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# Characterization of must and wine of six varieties of grapes by direct infusion electrospray ionization mass spectrometry

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Samples of must derived from six different varieties of grapes taken during the fermentation process, as well as the respective wine samples directly after the end of the malolactic fermentation, were analyzed by direct infusion negative ion mode electrospray ionization mass spectrometry (ESI-MS). Diagnostic ions for must were different from those of wine samples, although small variations for each of the grape varieties were also detected. The addition of unfermented must or sugar to wine could also be clearly detected. The spectra were acquired in a few minutes per sample, indicating that ESI-MS can be used for high-throughput analysis of samples and should prove useful for quality control during and after the fermentation process. Copyright © 2005 John Wiley & Sons, Ltd.

## INTRODUCTION

References to wine date back to biblical times and its production and consumption are characteristic of many cultures. Red wines are made into a variety of styles, based on differences in wine characteristics such as grape variety, color, flavor, body, mouth feel, and aging potential.[1–3] In addition to the many grape varieties available for red wine production,[4–6] factors such as soil, climate, growing conditions, and viticultural practices influence the composition of the fruit, and, therefore, the style of wine that can be produced. Varieties from the Vinifera group are most widely used for winemaking, although in regions where Vinifera grapes are not grown, French hybrids, Labrusca, and other varieties are often used.[7–9]

An important aspect of red wine is that, as the fermenting must consists of juice, skins and seeds, the composition is determined by constituents extracted from all these sources. In a highly competitive market, wineries need to invest in technology, increase productivity and improve average quality to remain competitive. Several compounds play a key role in problematic wine fermentations, such as sugars, nitrogen substrates and organic acids.[6–9] As

conventional chemical analysis is time consuming; currently, these compounds are not measured frequently enough. This problem is more acute in large wineries that operate hundreds of fermentation tanks simultaneously.

In regard to chemical composition, electrospray ionization mass spectrometry (ESI-MS) with direct infusion has appeared as a new alternative, offering a fast and robust technique for wine analysis. Recently, Cooper and Marsall[10] demonstrated that ESI-FTMS could be used to obtain the elemental composition of fingerprinting components of white and red wine. They noted that negative ion mode ESI-MS fingerprint showed greater variety in the composition and abundance of components and a lesser amount of salt adducts as compared to the positive ion mode. ESI-MS is a fast and sensitive method that can be used for at-line monitoring of the more polar components of wine without any need for sample preparation or component extraction. Direct infusion ESI-MS has also been applied with success as a fast fingerprint method for complex mixtures such as plant extracts,[11] propolis,[12] beer,[13] whisky[14] and vegetable oils.[15]

In this work, we therefore tested ESI-MS as a method for the fast characterization of samples of must derived from six different varieties of Brazilian grapes taken during the fermentation process, as well as the respective wine samples directly after the end of the malolactic fermentation. ESI-MS in the negative ion mode was used because acidic compounds such as phenols and flavonoids, as well as sugars, are easily ionized under these conditions. Chemometric analysis of the fingerprints obtained was used to group the samples and define the diagnostic ions of each group.

## EXPERIMENTAL

### Samples of must and wine

Samples of must and wine were obtained from Vinicola Velho Amâncio near Santa Maria; in the state of Rio Grande do Sul, Brazil. Samples of must were collected before the fermentation process began, during the fermentation at intervals of 6°Brix and at the end of the malolactic fermentation (wine). The term Brix refers to the sugar content of the grapes and wine as determined by a hydrometer, which indicates the liquid's specific density. Each degree Brix is equivalent to 1 g of sugar per 100 g of juice. The following varieties of grape were studied: Pinot Noir (V2 – must, V7 – wine), Cabernet Sauvignon (V3 – must, V8 – wine), Malbec (V4 – must, V9 – wine), Merlot (V5-must, V10 – wine), Isabel (V6 – must, V11 – wine), and Bordô (V12 – must, V13 – wine). The first four varieties are normally used for the production of wines, whereas the last two are more commonly used for grape juice.

