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Solid-phase extraction *versus* matrix solid-phase dispersion: Application to white grapes

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Abstract

The use of matrix solid-phase dispersion (MSPD) was tested to, separately, extract phenolic compounds and organic acids from white grapes. This method was compared with a more conventional analytical method previously developed that combines solid liquid extraction (SL) to simultaneously extract phenolic compounds and organic acids followed by a solid-phase extraction (SPE) to separate the two types of compounds. Although the results were qualitatively similar for both techniques, the levels of extracted compounds were in general quite lower on using MSPD, especially for organic acids. Therefore, SL-SPE method was preferred to analyse white "*Vinho Verde*" grapes. Twenty samples of 10 different varieties (Alvarinho, Avesso, Asal-Branco, Batoca, Douradinha, Esganoso de Castelo Paiva, Loureiro, Pedernã, Rabigato and Trajadura) from four different locations in Minho (Portugal) were analysed in order to study the effects of variety and origin on the profile of the above mentioned compounds. Principal component analysis (PCA) was applied separately to establish the main sources of variability present in the data sets for phenolic compounds, organic acids and for the global data. PCA of phenolic compounds accounted for the highest variability (77.9%) with two PCs, enabling characterization of the varieties of samples according to their higher content in flavonol derivatives or epicatechin. Additionally, a strong effect of sample origin was observed. Stepwise linear discriminant analysis (SLDA) was used for differentiation of grapes according to the origin and variety, resulting in a correct classification of 100 and 70%, respectively.

Keywords: MSPD; Multivariate analysis; Organic acids; Phenolics; "*Vinho Verde*"; White grapes

1. Introduction

Matrix solid-phase dispersion (MSPD) has been widely used in the last years for the isolation of a wide range of drugs, pesticides, naturally occurring constituents and other compounds from different complex plant and animal tissues providing, in many cases, equivalent or superior results to older official methods conducted by more classical extraction and/or SPE techniques (see reviews [1–5]).

Usually, for the analysis by conventional techniques of solid, semi-solid and/or highly viscous biological samples, several steps are necessary for their preparation, extraction and fractionation. However, MSPD enables to combine all these steps in just one, because the entire sample is homogeneously dispersed in a solid support, usually a C18 or C8-bonded silica, creating a unique chromatographic phase that is used as the stationary phase of a column. The extraction of the analytes and clean-up are carried out simultaneously with, generally, good recoveries and precision [3–4].

MSPD enables complete sample disruption and dispersal into particles of very small size, providing an enhanced surface area for subsequent extraction of the compounds [3], whereas in SL/SPE sample disruption must be conducted separately and many of the sample components must be discarded before an extractive solution is ready to be added to an SPE column. In SPE the extracted compounds are usually absorbed onto the top of the column packing material, not throughout the column as in MSPD. The physical and chemical interactions among the components of the system are greater in MSPD and different in many aspects from those that take

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place in classical SPE or other forms of liquid chromatography.

Although MSPD has been found to be a technique generally simpler, faster and requiring much less solvent than classical methods and has been widely used for the analysis of different analytes in plants (see review Barker [3]), only in a few studies phenolic compounds, as phenolic acids [6] or isoflavonoids, [7–8] have been analysed by this technique. However, these aforementioned studies do not show clearly that their complete extraction can be easily obtained by MSPD. Xiao et al. [7] compared the efficiency of MSPD with ultrasonic and Soxhlet methods to extract isoflavonoids from Radix astragali, the dried root of a medicinal Chinese plant. Four main isoflavonoids were identified, two aglycones and two glycosides, but while the amounts of the two aglycones were higher when using MSPD, the efficiency of the extraction was better for the glycosides if the conventional techniques, ultrasonic or Soxhlet (specially this last one) were used. De Rijke et al. [8] employed MSPD to extract and isolate isoflavone glucoside malonates from leaves of leguminous plants but, compared with solid liquid extraction, the efficiency for extracting the glucosides was found to be lower [4]. In the work of Ziaková et al. [6] that tried the extraction of phenolic acids by MSPD, from a medicinal plant, the results obtained were not quantitatively compared with any other conventional techniques.

Grapes are complex matrices and the complexicity of its study is increased by the numerous varieties of *Vitis vinifera* used over the various producing areas. Several studies have been carried out in order to try to correlate the variety with the chemical composition of the grapes and wines obtained from them. Among the metabolites used for such purpose, we can mention the phenolics, coloured or non-coloured [9–14], since, besides working as marker compounds, these substances are known to possess several interesting properties related with health [15–17]. In addition, these constituents contribute strongly to the organoleptic characteristics of grapes, and therefore of the wine obtained from them [18].

"*Vinho Verde*" is considered a QWPSR (Quality Wine Produced in a Specified Region) and is confined to the north-west region of Portugal. A search over the published literature showed that only few studies were carried out on the chemical composition of these wines [19–22] and even less on their producing grapes [23]. As far as we know, their content of phenolic compounds and organic acids has not been determined yet.

In a previous work [24] a conventional analytical method was developed to determine phenolic compounds and organic acids from white grapes. This method needed two steps, one for the simultaneously extraction of both types of compounds by solid liquid extraction (SL), and another for the separation and clean-up of organic acids and phenolic compounds by solid-phase extraction (SPE).

In this paper, the first objective was to test the ability of MSPD to, separately, extract phenolic compounds and organic acids from white grapes in just one step, simplifying the SL-SPE analytical procedure. The target compounds were determined using high performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) for phenolic compounds or to an UV detector for organic acids.

