woodinville, WA, USA.

2.7. Validation of method

After defining the best conditions for MS analysis and sample dilutions, the ESI-MS method was validated in terms of selectivity, accuracy, linearity, precision and limits of detection (LOD) and quantification (LOQ) (Ribani, Bottoli, Collins, Jardim, & Melo, 2004; INMETRO, 2010).

2.7.1. Selectivity

Two sets of standard solutions in the concentration range from 1.25 to 10.00 μg mL^{-1} were analyzed: the first group was prepared with standard solutions of malic acid, tartaric acid and citric acid in methanol and the second set of solutions was prepared in the de-acidified wine. Each solution for each concentration was prepared in triplicate to assess the matrix effect. A simple statistical test (the F test) was applied. The ratio between the variances of the two samples ($F = s_1^2/s_2^2$) was calculated and compared to the critical value of F at a given confidence level of 95% for three acids (INMETRO, 2010).

2.7.2. Accuracy

Three samples of wine were fortified with the organic acids in concentrations of 1.25 μg/ mL, 2.50 μg/ mL and 5.00 μg/ mL. These samples were also prepared and injected in triplicate. The accuracy of the analytical method was expressed by the ratio between the

4

experimentally determined average and the corresponding theoretical concentration, with the result given as recovery % (INMETRO, 2010).

2.7.3. Linearity

5   The linear range was established using five different solutions with concentrations obtained from dilutions of the stock solution of 1000 µg / mL of each acid (malic, tartaric and citric acids) de-acidified wine as follows: 1.25 µg / mL, 2.50 µg / mL, 5.00 µg / mL, 7.50 µg / mL and 10.00 µg / mL. The samples were prepared and analyzed in triplicate and the analytical curve for each acid was obtained by correlation between the concentration and area of the analytic signal through linear least squares model (Origin 6.0).

10 ### 2.7.4. Limits of detection (LOD) and quantification (LOQ)

The limit of detection (LOD) was calculated as the lowest detectable concentration (and not necessarily quantified) whereas the limit of quantification (LOQ) was the lowest concentration of the acid of interest that could be measured. The LOD and LOQ parameters were calculated based on the calibration curve, expressed as:

$LOD = 3, 3 \times s / S$; $LOQ = 10 \times s / S$

15 Where 's' is the estimated standard deviation of the regression line equation and S is the slope of the calibration curve (INMETRO, 2010).

2.7.5. Precision

Precision, as defined as the evaluation of the closeness of the results obtained in a series of measures of a multiple sampling of the 20 same sample, was herein considered at three levels: through repeatability, intermediate precision and reproducibility, and was expressed by the standard deviation and coefficient of variation.

2.7.6. Repeatability (intra-run precision)

The repeatability, which can be expressed quantitatively in terms of the characteristic dispersion of the results of areas, was obtained 25 by integration of the analytical signals. The intra-run precision was assessed by analysis of the standard solutions prepared in de-acidified wine at three concentration levels (2.0 µg / mL, 4.0 µg / mL and 8.0 µg / mL). Five replicates of each level of acid concentration were analyzed, obtaining values of five areas with their respective standard deviation (INMETRO, 2010).

2.7.7. Intermediate (inter-run) precision

30   To determine intermediate precision, 5 replicates were analyzed at three concentration levels: low (2 µg / mL), medium (4 µg / ml) and high (8 µg / ml) of each acid on different days with the same analyst. For each level of acid concentration, five values were calculated as well as their respective standard deviations (INMETRO, 2010).

2.8 High resolution MS data

   HRMS were acquired using a 7.2T LTQ FT Ultra mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a direct infusion electrospray ionization source (ESI) operating in the negative ion mode under the follow conditions: capillary voltage - 3.1 kV, tube lens - 160 V, temperature 280 °C. Data acquisition was performed along the *m/z* 100-1000 range by the Xcalibur 2.0 software. Wine samples were prepared by dissolving the wine in methanol 1:1. For ESI(-)-MS, an aqueous solution of 0.1 % ammonium hydroxide was added to facilitate deprotonation of the more acidic molecules (M) to form [M – H]⁻ ions.

2.9. Addition of citric acid to simulate adulteration.

   Using the stock solution of citric acid, pure wine samples were spiked to concentrations of citric acid between 1.0 and 100.0 mg / L. Since Brazilian legislation fails to determine a maximum amount of citric acid that may be added to wine, the purpose of this study was to determine the LOD of citric acid that could be detected by direct infusion ESI(-)-MS.

3. Results and discussion

3.1. Method validation

   The selectivity of direct infusion ESI(-)-MS was satisfactory for all three organic acids measured. The F test showed no significant difference between the variances of the set of samples diluted in methanol and diluted in de-acidified wine. The matrix effect failed to affect the results, since the F value calculated for the three acids was less than the critical F (Table S1). In complex matrixes, such as wine, the co-ionization of several molecules affects the ESI efficiency of each individual molecule, usually reducing the relative abundance of the respective ion. Although the average areas of the ion peaks in methanol were greater than those using de-acidified wine, the differences were not significant (F test). Although we ran our calibration curves using de-acidified wine, solutions of malic and tartaric acid standards in methanol/water could also be used to build the calibration curves, further simplifying the proposed method.