### General experimental procedure

Samples of wine and must were analyzed by direct infusion ESI by means of a syringe pump (Harvard Apparatus) at a flow rate of 10 µl/min. Negative ion mode ESI-MS) fingerprints and negative mode ESI-MS/MS for low energy collision-induced dissociation (CID) were acquired using a hybrid high-resolution and high-accuracy (5 ppm) Micromass Q-TOF mass spectrometer. Capillary and cone voltages were set to −3000 V and −50 V respectively, with a desolvation temperature of 100 °C. One milliliter of each sample was added to 10 ml of a solution containing 70% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA) and 30% (v/v) deionized water and 5 µl of ammonium hydroxide (Merck, Darmstadt, Germany) per milliliter. Fingerprint mass spectra were acquired in the range between $m/z$ 100 and 1400. No ions attributed to the solvents used were observed in this range.

### Statistical analysis of data

Principal component analysis (PCA) was performed using the 2.60 version of Pirouette software from Infometrix, Woodinville, WA, USA. The mass spectra were expressed as the intensities of individual $[M − H]^-$ ions (i.e. variables). Ions with relative intensities of less than 5% were excluded. The data was preprocessed using auto scale and the PCA method was run.

## RESULTS AND DISCUSSION

Figure 1 shows the ESI-MS fingerprints of must from the six varieties of grapes, sampled before the fermentation process began. When these spectra are compared, similarities between the samples are observed. All fingerprints display ions of $m/z$ 277, 313, 329, 359, 457, 539, 704 and 794 with variable intensities, which can be considered diagnostic ions for the unfermented must. The MS/MS spectra (not shown) of these ions show they are all clusters of a hexose ($m/z$ 179) with other low mass compounds present in the must. For example, $m/z$ 329 is a cluster of this hexose with a deprotonated organic acid of $m/z$ 149; $m/z$ 359 is the deprotonated dimer of the hexose. As sugar is consumed during the fermentation process, these ions disappear and
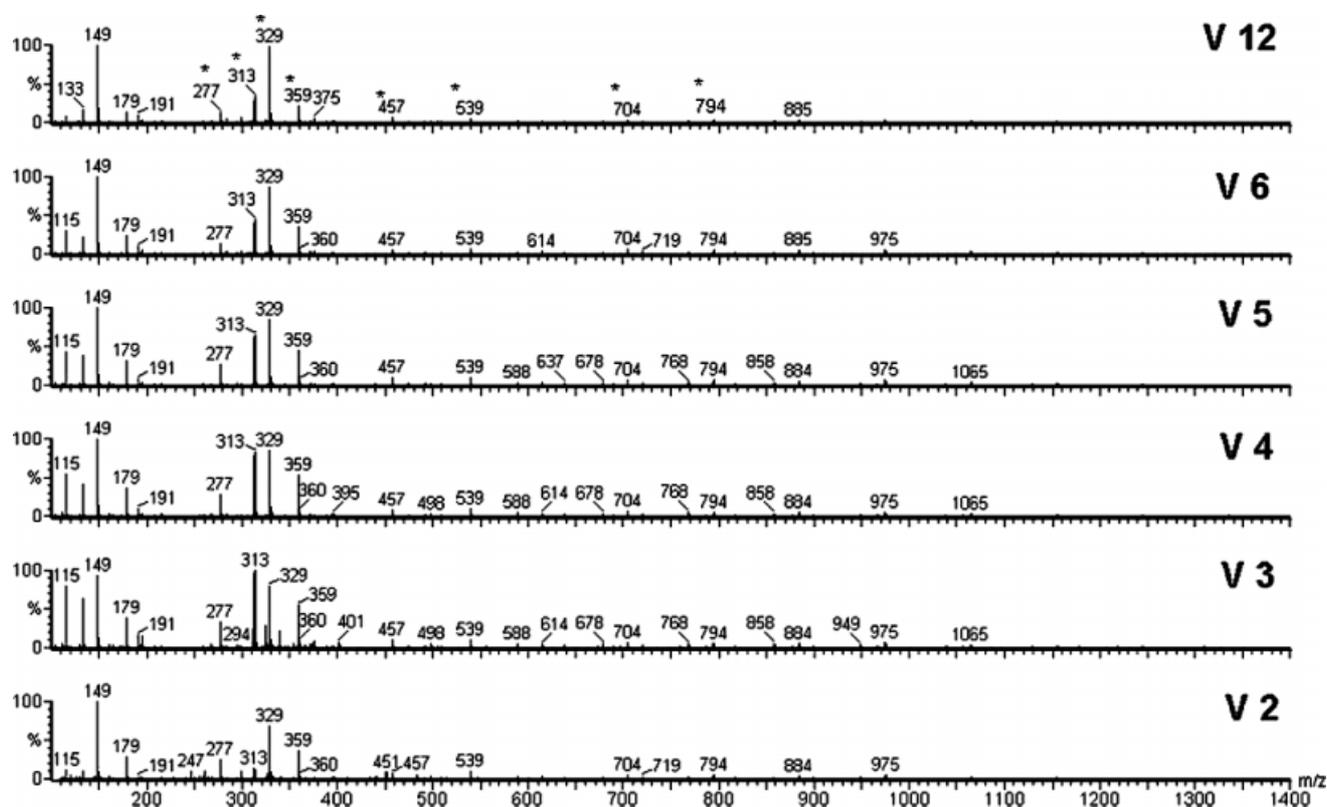