Once the advantages of the SL-SPE analytical method for the studied matrix were checked, samples of all the white grape varieties of the "*Vinho Verde*" recommended by "*Comissão de viticultura da região dos Vinhos Verdes*" [25] (i.e. Alvarinho, Avesso, Asal Branco, Batoca, Loureiro, Pedernã and Trajadura) and a few white grape varieties authorized (Douradinha, Esganoso Castelo Paiva and Rabigato), were analysed. Afterwards, multivariate techniques of data analysis, principal components analysis (PCA) and linear discriminant analysis (LDA) were employed in order to establish differentiation criteria as a function of the types of grapes. Although other factors such as polymorphism [26] can influence chemical composition of the grapes and, therefore, be important for the differentiation among them, in this study only the variety and origin of the samples have been considered for statistical analysis.

2. Experimental

2.1. Reagents and solvents

Methanol (MeOH), ethanol and formic acid were obtained from Merck (Darmstadt, Germany), hydrochloric acid from Pronalab (Lisboa, Portugal) and sulphuric acid from Fluka Sigma–Aldrich (Seetze, Germany). The water was treated in a Milli-Q water purification system (Millipore, Bedford, MA). The ultrasonic bath was from Bandelin (Berlin, Germany).

The phenolic compounds and the organic acids used as references were obtained from the following sources: oxalic, citric, fumaric, L(−)malic, (−)shikimic and DL-tartaric acids, quercetin and quercetin-3-*O*-glucoside from Sigma–Aldrich (Steinheim, Germany); (−)-epicatechin, kaempferol, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside and quercetin-3-*O*-rutinoside from Extrasynthése (Genay, France).

2.2. Procedures

2.2.1. Grape samples

Samples of different varieties of white "*Vinho Verde*" grapes were collected in different locations and vineyards: Famalicão (one vineyard), Felgueiras (one vineyard), Monção (three vineyards: Quinta de Menanços, Quinta de Moreira and Quinta de Ceivães) and Ponte de Lima (two vineyards: Quinta de Barreiros and Quinta da Facha) in September of 2005. The varieties under study were: Alvarinho, Asal Branco, Avesso, Batoca, Douradinha, Esganoso Castelo Paiva, Loureiro, Pedernã, Rabigato and Trajadura (Table 1). After harvest, the entire grapes were stored at −20 °C and freeze-dried in a Labconco 4.5 apparatus (Kansas City, MO).

2.2.2. Extraction and solid-phase extraction (SPE)

The solid-phase extraction columns used were Chromabond C18 non-endcapped (NEC) columns (50 μm particle size, 60 Å porosity; 10 g sorbent mass/70 mL reservoir volume) from Macherey-Nagel (Düren, Germany).

Table 1
Variety and origin of the "*Vinho Verde*" white grape samples studied

Observation	Identification	Variety	Geographical origin[a]
1	AlvCei	Alvarinho	Moncão (Ceivães)
2	AlvFel	Alvarinho	Felgueiras
3	AlvMen	Alvarinho	Moncão (Menanços)
4	AlvMor	Alvarinho	Moncão (Moreira)
5	AsFel	Asal Branco	Felgueiras
6	AvFel	Avesso	Felgueiras
7	BatFel	Batoca	Felgueiras
8	DouFel	Douradinha	Felgueiras
9	EsgFel	Esganoso Castelo de Paiva	Felgueiras
10	LouQBa	Loureiro	Ponte da Lima (Quinta Barreiros)
11	LouQFa	Loureiro	Ponte da Lima (Quinta Facha)
12	PedFam	Pedernã	Famalicão
13	PedFel	Pedernã	Felgueiras
14	PedQBa	Pedernã	Ponte da Lima (Quinta Barreiros)
15	PedQFa	Pedernã	Ponte da Lima (Quinta Facha)
16	RabFel	Rabigato	Felgueiras
17	TraFam	Trajadura	Famalicão
18	TraFel	Trajadura	Felgueiras
19	TraQBa	Trajadura	Ponte da Lima (Quinta Barreiros)
20	TraQFa	Trajadura	Ponte da Lima (Quinta Facha)

[a] When grapes come from different locations in the same geographical origin, it is noted in brackets.

The experimental procedure SL-SPE was described in a previous work [24]. Each sample (1 g) was accurately weighed and mixed with aqueous HCl (pH 2) containing 5% MeOH (5 × 50 mL) at 40 °C, in an ultrasonic bath during 20 min for each solvent fraction. The extracts were filtered under vacuum and combined. The aqueous solution was then passed through a Chromabond C18 (NEC) column, previously conditioned with 30 mL of MeOH and 70 mL of aqueous HCl (pH 2). The aqueous extract, containing the organic acids, was evaporated until dryness under reduced pressure (30 °C) and redissolved in 0.01N sulphuric acid (1 mL). Twenty microlitres of the redissolved extract were analysed by HPLC-UV.

After the elution of organic acids and other polar compounds with the aqueous solvent, the retained phenolic fraction was eluted with 150 mL of ethanol. The extracts were taken to dryness under reduced pressure (30 °C) and redissolved in MeOH (1 mL). This methanolic solution (20 μL) was analysed by HPLC-DAD.

2.2.3. Alkaline hydrolysis of the methanolic extract obtained by SL-SPE

Four millilitres of 2N NaOH were added to 0.4 mL of methanolic extract obtained as previously mentioned. The solution was kept in the dark for 4 h, acidified with HCl and passed through a C18 Bond Elut cartridge, preconditioned with MeOH and 2N HCl. The phenolic compounds were eluted with MeOH. This solution was taken to dryness under reduced pressure (30 °C), dissolved in 1 mL of MeOH, and 20 μL were analysed by HPLC.