   Table S2 shows the results of linearity, LOD, LOQ and accuracy, expressed as percent recovery. The ESI-MS method showed good linearity with R^2 values higher than 0.993 in all cases. LOD between 0.278 and 0.711 µg / mL and LOQ between 0.843 and 2.157 µg / mL were relatively low, and similar to values reported for these acids using other techniques (Zotou, Loukou, & Karava, 2004; Mato, Suárez-Luque, & Huidobro, 2007). The standard deviations were lower than 5%, which demonstrates proper repeatability and instrumental stability, providing values also similar to those obtained when applying more demanding methods (Mato, Súarez-Luque, & Huidobro, 2007; Zheng , Duan, Zhang, Pan, Li, & Huang, 2009).

   The accuracy of the ESI-MS method (Table S3) was also satisfactory and within the accepted limits of 70-120% (INMETRO, 2010). Also for all acids, repeatability and intermediate precision tests were within the acceptable values: 5 and 15%, respectively (Ribani, Bottoli, Collins, Jardim, & Melo, 2004). The intermediate precision of the method was evaluated by comparing the ion peak areas obtained for each acid on different days by F test and no significant difference between days was found (p <0.01) for all three organic

6

acids. The CV% (coefficient of variation) for intra-day precision ranged from 0.82 to 2.99% and for inter-day precision from 0.82 to 4.26%, indicating good precision.

3.2. Concentration of malic, tartaric and citric acids in wine

Table 1 shows the concentrations of malic, tartaric and citric acids found in wine samples using the direct infusion ESI-MS method. Overall in wine samples, higher content of tartaric acid was found compared to other acids, except in the RS/Brazilian (Merlot grape), RS/Brazilian (Isabel grape) and in the Chilean and Argentinian (both Syrah grape) wines. This variation is possibly due to the structure of the grapes, especially the skins, where a higher concentration of tartaric acid is found. Another aspect that may have contributed to the higher content of tartaric acid than malic acid in some wines is malolactic fermentation (MLF) responsible for the transformation of malic acid into lactic acid and carbon dioxide through the action of lactic acid bacteria. Other variables such as climatic conditions, grape maturation cycle, preparation process and storage conditions can also affect the acid content.

3.3. Validation of the procedure for extraction and analysis of organic acids in grapes

Table S3 shows the results of the linearity of the analytical curve, limit of detection and quantification accuracy for grapes, whose results were expressed as percentage of recovery. The extraction and dilution parameters were optimized and with linear coefficients of the calibration curves were R2=0.992 for malic acid and R2=0.998 for tartaric acid. The accuracy was considered satisfactory (within the limits accepted by INMETRO of 70 to 120% recovery), as well as the other parameters of CV%, recovery, linearity, LOD, and LOQ. The CV% values and percentage of recovery of the extracts fortified with standards at different concentrations showed good precision and accuracy, evaluated by the recovery tests.

3.4. Concentration of malic, tartaric and citric acids in grapes.

Quite opposite to the wine samples (Table 1), predominance of malic acid to tartaric acid was observed for all grape samples analyzed (Table 2). Grapes of American origin and hybrids (Bordô, Niagara, Carmen and Maximum BRS) have skins and pulp with higher levels of tartaric acid as comparison with *Vitis vinifera* (cultivar Syrah). For seeds, the reverse is observed and the *Vitis vinifera* cultivar has a higher content of tartaric acid than the group of American and hybrid grapes. Lower malic acid content was found in the skin, pulp and seeds of Bordô grapes.

3.5. PCA of the ESI-MS fingerprints

Wine ESI(-)-MS fingerprints were found to be similar to those of grape skins and therefore they are placed closely by PCA. When samples of wine and grape skins are individually evaluated, they formed however two distinct groups (Figure 1). The ESI(-)-MS fingerprints of samples skins, pulp and seed of grapes differ mostly in the abundance of common ions of *m/z* 115, 133, 149 and 179 (Figure 2). These fingerprints of the skins, pulp and seed also present other common ions of *m/z* 119, 179, 191, 341.113, 161, 195 and 321.

