Monitoring of wine aging process by electrospray ionization mass spectrometry

Monitoramento do processo de envelhecimento de vinho por espectrometria de massas com ionização por eletrospray

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Abstract

The characterization of wine samples by direct insertion electrospray ionization mass spectrometry (ESI-MS), without pre-treatment or chromatographic separation, in a process denominated fingerprinting, has been applied to several samples of wine produced with grapes of the Pinot noir, Merlot and Cabernet Sauvignon varieties from the state o Rio Grande do Sul, in Brazil. The ESI-MS fingerprints of the samples detected changes which occurred during the aging process in the three grape varieties. Principal Component Analysis (PCA) of the negative ion mode fingerprints was used to group the samples, pinpoint the main changes in their composition, and indicate marker ions for each group of samples.

Keywords: *electrospray ionization mass spectrometry; wine; aging process; Pinot noir; Merlot; Cabernet Sauvignon.*

Resumo

A caracterização de amostras de vinho, sem pré-tratamento ou separação cromatográfica, por espectrometria de massas com ionização por eletrospray e infusão direta, em um processo denominado de *fingerprinting*, foi aplicado a várias amostras de vinho produzidas com uvas das variedades Pinot noir, Merlot e Cabernet Sauvignon, provenientes do Estado do Rio Grande do Sul, Brasil. Os fingerprints por ESI-MS das amostras detectaram mudanças que ocorreram durante o processo de envelhecimento para as três variedades de uvas. Análise de componentes principais (PCA) dos fingerprints em modo negativo foi usada para agrupar as amostras, salientar as principais modificações em composição e indicar os íons marcadores para cada grupo.

Palavras-chave: *espectrometria de massas com ionização por eletrospray; vinho; processo de envelhecimento; Pinot noir; Merlot; Cabernet Sauvignon.*

1 Introduction

In order to improve the quality of wines, research in viticulture and enology is carried out in several fronts. Improved grape quality and ripening, the selection of yeast inoculums and enzymes, control of the conditions during the malolactic fermentation as well as the aging process, are all important aspects of the production process. A sound knowledge of wine chemistry is also necessary. Polyphenols that are released in the must, during the fermentation and pressing processes from different parts of the berry, undergo condensation and polymerization during winemaking and wine aging (FLAMINI, 2003).

These compounds not only play an important part in the organoleptic characteristics of wine, but have also been related to the benefits of dietary wine consumption. Wine polyphenolics interact with reactive oxygen species and increase post-prandial total antioxidant capacity. These protective effects are especially displayed in people most likely to be under oxidative stress conditions, such as smokers and coronary heart disease patients (COVAS et al., 2010). The phenolic compounds related to these effects can be divided into two main groups. Non-flavonoid phenols, such as gallic acid and caffeic acid, and stilbenes, like resveratrol, compose one group; whereas flavonoids, such as quercetin, cathechins and anthocyanidins, are the second group (GERMAN; WALTZEN, 2000).

Gas chromatography mass spectrometry (GC-MS) has been successfully used to study wine aroma. This technique, however, has not been successfully applied to the study of polyphenols, due to their low volatility. When derivatized to increase their volatility, their high molecular weight exceeds the mass range of most GC-MS systems (FLAMINI, 2003). Therefore, some of the first successful studies used hydrolysis and subsequent liquid chromatography (LC) methods to study wine phenolics. (WULF; NAGEL, 2000; HEBRERO et al., 1989) Later, the availability of effective interfaces for liquid chromatography mass spectrometric equipment (LC-MS) - some of which permitted multiple mass spectrometry experiments (MS/

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MS or MS^n) - supplied powerful tools for the analysis of wine and its components (GAMOH; NAKASHIMA, 1999; LA TORRE, et al., 2006).