2.2.4. Matrix solid-phase dispersion (MSPD)

The MSPD grade Isolute sorbents, MSPD C18 non-endcapped and MSPD C18 endcapped (EC) (mean particle size: 40–70 μm, average pore size: 60 Å) were from International Sorbent Technology Ltd. (Hengoed Mid Glam, UK). C18 EC sorbents present a structure of C18 silane and a trimethyl silyl group, covalently bonded to the surface of the silica particle, which reduce the polar secondary interactions associated with surface silanol groups. These columns exhibit non-polar retention mechanism. C18 NEC sorbents have a structure of only C18 silane covalently bonded to the surface of the silica particle, which provide additional polar interactions associated with surface silanol groups. These columns exhibit non-polar, polar and cation exchange retention mechanisms.

1.0 g portions of sample were weighted, mixed and blended carefully with 2.0 g of C18 sorbent in a mortar in order to obtain a mixed stationary phase. Then, the mixture was introduced into a 15 mL syringe body and packed using a syringe plunger.

Aqueous HCl (pH 2) or aqueous HCl (pH 2) containing 5% MeOH was passed through the mixture (at vacuum) to recover the organic acid fraction. This aqueous extract was evaporated until dryness under reduced pressure (30 °C), and the residue was redissolved in 1 mL of 0.01N sulphuric acid and analysed by HPLC-UV (20 μL).

After the elution of organic acids and other polar compounds with the aqueous solvent, the retained phenolic fraction was eluted with ethanol. This extract was taken to dryness under reduced pressure (30 °C), redissolved in MeOH (1 mL), and 20 μL were analysed by HPLC-DAD.

2.2.5. HPLC-UV analysis of organic acids

The chromatographic experiments were performed on an analytical HPLC unit (Gilson) as previously reported in Ref. [27]. The compounds were separated using an ion exclusion Nucleogel Ion 300 OA (300 mm × 7.7 mm) column, in conjunction with a column heating device at 30 °C. Elution was carried out at a solvent flow rate of 0.2 mL min^{-1}, isocratically, with 0.01N sulphuric acid as the mobile phase. Detection was performed with a UV detector set at 214 nm. Each compound was identified comparing its retention time with the corresponding peak in

a standard solution. Organic acids quantification was achieved using a calibration plot of external standard at 214 nm for all compounds.

2.2.6. HPLC-DAD analysis of phenolic compounds

The extracts were analysed on an analytical HPLC unit (Gilson), using a Spherisorb ODS2 column (25.0 cm × 0.46 cm; 5 μm particle size Waters, Milford, MA, USA) [27]. The solvent system used was a gradient of water/formic acid (19:1) (A) and MeOH (B), starting with 5% MeOH, followed by gradient steps of 15% B at 3 min, 25% B at 13 min, 30% B at 25 min, 35% B at 35 min, 45% B at 39 min, 45% B at 42 min, 50% B at 44 min, 55% B at 47 min, 70% B at 50 min, 75% B at 56 min and 100% B at 60 min, at a solvent flow rate of 0.9 mL min^{-1}. Detection was done using a Gilson diode array detector. The compounds quantified in each sample were identified comparing their retention times and UV–vis spectra in the 200–400 nm range with individual standards. Phenolic compounds quantification was achieved using a calibration plot of external standard at 350 nm for all compounds, except epicatechin, for which the calibration plot was at 280 nm. Quercetin-3-*O*-glucoside and quercetin-3-*O*-rutinoside were quantified together as quercetin-3-*O*-glucoside. Kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside were quantified together as kaempferol-3-*O*-rutinoside.

2.3. Statistical analysis

2.3.1. One-way analysis of variance (ANOVA)

Significant differences among the 10 white "*Vinho Verde*" grape varieties for each of the compounds were determined by ANOVA using a SPSS Program (version 14.0). Levene's test for homogeneity of variance was used to assess the validity of the ANOVA analysis. When variance homogeneity was not acceptable, the Welch test, a one-way robust test of equality of means, was employed instead of ANOVA.

2.3.2. Principal component analysis (PCA) and linear discriminant analysis (LDA)

PCA and LDA were performed by Statgraphics. PCA is a tool for data exploration which allows the reduction of the dimensionality of data facilitating the analysis of intersample relationships. New variables, so called principal components, are obtained to explain the greater part of total variance with a minimum of information loss [28,29]. LDA is employed as a classification tool to discriminate between two or more groups of samples.

PCA was performed separately for each chemical parameter studied (phenolic and organic acid profiles) and also for the global data. LDA was performed for the global data.

3. Results and discussion

3.1. Identification of compounds

White "*Vinho Verde*" grapes showed a phenolic profile composed by 10 identified phenolic compounds: caftaric acid, coutaric acid, fertaric acid, quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, quercetin, kaempferol and epicatechin. The HPLC chromatogram obtained at 350 nm is shown in Fig. 1.

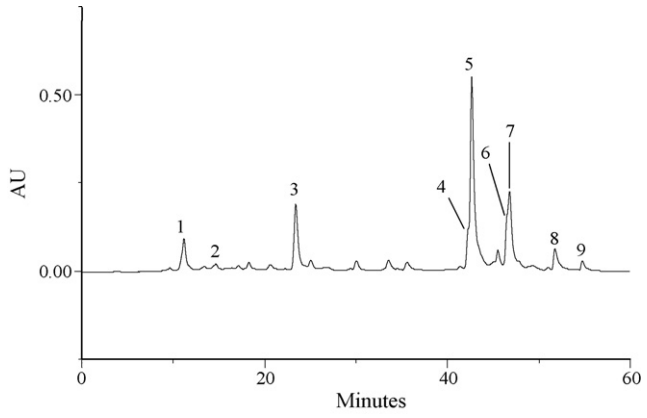

Fig. 1. HPLC chromatogram of phenolic compounds of a sample of white "*Vinho Verde*" grapes (AlvMen) at 350 nm. Peak identities: (1) caftaric acid; (2) coutaric acid; (3) fertaric acid; (4) quercetin-3-*O*-glucoside; (5) quercetin-3-*O*-rutinoside; (6) kaempferol-3-*O*-glucoside; (7) kaempferol-3-*O*-rutinoside; (8) quercetin; (9) kaempferol.

Quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, quercetin, kaempferol and epicatechin were identified by comparing their retention times and UV–vis spectra in the 200–400 nm range with individual standards. Other HPLC conditions were tried for the separation of coeluting compounds (pairs 4/5 and 6/7), namely different gradients and columns, but we decided for those described in Section 2, which allowed a better separation and quantification of the aglycons. Nevertheless, the compounds of each pair are structurally related: compounds 4 and 5 are both glycosidic derivatives of quercetin, while compounds 6 and 7 are kaempferol glycosides. In addition, the aglycone is the molecule's portion responsible for the UV–vis absorption spectrum of each compound. So, the quantification of the pair as a whole does not introduce great alterations in the quantitative information of the class of compounds in each sample.

Caftaric (Rt 11.2 min), coutaric (Rt 14.7 min) and fertaric (Rt 23.4 min) acids were identified after a chromatographic analysis under the conditions previously used in our laboratory [22] for an extract of white "*Vinho Verde*" grapes obtained by SL-SPE. Alkaline hydrolysis of this extract showed the absence of the chromatographic peaks corresponding to coutaric, caftaric and fertaric acids and the presence of *p*-coumaric, cafeic and ferulic acids, which confirmed the identity of these compounds. However, these compounds could not be quantified due to their high instability in the extracts.

The identified organic acids were oxalic, citric, tartaric, malic, shikimic and fumaric acids. The chromatographic peak between tartaric and malic acids was identified as fructose. The HPLC chromatogram obtained for organic acids is shown in Fig. 2.

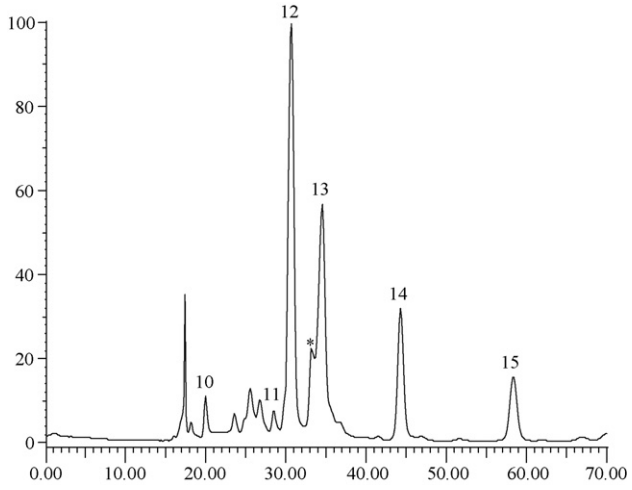

Fig. 2. HPLC chromatogram of the organic acids of a sample of white "*Vinho Verde*" grapes (BatFel) at 214 nm. Peak identities: (10) oxalic acid; (11) citric acid; (12) tartaric acid; (13) malic acid; (14) shikimic acid; (15) fumaric acid; (*) fructose.

3.2. MSPD versus SL-SPE for white grapes

The SL-SPE method previously developed [24] to extract phenolic compounds and organic acids from white grapes, consisted of a first simultaneous extraction of both types of compounds using aqueous HCl (pH 2) with 5% MeOH in ultrasonic bath and a second step of clean-up, where the organic acids and phenolic compounds were separated using a C18 NEC column. When the acidic aqueous extract was passed through the column, organic acids were eluted while phenolic compounds were retained on the C18 NEC sorbent and eluted later with ethanol. Finally, both fractions were analysed separately.

In this work, the potential use of MSPD to separately extract organic acids and phenolic compounds from white grapes was tested. Since in MSPD the entire sample is blended into the column, it is theoretically possible to perform the sequential elution of the sample to isolate several classes of compounds from it [1].

Some assays were planned based on the results previously obtained during the development of the SL-SPE method. The type of solvent and sorbent, have shown to be two of the most influential variables for the extraction and the separation of the analytes, respectively. C18 sorbent was chosen because of its wide use for MSPD [3,5] and, specifically, to extract phenolic compounds from plants [6–8]. Although C18 non-endcapped and endcapped did not show to influence the extraction of phenolic compounds from medicinal plants [6], both types of sorbents, or their combination, were tested because in our previous work using SL-SPE, they showed a different effect in the extraction of organic acids and phenolic compounds.

The solvents chosen to sequentially elute the analytes were those that have shown to be efficient to separate the compounds with the C18 SPE column: aqueous HCl (pH 2) with 5% MeOH was tested as first eluent to recover the organic acids and ethanol as second eluent to recover the phenolic compounds. In the development of the SL-SPE procedure [24], MeOH was added

Table 2
Experimental conditions employed for MSPD and SL-SPE

	1-MSPD	2-MSPD	3-MSPD	4-MSPD	5-MSPD	6-MSPD	SL-SPE
Type of sorbent	NEC	EC	NEC	EC	50% EC/50% NEC	NEC	NEC
First eluent[a]	150 mL aqueous HCl (pH 2) with 5% MeOH	150 mL aqueous HCl (pH 2) with 5% MeOH	150 mL aqueous HCl (pH 2)	150 mL aqueous HCl (pH 2)	150 mL aqueous HCl (pH 2)	150 mL aqueous HCl (pH 2) with 5% MeOH	150 mL aqueous HCl (pH 2) with 5% MeOH
Second eluent	70 mL ethanol	70 mL ethanol	70 mL ethanol	70 mL ethanol	70 mL ethanol	150 mL ethanol	150 mL ethanol

NEC: non-endcapped; EC: endcapped; HCl: hydrochloric acid; MEOH: methanol.
[a] pH was modified with HCl.

