

Short communication

Investigation of different pre-concentration methods for NMR analyses of Brazilian white wine

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Abstract

This work describes the investigation of different preparation methods of wine samples such as direct analysis, freeze-drying and nitrogen-flow concentration in the characterization of Brazilian Chardonnay wine composition by means of ^1H NMR spectroscopy. The direct analyses of the wine presented some limitations for the detection of “minor” compounds and substances whose signals were expected in the region from 3.6 to 4.0 and 0.8 to 1.3 ppm. The freeze-dried sample showed that a low quantity of ethanol remained in the lyophilisate. The freeze-dried process also revealed restrictions concerning long-time consumption and reproducibility. Diminution of the ethanol signal was observed in the ^1H NMR spectrum of wine samples which were concentrated under nitrogen-flow to allow the determination of compounds such as butylenglycol and alanine. The nitrogen-flow shows advantages in the identification of compounds present as minor compounds with less time consumption.

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1. Introduction

Most liquid foods have a complex structure and are composed of many different components: proteins, carbohydrates, fats, water, organic acids, flavor and others.

Wine is the result of the transformation of grape juice by alcoholic fermentation. However, with this single term we group a variety of wine products whose organoleptic characteristics are extremely different.

Another characteristic of vinicultural production is the vast hierarchy of quality and price that exists, differentiating wines of the same type from one another. This diversity expresses differences in chemical composition responsible for the difference in odor, color, viscosity and taste.

Analysis of the chemical composition can provide valuable information for the control of food origin,

process and overall quality. Many of the important substances are not always easily detected by classical analyses. Recently, nuclear magnetic resonance (NMR) spectroscopy has been used for the analysis of liquid food composition because it is capable of the simultaneous detection of a great number of organic compounds and enables a more rapid and non-invasive characterization (Ablett, 1992; Belton, Delgadillo, Helmes, Nicholson, & Spraul, 1996; Belton et al., 1998; Gall, Puaud, & Colquhoun, 2001; Gil et al., 2000).

It has been shown that ^1H NMR spectroscopy can be used for the characterization of amino acids and anthocyanins in wine samples (Kosir & Kidric, 2002). The composition of amino acids can be used as a fingerprint for differentiation of wines according to their geographical origin, vine variety and year of production. However, amino acids are present in wine only at low concentration (minor compounds) and therefore demand the pre-concentration of the sample

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(Arvanitoyannis, Katsota, Psarra, Soufleros, & Kallithraka, 1999; Brescia et al., 2001).

In this work, we describe the investigation of different methods of sample preparation: direct analysis, freeze-drying and nitrogen-flow concentration of wine samples to define the procedure that permits the characterization of Brazilian white wine composition by means of ^1H NMR spectroscopy.

2. Materials and methods

2.1. Preparation of samples

For our investigation, we selected Chardonnay variety white wines, vintage 2000 and 2003, produced in the southern region of Brazil (Rio Grande do Sul).

For the direct analysis of wine samples, 0.4 ml of wine was mixed with 0.1 ml D_2O (for NMR field/frequency lock) in a 5 mm NMR sample tube.

In the lyophilisation processes (freeze-drying method), 4.0 ml of wine was concentrated for 28 h and then dissolved in 0.4 ml of D_2O and transferred into a 5 mm NMR tube. Close attention was taken to minimize the contamination of the dried sample with atmospheric humidity.

An amount of 4.0 ml of wine was concentrated under nitrogen-flow for 2 h, until there was no further liquid in the sample. The dried sample was dissolved in 0.4 ml of D_2O and transferred into a 5 mm NMR tube. The nitrogen-flow was 20 ml/s. The purity of D_2O used was 99.9 at.% D.

In all samples, sodium 3-(trimethylsilyl) propionate d_4 (TSP) was used as a reference for chemical shift. The pH values for the sample were found to fall within the 3.3–3.8 range.

2.2. ^1H NMR measurements

All spectra were recorded on a Bruker AC 200 NMR spectrometer equipped with a 5 mm DUAL ^1H - ^{13}C probe, operating at 200.13 MHz for ^1H .

For one-dimensional proton spectra, one hundred and sixty scans of 16K data points were carried out with a spectral width of 5000 Hz, acquisition time of 1.63 s, and recycle delays of 3 s. The digital resolution is ± 0.61 Hz. In all experiments, the signal of water was suppressed by pre-saturation pulse sequence with irradiation at the water frequency (4.77 ppm), and the temperature during the experiments was set to 25 °C.