The characterization of whole samples by direct insertion electrospray ionization mass spectrometry (ESI-MS), without chromatographic separation, in a process denominated fingerprinting, is being applied to a constantly increasing array of analytes: natural products (MAURI; PIETTA, 2000; SAWAYA et al., 2004; ABREU et al., 2007); food and beverages (ARAÚJO et al., 2005; MOLLER; CATHARINO; EBERLIN, 2005, 2007; CATHARINO et al., 2005; DE SOUZA et al., 2007a,b); and petroleum and biodiesel (PORTER; MAYER; FINGAS, 2004; CATHARINO et al., 2007). It is a fast and reliable process especially applicable to the analysis of numerous samples and it is indicated for the qualitative distinction between samples. In the case of wine production, false information as to the type of grape, age and purity of wines could be used to mislead consumers. Therefore, a fast and robust method for the characterization of wine by grape variety and/or age could be applied to wine quality control.

Few studies of wine by direct insertion ESI-MS were carried out. Five samples of bottled wine were analyzed by direct insertion electrospray ionization Fourier transform mass spectrometry (ESI-FT-MS) in order to obtain the elemental composition of specific components (COOPER; MARSHALL, 2001). The authors noted that the negative ion mode fingerprint showed greater variety in the composition and abundance of components in the analyzed wines and a lesser amount of adducts, as well as higher resolution. Negative ion mode electrospray ionization mass spectrometry [ESI (-)-MS] is selective for compounds with acidic or phenolic sites, and therefore, adequate for studying wine phenols and polyphenols. Direct infusion ESI (-)-MS was used to analyze samples of must of five varieties of grapes and follow the transformations that occur during the malolactic fermentation process (CATHARINO et al., 2006). The ESI (-)-MS fingerprints clearly showed which samples were must and which came from the wine at the end of the malolactic fermentation, despite the variety of grape used. Furthermore, the addition of sugar or must to the wine could be easily detected in the fingerprints. In a more recent study, direct infusion ESI (-)-MS was used to characterize the oligosaccharides in two varieties of red wine (DUCASSE et al., 2010).

During aging, more subtle modifications occur, which are related to the final acceptability of mature wine. Changes in wine during maturation in new and used oak barrels or in tanks were followed using HPLC-UV and HPLC-MS, focusing on the anthocyanins (CANO-LOPEZ et al., 2010). Other authors have studied the effect of oxygenation on wine maturation using HPLC-UV and HPLC-MS (ATANASOVA et al., 2002). The question was whether direct infusion ESI (-)-MS (without chromatographic separation) would be capable of detecting differences in wine of different ages and different grape varieties. Therefore, in the present study, ESI (-)-MS is used to follow the transformations during the aging process of wine samples of three different varieties of grape, over a period of up to two years. Chemometric analysis of the negative mode fingerprints obtained was used to group the samples and pinpoint the main changes in their composition.

2 Materials and methods

2.1 Samples of wine

Samples of wine were obtained from 'Vinicola Velho Amâncio' winery near Santa Maria, in the State of Rio Grande do Sul, Brazil. Grape maturity was controlled by the winery (sugar content, acidity and pH) and the following varieties were used: Pinot noir, Cabernet Sauvignon and Merlot. The alcoholic fermentation was considered terminated when sugar content was equal to zero. After the malolactic fermentation, the wine was cooled, filtered and five replicate samples of each wine variety were collected (CS, ME, PN) in 2004. After one year's aging (CS1, ME1, PN1), five replicate samples of each wine variety were again taken. For Pinot noir, five replicate samples were taken after two years of aging (PN2). Aging was carried out in bottles at 12 °C. Aliquots of equal volumes of the PN1 and ME1 samples were mixed in the laboratory and analyzed (MIX).

2.2 General experimental procedure

Samples of wine were analyzed by direct infusion into the ESI source by means of a syringe pump (Harvard Apparatus) at a flow rate of 10 μL/minute. Negative mode electrospray ionization mass spectrometry [ESI(-)–MS] fingerprints and negative mode ESI-MS/MS (low energy CID) spectra were acquired using a hybrid high-resolution and high-accuracy (5 ppm) Micromass Q-TOF mass spectrometer; capillary and cone voltages were set to -3,000 V and -50 V, respectively, with a de-solvation temperature of 100 °C. Aliquots of 10 μL of each sample of wine were diluted in one mL of a solution containing 70 % (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA) and 30% (v/v), deionized water, and 0.5% of ammonium hydroxide (Merck, Darmstadt, Germany). The negative ion mode was used because it is considered to be more adequate for the analysis of phenolic compounds in wine (COOPER; MARSHALL, 2001; CATHARINO et al., 2006).