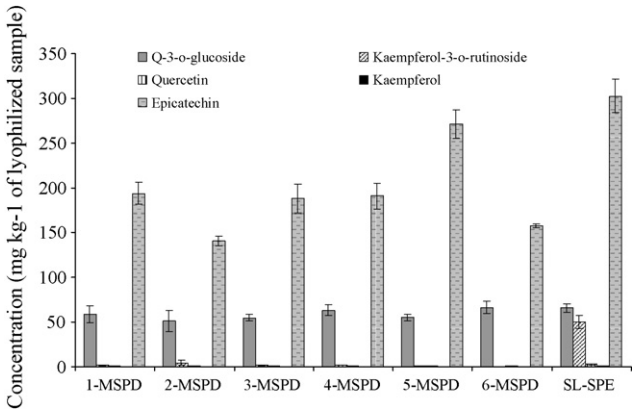

Fig. 3. Comparison between MSPD and SL-SPE for the extraction of phenolic compounds from a sample of white grapes. Experimental conditions of each assay according to Table 2.

to aqueous HCl (pH 2) to improve the recovery of the analytes. Therefore, the addition of MeOH to the acid water was also tested.

In literature, sample/sorbent ratios typically range from 1:1 to 1:4 [5]; therefore a ratio of 1 g sample:2 g of sorbent was chosen.

For these preliminary assays, large volumes of solvents (150 mL of aqueous HCl (pH 2) and 70 mL of ethanol) were employed to guarantee the complete elution of the analytes. This precaution was taken because although small volumes are usually reported to be sufficient for MSPD procedures, the study carried out by Ziaková et al. [6] showed that 20–25 mL of some eluents were necessary to get quantitative recoveries of some phenolic acids.

Considering all these variables, five different assays (1-MSPD to 5-MSPD) were carried out. One more assay (6-MSPD) was planned using the same sorbent, volume and type of eluents as in the SL-SPE method, although these volumes are much higher than the ones usually employed for MSPD. Their experimental conditions are shown in Table 2.

Finally, the grapes were also analysed by the SL-SPE method (Table 2) to compare the results obtained with both techniques. The obtained results as mean of three determinations are shown in Figs. 3 and 4 for phenolic compounds and organic acids,

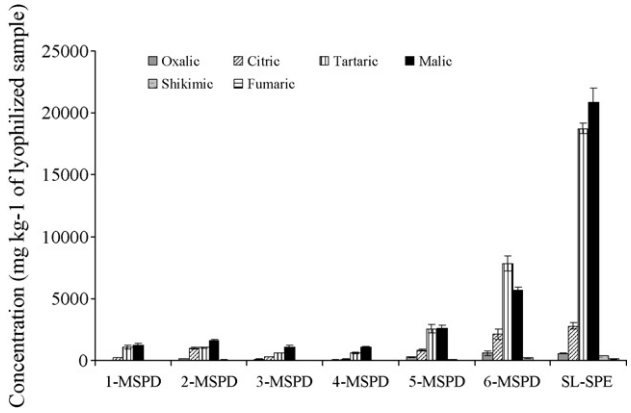

Fig. 4. Comparison between MSPD and SL-SPE for the extraction of organic acids from a sample of white grapes. Experimental conditions of each assay according to Table 2.

respectively. Results are given as concentration of each compound in the lyophilized sample.

3.2.1. Phenolic compounds

As can be seen in Fig. 3, recoveries obtained for phenolic compounds were, in general, lower for MSPD than for SL-SPE. Only the recoveries obtained for the pair quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside and for epicatechin were similar, in some assays, to those obtained using SL-SPE. So, for the pair quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside, the signal obtained with the assay 6-MSPD was similar to the one obtained with SL-SPE (101%), but a very high volume of solvents (150 mL of ethanol), not usual in MSPD, was necessary. The best results for epicatechin, on using MSPD, were obtained with experiment 5-MSPD when a sorbent obtained mixing 50% NEC C18 and 50% EC C18 was employed. The amount of epicatechin obtained was 90% in relation to SL-SPE.

For the rest of the phenolic compounds the recoveries obtained for MSPD were very poor in comparison to those obtained for SL-SPE. The pair kaempferol-3-*O*-glucoside/kaempferol-3-*O*-rutinoside was not recovered at all in experiment 6-MSPD, even using the highest elution volumes. This indicates that probably these compounds were eluted with the first solvent (aqueous HCl (pH 2)) instead of ethanol. Another assay using 45 mL of aqueous HCl (pH 2) and 70 mL of ethanol was carried out to check this hypothesis (data not shown). As was expected, the amount of kaempferol-3-*O*-glucoside/kaempferol-3-*O*-rutinoside increased, although only 52% of the amount recovered with SL-SPE method was obtained while the recoveries of the other compounds were negatively affected.

Recoveries of quercetin and kaempferol, in all the assays carried out by MSPD, were always much lower than by SL-SPE, with the best recoveries being around only 30%.

3.2.2. Organic acids

As can be seen in Fig. 4, recoveries obtained for organic acids were, in general, much lower for MSPD than for SL-SPE, and much poorer than those obtained for phenolic compounds. The best results with MSPD were obtained with assay 6-MSPD that uses the highest volumes of eluents. In these conditions, the best recoveries were obtained for oxalic and citric acids, for which the obtained area was 103 and 77%, respectively, of the signal obtained with SL-SPE. For the other acids the obtained signal was much lower on using MSPD: 55% for shikimic acid, 42% for tartaric acid, 27% for malic acid and 6% for fumaric acid.