**Figure 1.** ESI-MS fingerprints in the negative ion mode of must from the following six varieties of grapes before fermentation: Pinot Noir (V2), Cabernet Sauvignon (V3), Malbec (V4), Merlot (V5), Isabel (V6) and Bordô (V12). *Diagnostic ions for must.

are no longer observed in the fermented wine. Fingerprints of different varieties of grape show slight individual variations. For example: for the sample of Pinot Noir must (V2), the ion of $m/z$ 313 is much less intense and a unique ion of $m/z$ 451 is present; the ion of $m/z$ 588 is only clearly detected in the fingerprints of Cabernet Sauvignon (V3), Malbec (V4) and Merlot (V5) must samples being practically absent in the other samples of must. These differences cannot be considered diagnostic of the grape variety, as only one sample of each was analyzed, and are presented only for illustrative purposes.

The ESI-MS fingerprints of the samples of wine taken at the end of the malolactic fermentation also show similarities and some important differences (Fig. 2). In the fingerprints of all these samples, the ions of $m/z$ 439 and 559 can be observed, and are therefore identified as diagnostic ions for wine. These ions were also observed in the negative mode fingerprints of several wine samples analyzed by ESI-FTMS, with that of $m/z$ 439 being the most intense for the California Red wine and that of $m/z$ 559 for Beaujolais.[10] The ion of $m/z$ 491, present in fingerprints of five of the wine samples analyzed by us was also observed in three wine samples by Cooper and Marshall (2001).[10] What is more important is that none of the marker ions for the unfermented must are seen in the fermented wine, as they are all related to sugars consumed during the fermentation process. Differences for each variety of wine are also observed. Pinot Noir (V7) is the only one to show intense ions of $m/z$ 245, 289 and 311, whereas Cabernet Sauvignon (V8) shows intense ions of $m/z$ 267 and 283, which can also be observed as less intense ions for the other four

samples. Ions of $m/z$ 299 and 369 are detected for Malbec (V9), Merlot (V10), Isabel (V11), and Bordô (V13). Merlot (V10) displays unique ions of $m/z$ 301 and 621, as well as the ion of $m/z$ 735 also displayed by Isabel (V11). Isabel (V11) and Bordô (V13) display similar fingerprints that differ only in relation to the ions of $m/z$ 745 and 925 for Isabel (V11) and $m/z$ 783 for Bordô (V13). Therefore, ESI-MS fingerprints are able to detect common diagnostic ions for wine as well as reveal differences between wines made from different varieties of grapes. Further studies with more samples of wine derived from the same grape variety could confirm diagnostic ions for each variety.