3.6. High resolution ESI(-)-MS fingerprints

High resolution ESI(-)-MS data of a wine sample permitted the separation of the isobaric ions of m/z 191 from quinic acid and citric acid (Figure 3 and Table 3). Although detected together in low resolution ESI(-)-MS as a single ion, both of these isobaric acids normally produce less abundant ions of m/z 191 in wines than the ion of m/z 193 from galacturonic acid. The m/z 191:193 ratio can be used therefore to monitor wine adulteration by the addition of citric acid. Figure 4 shows a typical ESI(-)-MS fingerprint of wine with (A) a normal m/z 191:193 ratio as well as the same sample spiked with citric acid. Note that at already at a concentration of 18 μg/ mL (less than 20 ppm), this ratio inverts. The presence of malic and tartaric acids in the wine sample was also confirmed by high resolution MS (Figure 5). Note that no other ions of the same nominal m/z are present, therefore the low resolution ESI(-)-MS is capable of quantifying these acids without interference of isobaric compounds.

4. Conclusion

Direct infusion ESI-MS is a method that provides quite comprehensive wine analysis. As shown herein, it can be successfully applied to quantitate organic acids in wine and grapes. Main advantages are its speed (less than 5 min per sample of total analysis time) and simplicity and the analysis of the intact sample with no pre-separation or pre-derivatization procedures. Linearity, precision, sensitivity and recuperation were as good as those of chromatographic methods using pre-separation via liquid chromatography (Zotou,Loukou, e Karava, 2004; Zheng, Duan, Zhang, Pan, Li, e Huang, 2009) or capillary electrophoresis (Peres, Moraes, Micke, Tonin, Tavares, e Rodriguez-Amaya, 2009; Mato, Suárez-Luque, e Huidobro, 2007). Additionally, direct infusion ESI-MS also provides wine typification as demonstrated herein via PCA analysis, which grouped samples according to similar concentrations of organic acids. Two groups were formed, the first consisting of wines originating from American grapes (Merlot and Bordô) and the second group consisting of wines from national and hybrid grapes (Syrah, Maximum and Carmen). Tartaric acid was found to be predominant in most national and imported wines analyzed.

The method is shown to be also able to monitor wine adulteration by the addition of exogenous acids, and citric acid with a LOD of ca. 20 ppm could be detected. Direct Infusion ESI-MS was also shown applicable to monitoring different stages of ripeness in grapes and to establish the ideal time of the grape harvest as the concentrations of tartaric and malic acid cam be quantified. All national varieties and hybrid grapes were shown to display similar chemical ESI-MS profiles but differ in abundances of ions from malic and tartaric acids, making therefore possible to simultaneously evaluate, both qualitatively and quantitatively, the presence of these key analytes in different samples of grape skins, pulps and seeds.

8

5. References

Amorin, A.C.L., Hovell, A.M.C., Pinto, A.C., Eberlin, M.N., Arruda, N.P., Pereira, E.J., Bizzo, H.R., Catharino, R.R., Moraes Filho, Z.B., Rezende, C.M. (2009). Green and roasted arabica coffees differentiated by ripeness, process and cup quality via electrospray ionization mass spectrometry fingerprinting. *Journal of the Brazilian Chemical Society,* 20 (2), 313-321.

Andrade Lima, L.L., Schuler, A., Guerra, N.B., Pereira, G.E., Andrade Lima, T.L., & Rocha, H. (2010). Otimização e validação de métodos para determinação de ácidos orgânicos em vinhos por cromatografia líquida de alta eficiência.*Quimica Nova*, 33 (5), 1186 -1189.

Catharino, R.R., Cunha, I.B.S., Fogaça, A.O., Facco, E.M.P., Godoy, H.T., Daudt, C.E., Eberlin, M.N., & Sawaya, A.C.H.F. (2006). Characterization of must and wine of six varieties of grapes by direct infusion electrospray ionization mass spectrometry. *J. Mass Spectrometry*, 41,185-190.

Catharino, R.R., Milagre, H.M.S., Saraiva, S.A., Garcia, C.M., Schuchardt, U., & Eberlin, M.N. (2007). Biodiesel typification and quality control by direct infusion electrospray ionization mass spectrometry fingerprinting. *Energy & Fuels*, 21, 3698-3701.

Cooper, H.J., & Marshall, A.G. (2001). Electrospray Ionization Fourier Transform Mass Spectrometric Analysis of Wine. *J. Agric. Food Chem*, 49, 5710-5718.

Coordenação Geral de Acreditação. Orientação sobre Validação de métodos analíticos. DOQ-CGCRE-008-Revisão 03 – Fev/2010.

Cottica, S.M., Sawaya, A.C.H.F., Eberlin, M.N., Franco, S.L., Zeoula, L.M., Visentainer, J.V. (2011). Antioxidant activity and composition of propolis obtained by different methods of extraction. *Journal of the Brazilian Chemical Society*, 22 (5), 929-935.

Ehling, S., & Cole, S. (2011). Analysis of organic acids in fruit juice by liquid chromatography-mass spectrometry: an enhanced tool for authenticity testing. *Journal of Agricultural and Food Chemistry*, 59, 2229-2234.

Flores, P., Hellín, P., & Fenoll J. (2012). Determination of organic acids in fruits and vegetables by liquid chromatography with tandem-mass spectrometry. *Food Chemistry*, 132, 1049-1054.