2.3 Statistical analysis of data

Principal Component Analysis (PCA) was performed using the 2.60 version of Pirouette software, 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 10% were not included. The data was preprocessed using auto scale and the PCA method was run.

3 Results and discussion

Figure 1 shows the representative ESI-MS fingerprints of wine samples PN, ME and CS originated from the three varieties of grapes directly after the malolactic fermentation. The two diagnostic ions (*m/z* 439 – $C_{33}H_{12}O_2$ and *m/z* 559 – a trimeric sugar $C_{19}H_{28}O_{19}$) observed in all the wine samples at the end of the malolactic fermentation, mentioned in a previous study (CATHARINO et al., 2006), are also present in these samples.

These ions were also observed in negative ion fingerprints of wine by Cooper and Marshal (2001) and had the following structures assigned: *m/z* 439 – CHO and *m/z* 559 – a trimeric sugar CHO. Another oligosaccharide found in several samples of wine was the ion at *m/z* 605, found commonly in Merlot wine (DUCASSE et al., 2010).The ions of *m/z* 115, 133 and 149 are also very intense in the fingerprints of wine before aging, they were identified as organic acids by the loss of 44 Da in their MS/MS. Comparing their fragmentation pattern to previous studies; *m/z* 133 was identified as malic acid, *m/z* 149 as tartaric acid, *m/z* 191 as quinic acid, and *m/z* 193 as ferrulic acid (ROESLER et al.,