The components detected as the ions of $m/z$ 115, 133, 149 and 191, found in the fingerprints of both the must and fermented wine are apparently unaffected by fermentation. The ions of $m/z$ 115, 133, 149 are likely to be de-protonated organic acids owing to their CID patterns (neutral loss of 44 corresponding to $CO_2$), whereas that of $m/z$ 191 was identified as de-protonated quinnic acid by comparison with a standard. The ion of $m/z$ 179 found in all fingerprints of must was identified via ESI-MS/MS as a mixture of the deprotonated molecules of both caffeic acid and a hexose. In the samples of wine, this ion corresponds only to deprotonated caffeic acid, confirming that the sugar was consumed in the fermentation process.

To show more clearly how the composition changes during fermentation, Fig. 3 compares the fingerprints of samples taken during fermentation at intervals of 6°Brix. Random samples are presented to demonstrate that the change is similar for all the varieties analyzed. The high mass
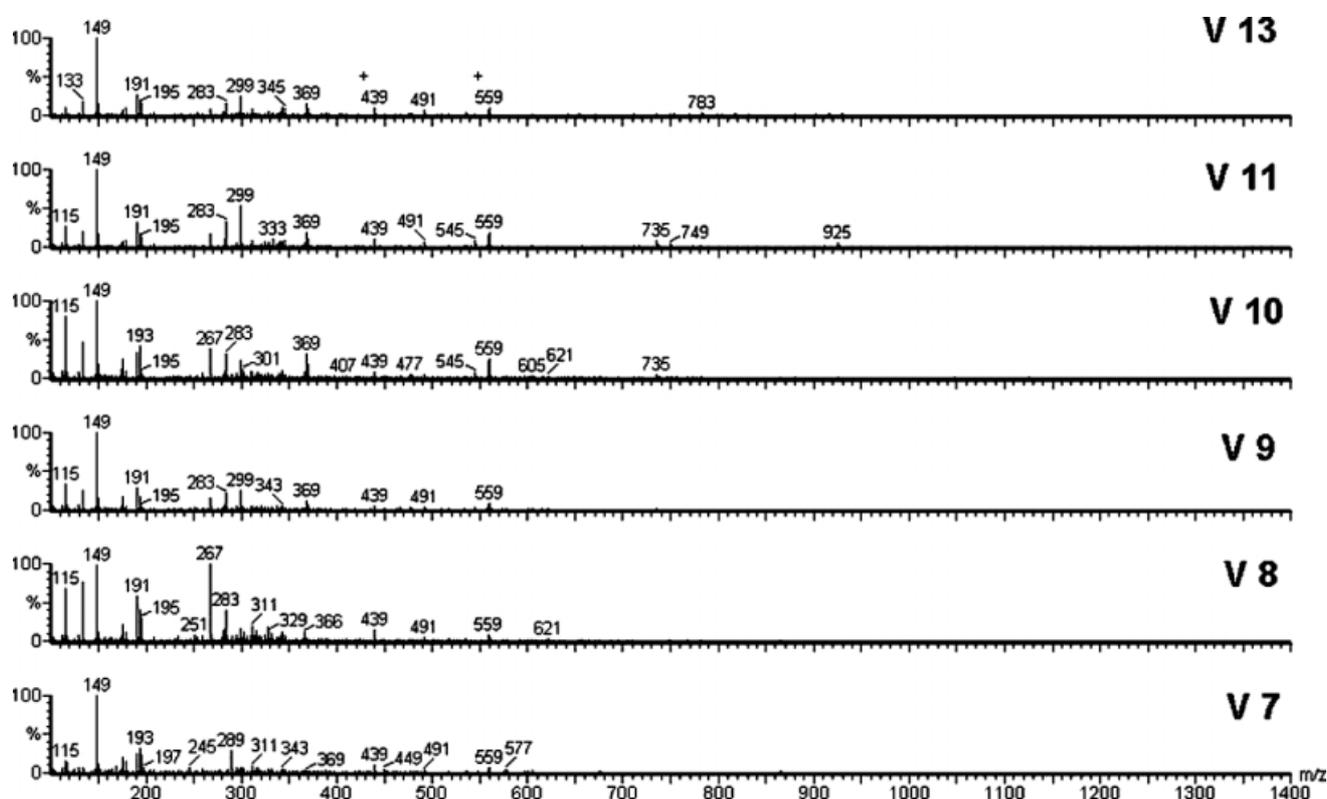