Frayne, R.F. (1986). Direct analysis of the major organic components in grape must and wine using high performance liquid chromatography. *American journal of enolology and viticulture*, 37 (4), 281- 287.

Mato, I., Suárez-Luque, S., Huidobro, J.F. (2005). A review of the analytical methods to determine organic acids in grape juice and wines. *Food Research International*, 38,1175-1188.

Mato, I., Suárez-Luque, S., & Huidobro, J.F. (2007). Simple determination of main organic acids in grape juice and wine by using capillary zone electrophoresis with direct UV detection. *Food Chemistry*, 102, 104-112.

Møller, J.K.S., Catharino, R.R., & Eberlin, M.N. (2007). Electrospray ionization mass spectrometry fingerprinting of essential oils: Spices from the labiatae family. *Food Chemi*stry, 100, 1283.

5 Palma, M., & Barroso, C.G. (2002). Ultrasound-assisted extraction and determination of tartaric and malic acids from grapes and winemaking by products. *Analytica Chimica Acta*, 458, 119-130.

Ribani, M., Bottoli, C.B.G., Collins, C.H., Jardim, I.C.S.F., & Melo, L.F.C. (2004). Validação em métodos cromatográficos e eletroforéticos. *Química Nova*, 27, 771-780.

10

Roesler, R., Catharino, R.R., Malta, L.G., Eberlin, M.N., & Pastore, G. (2008). Antioxidant activity of caryocar brasiliense (pequi) and characterisation of components by electrospray ionization mass spectrometry. *Food Chemistry*, 110, 711-717.

Sawaya, A.C.H.F., Catharino, R.R., Facco, E.M.P., Fogaça, A.O., Godoy, H.T., Daudt, C.E., Eberlin, M.N. (2011). Monitoring of wine aging process by

15 electrospray ionization mass spectrometry. *Ciência e Tecnologia de Alimentos*, 31 (3), 730-734.

Schiozer, A.L., Cabral, E.C., Godoy, L.A.F., Chaves, F.C.M., Poppi, R.J., Riveros, J.M., Eberlin, M.N., & Barata, L.E.S. (2012). Electrospray ionization mass spectrometry fingerprinting of extracts of the leaves of arrabidaea chica. *Journal of the Brazilian Chemical Society,* 23 (3), 409-414.

20 Villaga, E., Santos, L.S., Vaz, B.G., Eberlin, M.N., & Laurie, V.F. (2012). Varietal discrimination of Chilean wines by direct injection mass spectrometry analysis combined with multivariate statistics. *Food Chemistry*,131, 692-697.

Villiers, A., Alberts, P., Tredoux, A.G.J., Nieuwoudt, H.H. (2012). Analytical techniques for wine analysis: An African perspective; a review. *Analytica Chimica Acta*, 730, 2-23.

25

Zen, C., Zhou, Y., Zhang, N., Wang, J., & Xiong, C. (2013). Differentiation of Chinese liquors by using ambient glow discharge ionization mass spectrometry. Analyst, 138, 3830-3835.

Zheng, Y.J., Duan, Y.T., Zhang, Y.F., Pan, Q.H., Li, J.M., & Huang, W.D. (2009). Determination of Organic Acids in Red Wine and Must on Only One

30 RP-LC-Column Directly After Sample Dilution and Filtration. *Chromatographia*, DOI: 10.1365/s10337-009-1085-0.

Zotou, A., Loukou, Z., & Karava, O. (2004). Method Development for the Determination of Seven Organic Acids in Wines by Reversed-Phase High Performance Liquid Chromatography. *Chromatographia*, 60, 39-44

10

Lago-Vanzela, E. S., Da-Silva, R., Gomes, E., Garcia-Romero, E., Hermosin-Gutiérrez, I. (2011). Phenolic Composition of the Edible Parts (Flesh and Skin) of Bordô Grape (Vitis labrusca) Using HPLC-DAD-ESI-MS/MS. J. Agric. Food Chem., 59 (24), 13136-13146. DOI: 10.1021/jf203679n.

5

Table 1. Concentrations of tartaric, malic and citric acids in samples of Brazilian and imported red wines as measured by direct infusion ESI(-)-MS (mg/mL)