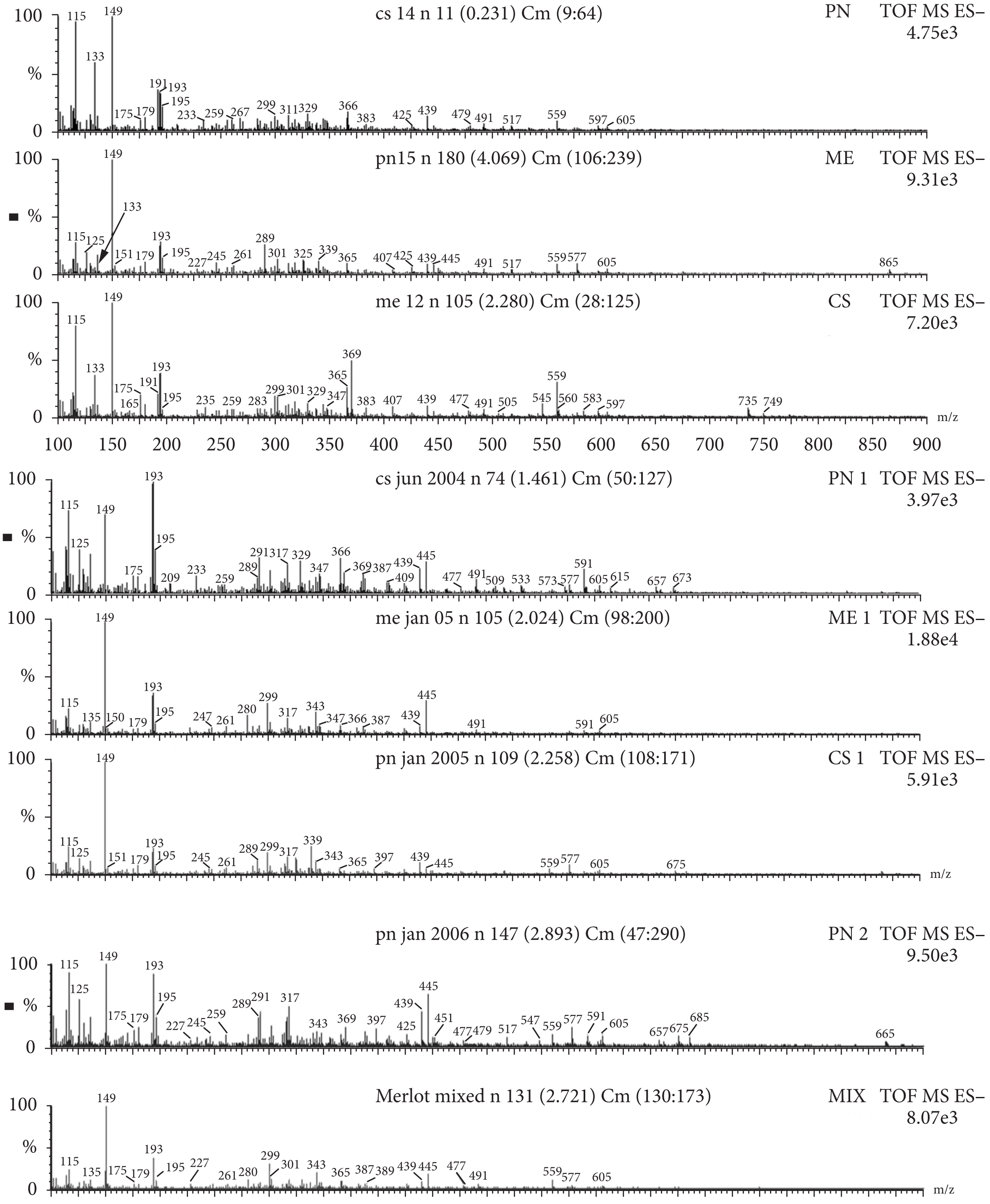

Figure 1. ESI-MS fingerprints in the negative ion mode of the wine samples directly after the malolactic fermentation (PN, Pinot Noir; ME, Merlot; CS, Cabernet Sauvignon), after one year's aging (PN1, Pinot Noir one year; ME1, Merlot one year; CS1, Cabernet Sauvignon one year) and after two year's aging (PN2, Pinot Noir two years). MIX is a mixture of ME1 and PN1.

2007). These are phenolic compounds commonly found in red wine (VALENTÃO et al., 2007).

In the ESI-MS fingerprints of samples of one-year-old wines of the same three grape varieties (CS1, ME1, PN1), the diagnostic ion of *m/z* 439 can still be observed in all three varieties, but the ion of *m/z* 559 can be observed only in the CS1 fingerprint. The ions of *m/z* 115 and 133 are much less intense and *m/z* 149 (although still the most intense in the fingerprints of PN1and ME1) is relatively less intense in relation to other ions in the fingerprint. The ion of *m/z* 191 (quinic acid) is less intense and the ions of *m/z* 193 (ferrulic acid) and *m/z* 195 are relatively more intense. The ions of *m/z* 445 and 317 are now clearly present in the fingerprints of the three varieties.

In the ESI-MS fingerprint of the two-year-old Pinot noir wine (PN2), the diagnostic ions of *m/z* 439 can still be observed, but not the ions of *m/z* 133 and 191. Although the ions of *m/z* 115 and 149 are still the most intense in the fingerprints, they are now relatively less intense in relation to other ions in the fingerprint, such as *m/z* 193, 195, 439 and 445.

The importance of these modifications during the aging process can be confirmed by the PCA. In Figure 2a, the scores plot, the samples were clearly grouped according to the variety of grape and age. The samples of young wine (CS, ME and PN) are all placed on the top left side of the plot, whereas the one-year-old samples are placed in the center and at the bottom (CS1, ME1 and PN1), and the two-year-old sample (PN2) is on the right. The loadings plot (Figure 2b) indicates that the ions responsible for grouping the young wines are *m/z* 115, 133 and 191, which are also evident in the fingerprints. As the wines mature, the decrease in the ions above, plus the increase in the intensity of the ions of *m/z* 193, 445 and (to a lesser extent) *m/z* 317, which are also observed in the fingerprints, place these samples closer to the center. The two-year-old sample is placed on the right due to the relatively lower intensities of *m/z* 149 and 115 and to the greater intensity of other ions of higher mass. The marker ions that denote the end of the malolactic fermentation (*m/z* 439 and 559) are present in all fingerprints. These results characterize the transformation which occurs during the aging process, that is: the reduction of low mass organic acids and phenolic compounds and the formation of high mass polyphenols. Most studies of wine aging have focused on the changes that occur in oak barrels and under micro-oxygenation conditions (ALCALDE-EON et al., 2006; CANO-LOPEZ et al., 2010), which may not reflect the conditions of bottle-aged wines. Furthermore, in fingerprinting studies, the separation of different types of sample, due to characteristic fingerprints, is of greater importance than the identification of individual compounds. Nevertheless, the possibility of identifying compounds by MS/MS fragmentation can add further information about the samples.