**Figure 2.** ESI-MS fingerprints in the negative ion mode of wine from the following six varieties of grapes after the malolactic fermentation: Pinot Noir (V7), Cabernet Sauvignon (V8), Malbec (V9), Merlot (V10), Isabel (V11) and Bordô (V13). + diagnostic ions for wine.
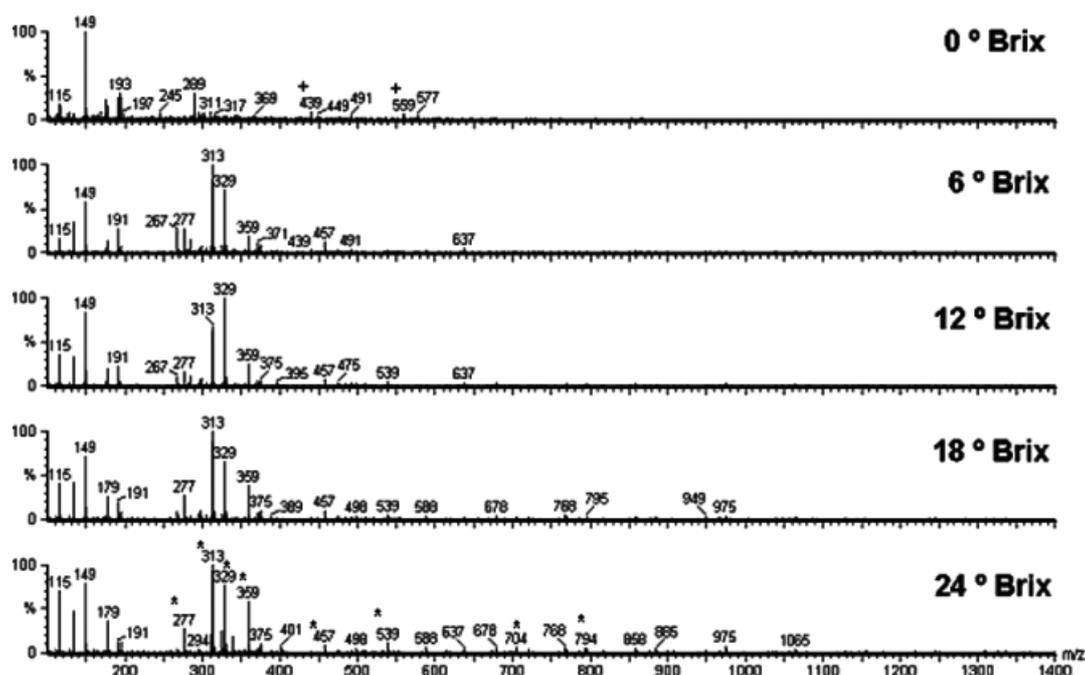
**Figure 3.** ESI-MS fingerprints in the negative ion mode of random samples of must and wine taken at intervals of 6°Brix. The 24°Brix sample is representative of unfermented must, whereas, 0°Brix indicates the end of the malolactic fermentation. *Diagnostic ions for must and + diagnostic ions for wine.
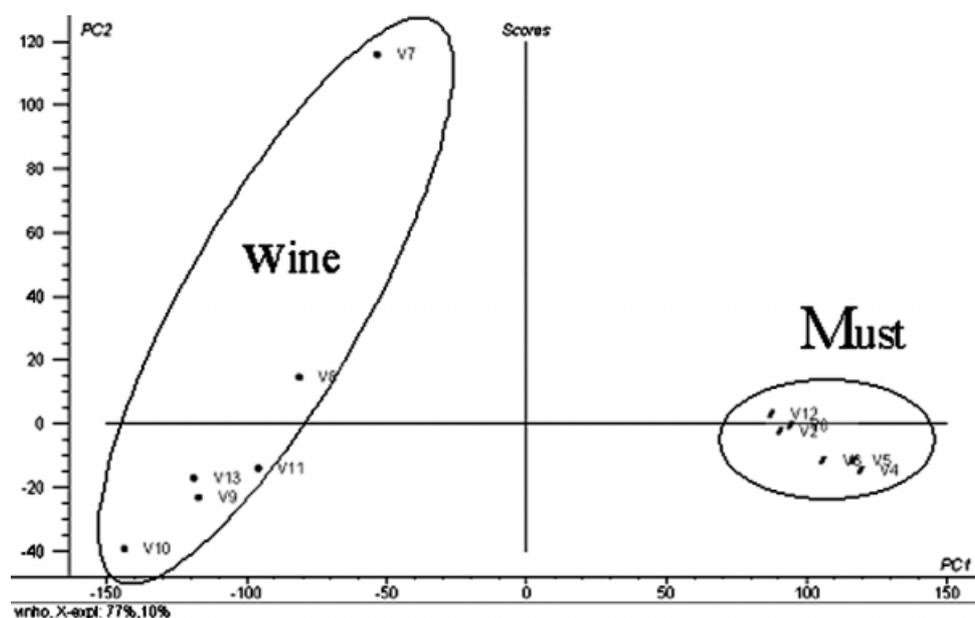