wine	grape	origin	malic acid	tartaric acid	citric acid
2	Bordô*	RS/ Brazil	2.79	7.63	1.82
3	Bordô*+Merlot	RS/Brazil	2.88	9.94	3.88
4	Bordô*+Merlot	RS/Brazil	2.05	2.58	1.77
5	Syrah	New Zealand	4.18	5.48	1.70
6	Isabel+Bordô*	RS/Brazil	5.07	5.93	2.77
7	Syrah	France	5.38	4.91	2.62
8	Carmem	RS/Brazil	2.75	4.24	2.48
9	Merlot	RS/Brazil	6.30	3.01	1.49
10	Syrah	Australia	2.99	3.47	1.47
11	Syrah	Italy	4.56	3.49	1.61
12	Syrah	Chile	5.01	4.60	1.74
13	Syrah	Greece	4.58	2.83	3.59
14	Syrah	Portugal	3.61	5.55	1.78
15	Bordô*	SP/Brazil	4.16	4.52	1.35
16	Isabel	SP/Brazil	8.72	3.56	1.43
17	Syrah	Argentina	4.49	6.73	2.85
18	Syrah	Portugal	4.57	5.52	2.19
19	Syrah	South Africa	2.81	4.34	1.82
20	Syrah	Vale São Francisco, Brazil	4.20	3.74	1.39
21	Syrah	Chile	4.79	6.32	2.46
22	Syrah	Argentina	3.38	4.44	1.54
23	Maximum	SP/Brazil	2.90	3.66	1.28
24	Syrah	Chile	3.16	4.44	1.45
25	Maximum	SP/Brazil	3.15	4.61	1.21
26	Syrah	France	7.46	4.23	4.32
27	Merlot	RS/Brazil	6.37	3.02	1.67

Bordô*- Brazilian denomination for *Vitis labrusca* grapes cultivated in the south of Brazil.

Table 2. Concentrations of malic and tartaric acids in national samples of grapes and hybrids grown in Santa Catarina, Paraná and São Paulo.

sample	Concentration of the tartaric acid			Concentration of the malic acid		
	diluted sample μg/mL	CV%	μg/100mg lyophilized material	diluted sample μg/mL	CV%	μg/100mg lyophilized material
Carmem skins	4.66	1.14	18.65	9.82	0.77	39.30
Niagara skins	4.41	2.61	17.63	8.41	2.86	33.65
Bordô* skins	2.91	0.64	11.63	4.88	1.32	19.51
Syrah skins	1.73	6.34	6.90	8.19	4.54	32.77
Maximum skins	4.55	6.29	18.18	6.05	3.26	24.22
Bordô* pulp	6.69	1.15	334.41	7.25	1.59	725.48
Carmen pulp	2.83	1.21	141.36	8.13	12.83	813.15
Maximum pulp	4.72	1.65	236.02	9.55	6.05	954.79
Niagara pulp	6.04	2.36	301.85	8.04	6.15	803.96
Syrah pulp	4.53	0.79	226.54	7.63	8.26	762.78
Bordô* seeds	2.02	20.61	40.39	1.22	9.74	48.99
Niagara seeds	4.05	13.13	81.07	4.60	11.78	184.15
Syrah seeds	3.32	4.70	132.72	7.86	10.04	314.31
Carmem seeds	0.94	3.42	37.55	5.51	6.56	220.25
Maximum seeds	2.60	7.85	104.03	5.24	9.21	209.77

Bordô*- Brazilian denomination for *Vitis labrusca* grapes cultivated in the south of Brazil.

Table 3. Compounds identified in the wine and grape analyzed by ESI (-) FT-ICR MS.

Acids	Formula	Theoretical *m/z*	Experimental *m/z*	Error (ppm)
Malic	$C_4H_5O_5$	133.01430	133.01425	0.38552
Tartaric	$C_4H_5O_6$	149.00924	149.00916	-0.10995
Citric	$C_6H_7O_7$	191.01970	191.01973	-0.10995
Quinic	$C_7H_{11}O_6$	191.05611	191.05611	-0.01150
Galaturonic	$C_6H_9O_7$	193.03537	193.03538	-0.01547