Addition of MeOH to the aqueous HCl (pH 2), or the use of C18 NEC or EC did not show to be very influential, although better results were obtained for some compounds (epicatechin and organic acids) when both sorbents were combined 1:1 (5-MSPD). The best results for MSPD were obtained using the highest volume of eluent, which is considerably higher than usually employed with this technique.

In conclusion, MSPD can be useful for the qualitative analysis of phenolic compounds and organic acids from white grapes but, for the quantitative analysis, SL-SPE has shown to have higher extractive capacity, especially for organic acids. This much lower extractive capacity of MSPD for organic acids

was probably related to the high content in sugars of the white grapes that coelute with them making their complete extraction difficult. On the other hand, results obtained for phenolic compounds agree with those obtained by Xiao et al. [7] who got lower extraction levels of glycoside phenolic compounds with MSPD than with other conventional techniques (Soxhlet, ultrasonic).

In a more specific point of view, with the exception of oxalic acid, the recuperation of organic acids was very small. For phenolic compounds, only quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside and epicatechin presented similar recuperation values for both methods, but for the remaining ones it was very low and kaempferol-3-*O*-glucoside and kaempferol-3-*O*-rutinoside even disappeared with MSPD.

Considering these results, the previously developed SL-SPE was chosen to analyse white "*Vinho Verde*" grapes. This analytical method was validated in a previous work [24]. The analytical method was precise since relative standard deviation (R.S.D.) was 2.2–9.1% for organic acids and 2.8–15% for phenolic compounds. Limits of detection were between 1.5 and 86 mg kg^{-1} for organic acids and 0.55 and 3.5 mg kg^{-1} for phenolic compounds while limits of quantification ranged between 5.1 and 286, and 1.8 and 12 mg kg^{-1} for organic acids and phenolic compounds, respectively. Recovery values of the method were between 72 and 95% for phenolic compounds and between 72 and 103% for organic acids which demonstrates the effectiveness of the extraction.

Under the described assay conditions, a linear relationship between the concentration of each compound and their UV absorbance was obtained, at 214 nm for oxalic, citric, L-tartaric, L-malic, shikimic and fumaric acids, at 280 nm for epicatechin and at 350 nm for quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin and kaempferol. The correlation coefficient for the standard curves invariably exceeded 0.99 for all studied compounds. The regression equations for oxalic, citric, L-tartaric, L-malic, shikimic and fumaric acids were $y = 1.5 \times 10^3 x + 5.5 \times 10^4$, $y = 2.4 \times 10^2 x + 7.0 \times 10^3$, $y = 8.6 \times 10^2 x + 7.8 \times 10^3$, $y = 2.5 \times 10^2 x - 1.5 \times 10^4$, $y = 1.3 \times 10^4 x + 2.6 \times 10^4$, $y = 2.6 \times 10^4 x + 1.6 \times 10^4$, respectively. The regression equations for epicatechin, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin and kaempferol were $y = 3.3 \times 10^5 x - 8.1 \times 10^4$, $y = 1.0 \times 10^6 x - 3.6 \times 10^5$, $y = 8.4 \times 10^5 x - 7.4 \times 10^5$, $y = 1.2 \times 10^6 x - 4.8 \times 10^5$, $y = 1.5 \times 10^6 x + 1.1 \times 10^5$.

The repeatability and reproducibility of the chromatographic method was evaluated by measuring the peak chromatographic area of each compound six times on the same standard solution in the same day and in different days, respectively. The chromatographic method is precise: the repeatability study showed relative standard deviation (R.S.D.) between 1.6 and 4.4% for organic acids, and between 3.5 and 4.7% for phenolic compounds. The reproducibility study showed that R.S.D. ranged from 6.7 to 9.9% for organic acids and from 3.8 to 4.9 for phenolic compounds.

3.3. Analysis of white "Vinho Verde" grapes

3.3.1. Phenolic compounds

Seven phenolic compounds were quantified in white "*Vinho Verde*" grapes: quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, quercetin, kaempferol and epicatechin (Table 3).

Table 3
Phenolic composition of white "*Vinho Verde*" grape samples (mg kg^{-1} of lyophilized sample)

		Q3gluc (Rt 42.2) + Q3rutin (Rt 42.7)		Kaemp3rutin (Rt 46.5) + Kaemp3gluc (Rt 46.8)		Quercetin (Rt 51.8)		Kaempferol (Rt 54.8)		Epicatechin (Rt 18.6)		Total
		Mean	*S*	Mean	*S*	Mean	*S*	Mean	*S*	Mean	S	
1	AlvCei	247	2.4	65	0.86	6.8	0.023	1.8	0.017	91	4.7	411
2	AlvFel	228	3.4	308	5.2	16	1.3	3.5	0.43	350	6.3	906
3	AlvMen	281	13	138	9.4	29	3.8	5.4	0.87	106	8.3	559
4	AlvMor	278	5.8	209	2.2	28	2.1	5.9	0.42	175	5.6	695
5	AsFel	234	3.3	76	6.3	4.9	0.55	nq		97	6.8	412
6	AvFel	92	1.2	79	2.1	4.9	0.14	nq		281	1.8	457
7	BatFel	44	0.26	52	2.0	nq		nq		331	9.8	426
8	DouFel	439	9.1	427	29	11	1.3	nq		174	9.3	1051
9	EsgFel	79	4.8	58	3.0	nd		nd		152	26	290
10	LouQBa	69	6.9	18	0.88	nq		nd		644	59	731
11	LouQFa	171	7.0	124	1.3	10	0.61	2.0	0.039	158	0.086	465
12	PedFam	191	13	76	2.9	nq		nq		174	4.6	441
13	PedFel	100	7.3	45	6.5	10	0.92	1.8	0.11	116	24	273
14	PedQBa	87	6.6	12	1.2	nd		nd		450	0.90	549
15	PedQFa	288	8.0	145	22	9.5	0.85	nq		289	18	732
16	RabFel	395	22	159	17	16	2.4	2.2	0.25	nd		573
17	TraFam	121	3.2	17	1.3	nd		nd		274	5.8	412
18	TraFel	127	11	52	2.5	9.3	0.16	nq		258	16	446
19	TraQBa	74	1.7	6.5	0.92	5.5	0.19	nq		263	7.7	349
20	TraQFa	149	10	58	2.6	50	2.2	4.0	0.42	179	2.2	440