3. Results and discussion

Fig. 1 shows the ^1H NMR spectrum of the wine samples that were directly analyzed. The identification of ^1H

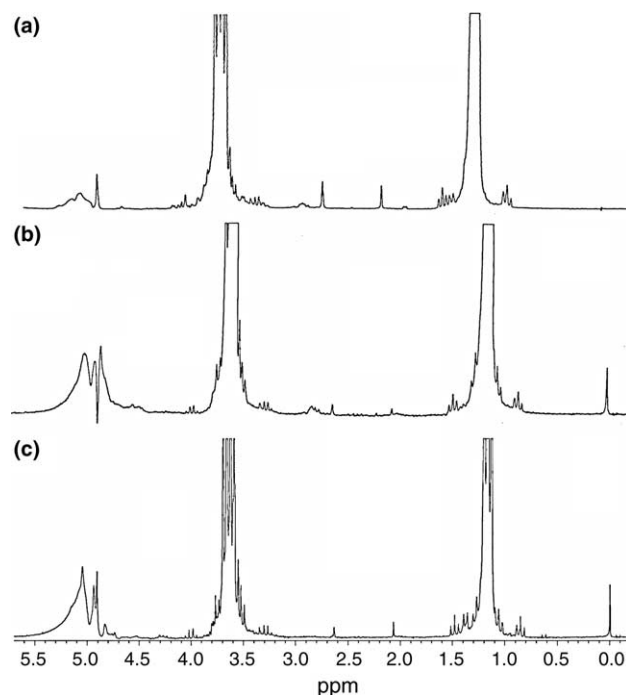


Fig. 1. ^1H NMR spectrum of the Brazilian Chardonnay wines: Marson vintage 2000 (a), Marson vintage 2003 (b) and Aurora vintage 2003 (c).

assignments of the main compounds was done on the basis of previous works (Duarte et al., 2002; Kosir & Kidric, 2001).

The shielding region of the spectrum (0.5–2.5 ppm) contains the signals from many species including ethanol, acetic acids and acetates (Table 1). A strong ethanol signal at $\delta = 1.17$ ppm shows overlapping, and the side bands at $\delta = 0.84$ and 1.43 ppm cause difficulties for the assignment in this region.

Between 2.5 and 5.5 ppm, the spectrum shows signals related to the principal organic acids present in wine (malic, succinic, lactic and tartaric) as shown in Table 1. However, the tails of the dominant frequencies of ethanol at $\delta = 3.64$ ppm and glycerol at $\delta = 3.62$ and 3.79 ppm obscure the weak signals near the strong ones. This fact makes the assignment of ^1H NMR signals of amino acids and sugars extremely difficult.

The ^1H spectrum of the freeze-dried samples (Fig. 2) shows signals at 1.17 and 3.64 ppm, demonstrate that a low quantity of ethanol remained in the lyophilisate. Differences in signal intensity were also observed, especially for compounds such as acetic acids and acetates at 2.04 ppm when compared to the ^1H spectrum of wine without pre-concentration (Fig. 1).

The conditions of the freeze-drying method are not easily controlled, and the temperature and pressure must be well monitored in order to reach a good state of reproducibility.

Table 1
 ^1H chemical shift and proton multiplicities for assignments in wine samples

Compound	Assignment	$\delta^1\text{H}$ (ppm)	Multiplicity
Acetic acid/acetates	CH_3	2.04	s
Alanine	CH_3	1.49	d
Arginine	CH_2	3.26	m
Proline	CH_2	2.35	m
Butylenglycol	CH_3	1.13	d
Ethanol	CH_3	1.17	t
	CH_2	3.64	q
Glycerol	CH_2	3.62	m
	CH	3.79	m
Lactic acid	CH_3	1.35	d
Malic acid	CH_2	2.63	m
	CH_2	2.82	m
Succinic acid/succinates	CH_2	2.59	s
Tartaric acid	CH	4.79	s

s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets and m, multiplet.

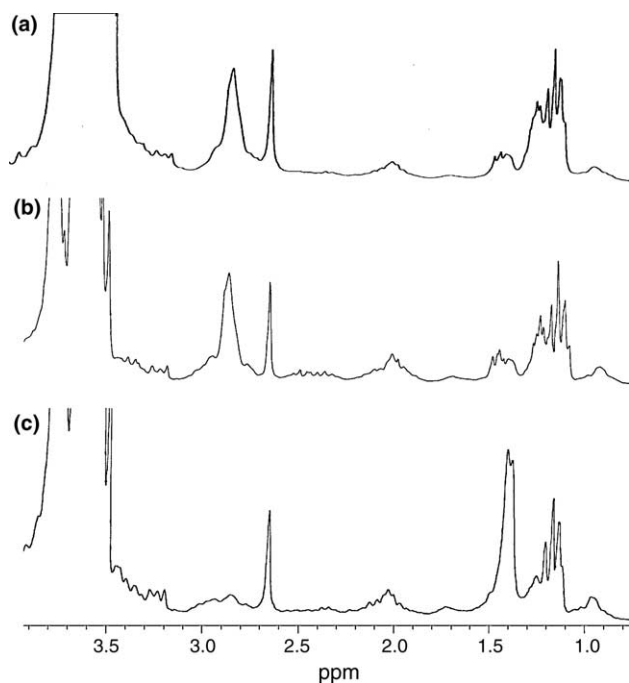


Fig. 2. ^1H NMR spectrum of the freeze-dried Brazilian Chardonnay wines: Marson vintage 2000 (a), Marson vintage 2003 (b) and Aurora vintage 2003 (c).