14
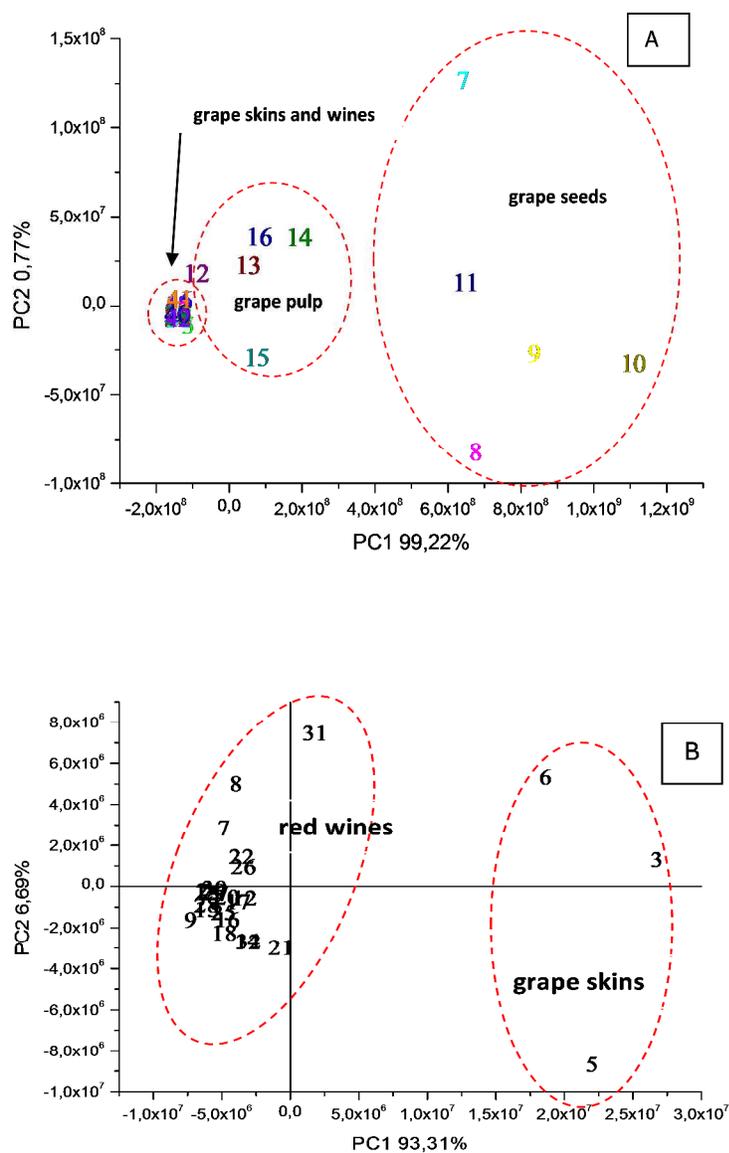
Figure 1. Principal component analysis (PC1 x PC2) applied to the ESI(-)-MS data: (**A**) samples of skin, pulp(12-16) and seeds (7-11) of grapes as well as wine samples; (**B**) samples of red wines (7-32) and skins from different varieties of grapes: Niagara (3), Syrah (5) and Maximum hybrid (6).
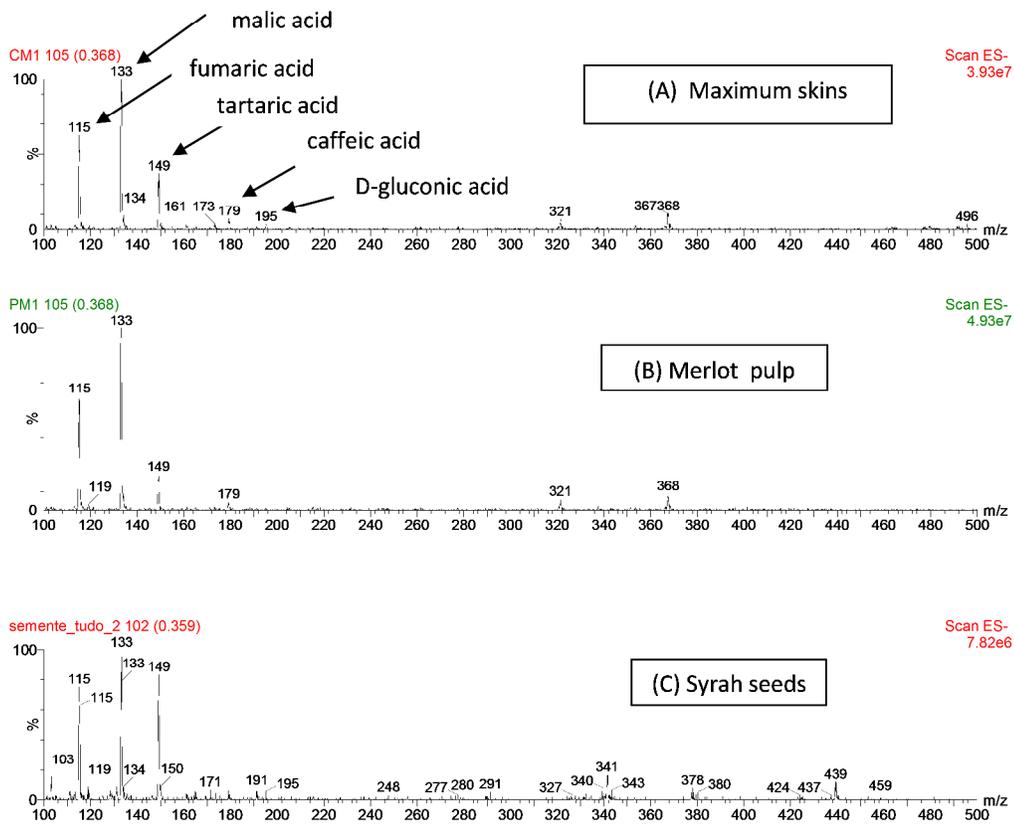
Figure 2. ESI(-)-MS fingerprints of samples of skins (A), pulp (B) and seed (C) of different grapes. Major organic acids detected as [M − H]⁻ ions are indicated in (A).
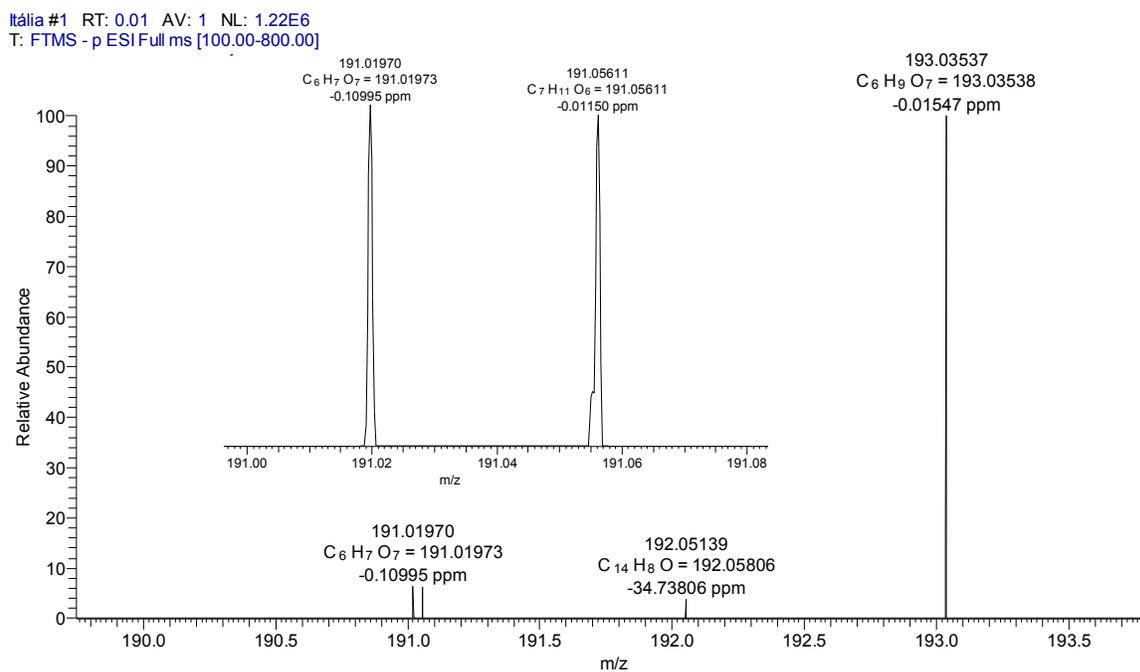
5