Q3gluc: quercetin-3-*O*-glucoside; Q3rutin: quercetin-3-*O*-rutinoside; Kaemp3rutin: Kaempferol-3-*O*-rutinoside; Kaemp3gluc: Kaempferol-3-*O*-glucoside. Results are expressed as mean of three determinations and standard deviation. nd = not detected (concentration sample < detection limit). nq = not quantified (detection limit < concentration sample < quantification limit).

Table 4
Eigenvalues, percentage of variance and cumulative percentage explained by the most important principal components for each chemical group under study

Group	Principal component	Eigenvalue	Percent of variance (%)	Cumulative percentage (%)
Phenolic compounds	1	2.75	55.1	55.1
	2	1.14	22.8	77.9
Organic acids	1	2.12	35.3	35.3
	2	1.58	26.4	61.6
	3	1.06	17.7	79.4
Global data	1	3.46	31.4	31.4
	2	2.19	19.9	51.4
	3	1.64	15.0	66.4
	4	1.14	10.3	76.7

The most abundant phenolic compounds found in all the studied grapes were epicatechin (39–82%) or the pair quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside (37–69%).

Epicatechin was the most abundant phenolic compound for the grapes of the varieties Avesso, Batoca, Esganoso, Castelo de Paiva and Trajadura while quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside was the most abundant for Alvarinho (except those from Felgueiras), Asal Branco, Douradinha and Rabigato. For the varieties Loureiro and Pedernã the amounts of epicatechin and quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside were similar, except for grapes from Quinta de Barreiros, which content of epicatechin was considerably higher.

Kaempferol-3-*O*-rutinoside and kaempferol-3-*O*-glucoside appeared in all samples in lower proportion (1.9–41%). Quercetin and, in particular, kaempferol were found in such low amounts, that they could not be quantified in most samples.

This phenolic profile with high content of glycosylflavonols or epicatechin and low levels of quercetin and kaempferol agrees with results obtained for other grapes. Quercetin-3-*O*-glucoside was the most abundant non-coloured phenolic compound in different red and white varieties of grapes studied by Cantos et al. [9] (who also quantified it together with quercetin-3-*O*-rutinoside), and Amico et al. [11] and it was the second most abundant in skin of white Weisser Riesling grapes (where quercetin-3-*O*-glucuronide was the most abundant) [12]. On the other hand, epicatechin was the most abundant compound in seeds of muscadine grapes [10] and in some red grapes [30]. Kaempferol-3-*O*-glucoside was detected in low quantity in white grapes [9] and red [11,13] and in higher concentration in the skin of Weisser Riesling grapes [12].

Quercetin or kaempferol were not detected in other grape samples such as in Weisser Riesling [12], or were found in only low quantities in muscadine grapes [10].

ANOVA showed significant differences between the phenolic profiles among samples of different varieties, in terms of kaempferol-3-*O*-rutinoside/kaempferol-3-*O*-glucoside ($p < 0.05$). Similarly, the Welch test showed differences in terms of quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside ($p < 0.05$). ANOVA also showed the influence of geographical origin on kaempferol ($p < 0.05$).

3.3.1.1. Principal component analysis. The differences among the samples, according to the variety and origin, were emphasized by the PCA. For phenolic compounds, PCA yields two principal components explaining 77.9% of the total variance in the data (Table 4). Fig. 5a shows the corresponding loading plots that establish the relative importance of each variable and it is therefore useful for the study of relations among the variables and grapes. The first PC, which explains 55.1% of the variance, correlates positively with epicatechin and negatively with the rest of the phenolic compounds. The second PC correlates positively

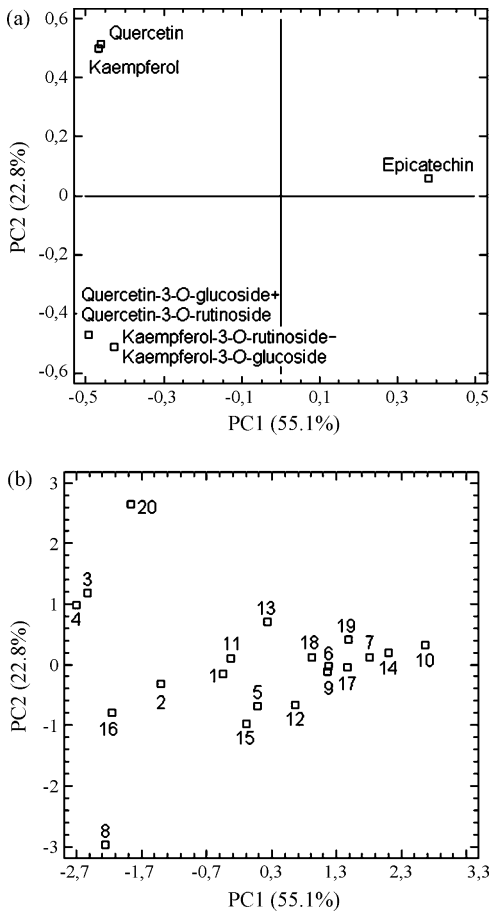

Fig. 5. PC1 vs. PC2 scatter plot of the main sources of variability between the "*Vinho Verde*" white grapes (a) relation between the phenolic compounds (loadings); (b) distinction between the samples (scores). Observations identities are listed in Table 1.

with epicatechin, kaempferol and quercetin, and negatively with kaempferol-3-*O*-rutinoside/kaempferol-3-*O*-glucoside and quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside.