The pre-concentration of the Brazilian Chardonnay wines by nitrogen-flow revealed signals of compounds from 1.0 to 1.5 ppm in the ^1H NMR spectrum. The diminution of the ethanol signal is clearly observed in the ^1H NMR spectrum of a wine samples concentrated under nitrogen flow (Fig. 3). This allows the determination of butylenglycol and alanine at 1.13 and 1.49 ppm, respectively. When the malolatic fermentation is completed, the presence of significant quantity of lactic acid (1.35 ppm) difficult the assignment of alanine signals as shown in Fig. 3(c).

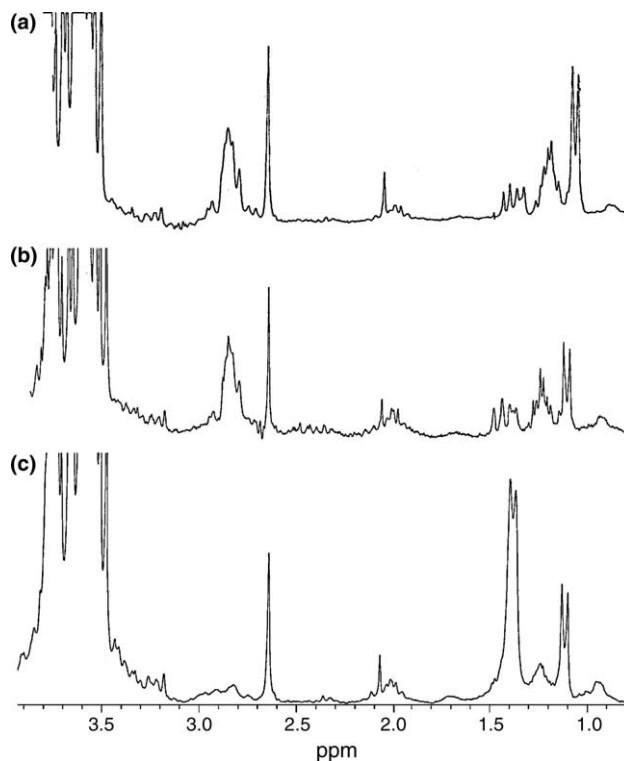


Fig. 3. ^1H NMR spectrum of the Brazilian Chardonnay wines concentrated by nitrogen-flow: Marson vintages 2000 (a), Marson vintage 2003 (b) and Aurora vintage 2003 (c).

From 2.0 to 3.4 ppm, peaks from organic acids, arginine and proline are also observed in the ^1H NMR spectrum of the Brazilian Chardonnay wines concentrated by nitrogen-flow. The 3.5–4.0 ppm region of the spectrum still shows a strong contribution of the glycerol. The spectral distinction of carbohydrate is extremely difficult due to peak overlap. In the nitrogen-flow and freeze-drying concentration, it is not possible to make

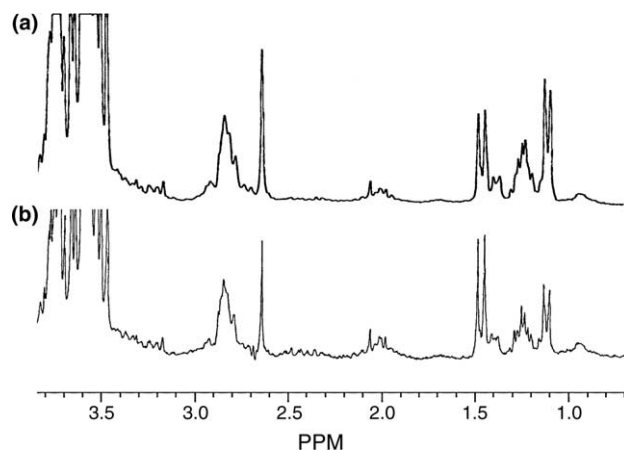


Fig. 4. ^1H NMR spectrum of the Brazilian Chardonnay wines concentrated by nitrogen-flow with addition of alanine: Marson vintage 2000 (a) and Marson vintage 2003 (b).

quantitative analyses of the volatile compounds due to evaporation.

These signals were also confirmed by the addition of internal standards. Fig. 4 shows the increase in the signal intensity of alanine by standard addition.

4. Conclusion

The results presented in this work demonstrate that the direct analysis of the Brazilian Chardonnay wine is not suitable for detection of “minor” compounds such as amino acids and substances whose signals are expected in the regions from 3.5 to 4.0 and 0.8 to 1.3 ppm when working with an ^1H NMR at 4.7 Tesla.

The freeze-drying method also reveals the same restriction, and presents long-time consumption and problems of reproducibility. Nitrogen-flow was a better concentration method compared to the freeze-drying method. It shows its advantages especially concerning the identification of substances present as minor compounds, and it is also less time-consuming.

The nitrogen-flow method of pre-concentration amplifies the possibilities of the ^1H NMR's use for analyses of wines. High spectral complexity can be resolved by the ^1H NMR spectroscopy and pre-concentration method, allowing the identification of different compounds in different concentrations.

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