16

Figure 3. High resolution ESI FT-ICR MS in the *m/z* 190-194 range showing resolution and accurate *m/z* values for [M − H]⁻ ions of citric (*m/z*

191.01989), quinic (*m/z* 191.05611) and galacturonic *(m/z* 193.03547) acids.
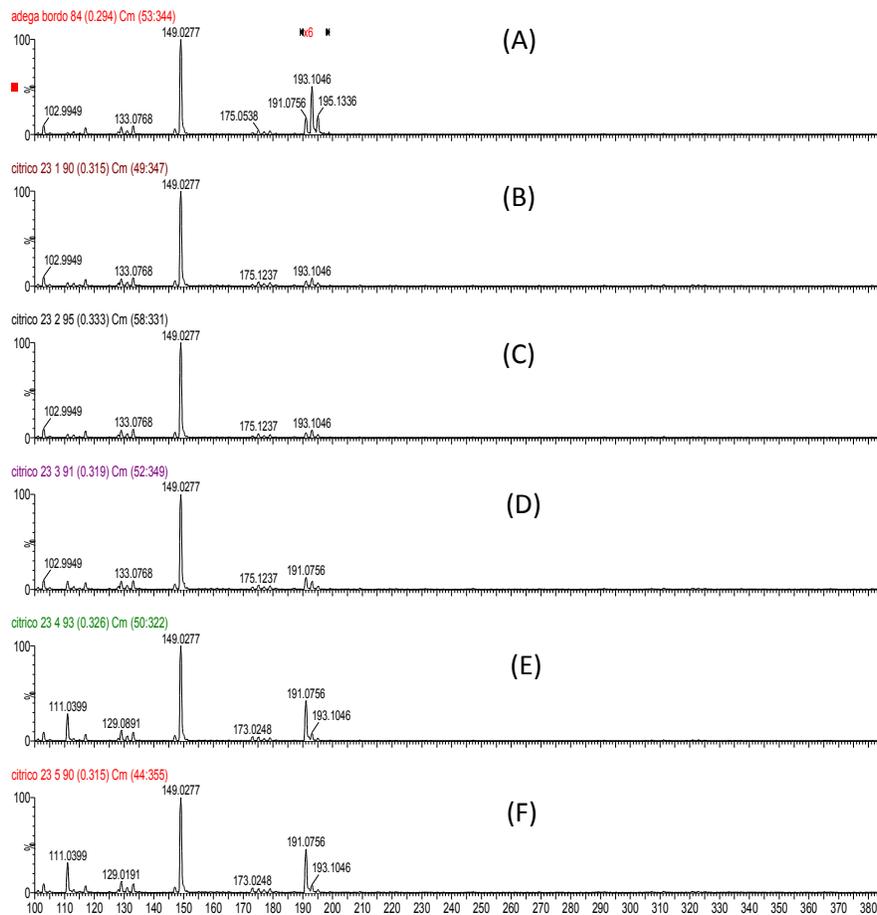
5