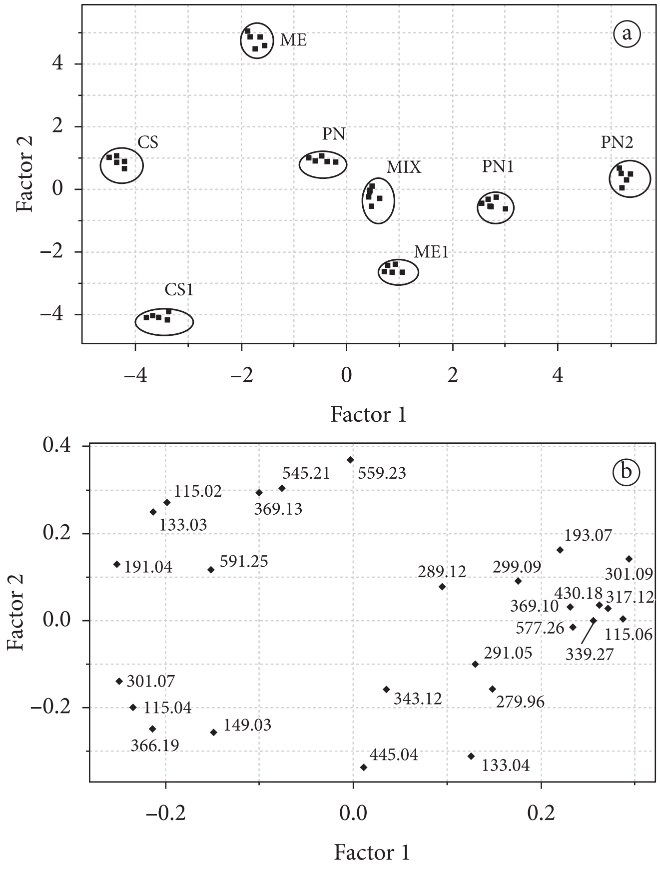

Figure 2. A. scores plot and B. loadings plot of the PCA analysis of the ESI-MS data for the wine samples (for abbreviations see Figure 1). The first two components (PC1 and PC2) retained 86% of the variation.

The analysis of the fingerprints also indicates differences for each variety of grapes. For example, the fingerprints of Pinot noir wine contained the ions of: *m/z* 289 (catechin or epicatechin), *m/z* 577 (dimeric procianidin) and *m/z* 865 (trimeric procianidin), identified by comparison with literature (PÉREZ-MAGARIÑO et al., 1999; MONAGAS et al., 2003), as well as the ion of *m/z* 245, observed in the previous study (CATHARINO et al., 2006). Mixtures of one-year-old samples of Pinot noir and Merlot presented fingerprints as shown in Figure 1 MIX, contained ions observed in both varieties of wine. PCA placed these samples among those of the individual wines in the scores plot (Figure 2a), indicating that the fingerprint came from a wine of intermediate composition.

Other compounds identified in several samples comparing their MS/MS spectra to those found in the literature were: *m/z* 227 – resveratrol, *m/z* 301- quercetin, and m/z 317 – myrcetin (LA TORRE et al., 2006). Flavonoids and other phenolic compounds, such as resveratrol, are important components of wine due to their beneficial antioxidant effects (COVAS et al., 2010).

4 Conclusions

Direct insertion ESI-MS fingerprints in the negative ion mode were able to detect transformations which occur during the aging process, as well as to detect variations in composition between wine made from different grape varieties and a mixture of two varieties prepared in laboratory. The analysis of large amounts of samples, in order to confirm the used grape variety and the age, is feasible through this fast and reliable process. Further studies with larger number of wine samples and other grape varieties of diverse geographic origin could be used to compile a library of fingerprints for the certification of origin of the individual samples.

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