**Figure 4.** PCA analysis of the ESI-MS data for the must and wine samples from the following grape varieties: Pinot Noir (V2 – must, V7 – wine), Cabernet Sauvignon (V3 – must, V8 – wine), Malbec (V4 – must, V9 – wine), Merlot (V5 – must, V10 – wine), Isabel (V6 – must, V11 – wine) and Bordô (V12 – must, V13 – wine).

diagnostic ions found in the fingerprint of must disappear completely at about 6°Brix. The diagnostic ions for wine are only observed at 0°Brix. This finding indicates that major changes in composition as related to the more polar components occurs between 6 and 0°Brix, at the end of the malolactic fermentation.

PCA of the fingerprints (Fig. 4) clearly separates must and wine in two well-defined groups, confirming the suitability of ESI-MS characterization. PCA also confirmed that less

variation occurs for must fingerprints, whereas wine shows greater dispersion, with sample V7 (Pinot Noir) clearly standing out.

Addition of sugar and unfermented must to wine were also tested. Figure 5(A) shows the ESI-MS fingerprint of Pinot Noir wine (V7). The fingerprint of a 1% (v/v) solution of sucrose in water (Fig. 5(B)) shows the ions of $m/z$ 341, 377, 683 and 719 as the most intense and characteristic, and 3 of these ions are still clearly seen when the sucrose solution
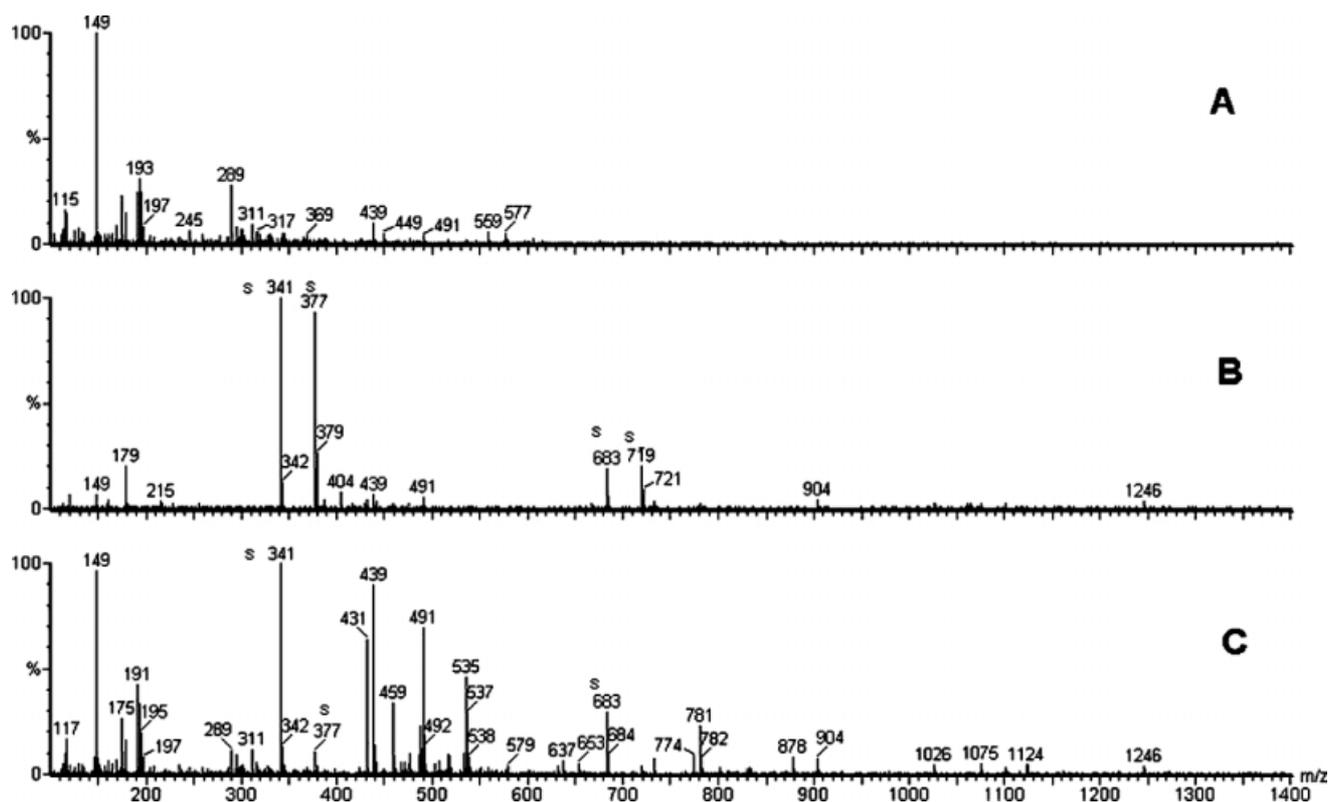
**Figure 5.** ESI-MS fingerprints in the negative ion mode of samples of: (A) Pinot Noir wine at the end of the malolactic fermentation; (B) a solution of sucrose in water; (C) Pinot Noir wine admixed with sucrose aqueous solution. +: diagnostic ions for wine and ˢ: diagnostic ions for sucrose.
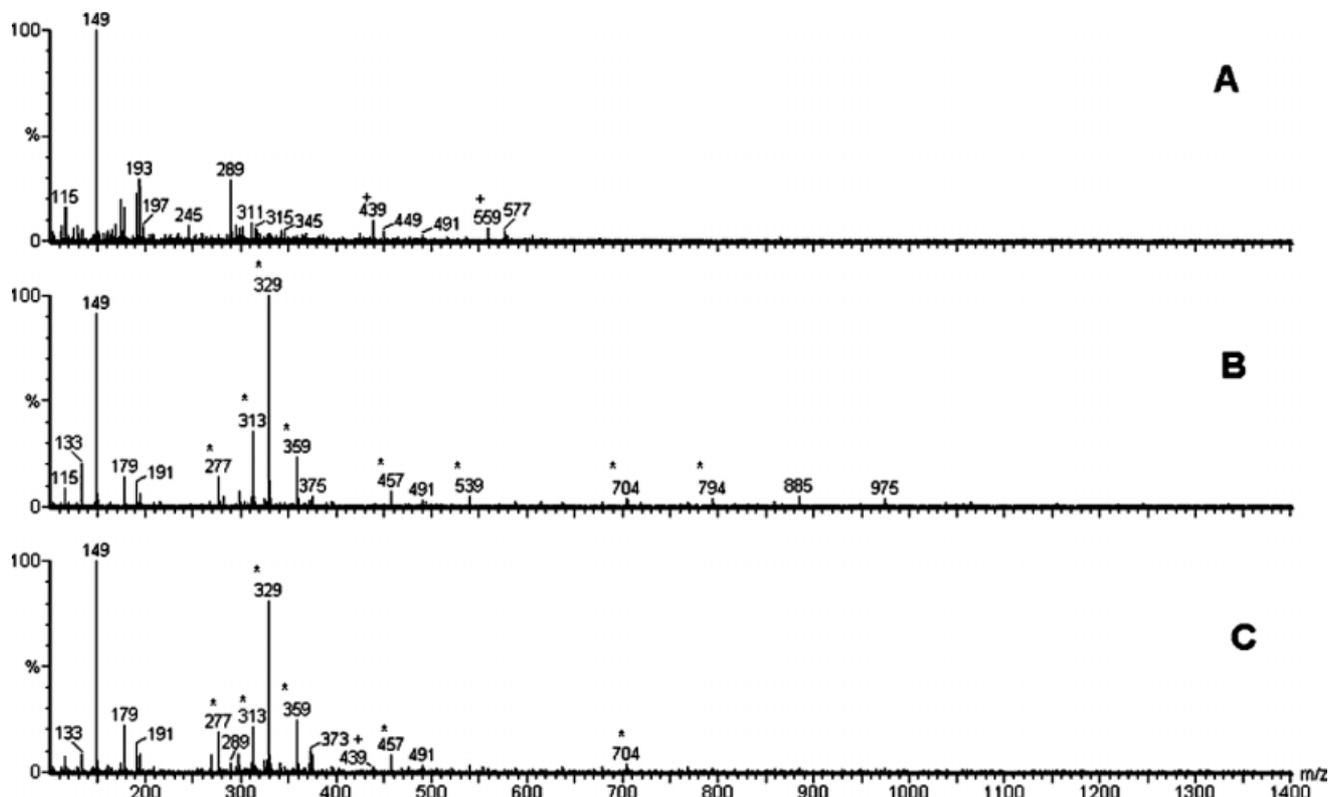