Figure 4. Direct infusion ESI(-)-MS fingerprints of: (A) a pure wine sample as well as the same sample adulterated with citric acid to the concentration of (B) 1.0 µg/mL, (C) 3.0 µg/mL, (D) 18 µg/mL, (E) 70.0 µg/mL and (F) 100 µg/mL. Observe relative intensities of *m/z* 149- tartaric acid, *m/z* 191- citric acid an *m/z* 193 galacturonic acid.
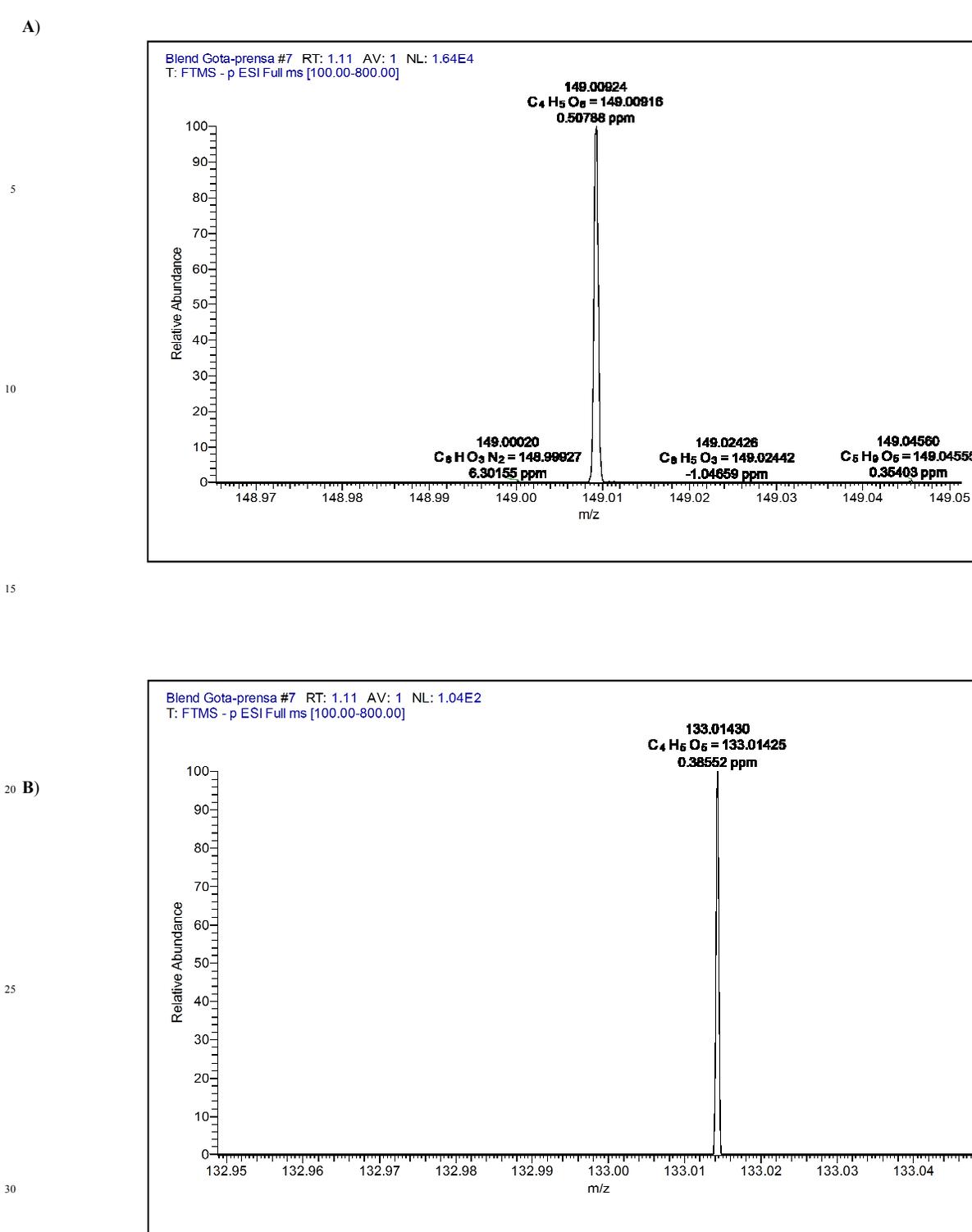
5

18

A)

B)

Figure 5. High resolution ESI(-) FT ICR MS data showing accurate *m/z* values for [M − H]⁻ ions of: (A) tartaric acid and (B) malic acid found in wines and grapes.