**Figure 6.** Fingerprints in the negative ion mode of samples of: (A) Pinot Noir wine after the malolactic fermentation; (B) unfermented Bordô must; (C) (1 : 1) mixture of Bordô must and Pinot Noir wine. *Diagnostic ions for must and + diagnostic ions for wine.

is added to wine (Fig. 5(C)). Concentrations of sucrose at nanomolar level could be detected by this analysis.

In the same way, the diagnostic ions for unfermented Bordô must of $m/z$ 277, 313, 329, 359, 457, 539 and 704 (Fig. 6(B)) also stand out clearly in the fingerprint of a mixture of equal parts of must and Pinot Noir wine (Fig. 6(C)). Note that the ion of $m/z$ 289, observed in the fingerprint of Pinot Noir and the diagnostic wine ion ($m/z$ 439) are also seen. The detection of diagnostic ions of must in wine indicates therefore addition of unfermented must to wine. In fact, both forms of sugar addition (must or sucrose) can be identified by ESI-MS fingerprint.

## CONCLUSION

ESI-MS fingerprints in the negative ion mode are able to identify diagnostic ions for both wine and must. Addition of sugar or unfermented must can also be detected. ESI-MS fingerprints are acquired in a few minutes per sample, hence direct infusion technique provides high-throughput characterization of both must and wine and should prove useful for quality control during and after fermentation, especially in large wineries with many concurrent batches. Sugar consumption during the fermentation process can be easily visualized. Further studies are being undertaken to evaluate the suitability of ESI-MS to accompany wine maturation and monitor the changes in chemical composition.

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