As can be seen in Fig. 5b phenolic composition was strongly related to both origin and variety of the grapes: the grapes of the varieties Douradinha, Rabigato and Alvarinho (observations 1, 2, 3, 4, 8 and 16) appeared in the third/fourth quadrants, separated from the rest of the samples, due to their higher content of glycosyl flavonols and flavonols. The scores of the rest of the samples fall along the second PC. These last grapes belonged to the varieties Loureiro, Asal Branco, Pedernã, Esganoso Castelo de Paiva, Trajadura, Avesso and Batoca. In general, these grapes show higher content in epicatechin and lower content in flavonols.

However, differences among the varieties were observed according to their different geographical origin. In this way, for the first group, grapes from Felgueiras (observations 2, 8 and 16) showed the highest content in the glycosyl flavonols while the rest of samples (1, 3 and 4, all Alvarinho) showed lower levels of these compounds. Even the Alvarinho grapes from Monção appeared separated in two subgroups, one with grapes with higher content of quercetin and kaempferol (Menanços and Moreira) and another with lower content (Ceivães) of those compounds. This may be explained because Menanços and Moreira are geographically closer.

In the second group, if only the variety is considered, the samples are strongly mixed. However, it could be observed that samples of different varieties with the same origin appeared quite close in the PC plot, like Trajadura and Pedernã from Famalicão (observations 12 and 17), Avesso, Batoca, Esganoso, Pedernã and Trajadura from Felgueiras (6, 7, 9, 13 and 18) and Trajadura, Loureiro and Pedernã from Quinta de Barreiros (10, 14 and 19), showing that both origin and variety influenced the phenolic composition of the studied grapes. It could be noticed again that samples of the same variety (Loureiro and Pedernã) and the same geographical origin (Ponte de Lima) but from different vineyards (observations 11 and 15, 10 and 14) showed differences in their phenolic profiles.

3.3.1.2. Phenolic profile of varieties with the same origin. If only the data of grapes from Felgueiras are considered (Table 3), the phenolic profile of grapes of nine different varieties with the same origin can be compared. Varieties Douradinha and Alvarinho showed the higher total content in phenolic compounds, followed by Trajadura, Asal Branco, Avesso and Batoca with a similar content, while the lowest levels were found in Esganoso Castelo Paiva and Pedernã. Quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside was the most abundant compound for Asal Branco (57%), Douradinha (42%) and Rabigato (69%), whereas epicatechin was the most abundant for Alvarinho (39%), Avesso (62%), Batoca (78%), Esganoso (52%), Pedernã (43%) and Trajadura (58%), the sum of glycosylflavonols was more abundant for Rabigato (97%) and Douradinha (82%) followed by Asal Branco (75%), while Alvarinho, Avesso, Esganoso, Pedernã, and Trajadura showed lower levels, between 37 and 59%, and Batoca the lowest content (22%). All the varieties showed very low contents in quercetin, with a slightly higher content for Pedernã and Rabigato. Kaempferol was only quantified in Pedernã. If the ratios between the compounds are considered to design the phenolic fingerprint, which can be easily observed drawing the graphs, it can be observed that Rabigato, Douradinha, Alvarinho, Esganoso and Batoca have unique and distinct profiles while those of Pedernã, Trajadura and Avesso show more similar profiles.

Table 5
Organic acids composition of white "*Vinho Verde*" grape samples (mg kg^{-1} of lyophilized sample)

		Oxalic (Rt 19.8)		Citric (Rt 28.3)		Tartaric (Rt 30.5)		Malic (Rt 34.4)		Shikimic (Rt 44.2)		Fumaric (Rt 58.2)		Total
		Mean	*S*	Mean	*S*	Mean	*S*	Mean	*S*	Mean	*S*	Mean	*S*	
1	AlvCei	733	10	459	48	3602	30	3288	245	17	2.3	nq		8100
2	AlvFel	1371	64	1167	17	3990	135	5822	345	43	4.9	nd		12392
3	AlvMen	721	83	527	35	3999	215	5505	106	19	1.6	nq.		10771
4	AlvMor	694	42	593	27	2738	397	6061	465	18	0.036	9.1	1.3	10114
5	AsFel	630	63	646	38	3025	12	4960	463	45	0.78	nq		9305
6	AvFel	409	13	507	21	3542	151	4314	537	51	2.0	nq		8822
7	BatFel	363	44	588	50	3395	75	5845	425	101	9.0	7.1	1.0	10299
8	DouFel	543	2.5	650	97	4139	147	4850	72	147	5.2	nq		10329
9	EsgFel	606	13	1110	89	5172	445	6731	556	193	5.7	nq		13811
10	LouQBa	731	31	2014	21	4827	659	11269	161	57	4.8	9.2	0.1	18907
11	LouQFa	417	10	529	99	4433	92	4335	186	18	2.1	8.2	0.16	9740
12	PedFam	465	69	nq		3105	82	3273	258	58	2.2	11	1.3	6911
13	PedFel	228	14	710	80	4017	307	5154	750	99	6.8	nq		10208
14	PedQBa	434	4.1	731	19	3543	27	5340	626	126	4.8	nq		10172
15	PedQFa	422	28	nq		4733	105	1857	105	37	1.7	nq		7050
16	RabFel	231	24	692	106	3015	222	5223	350	158	9.3	nq		9319
17	TraFam	544	28	1515	133	3754	623	4394	791	192	24	9.4	1.0	10410
18	TraFel	500	39	505	146	3158	93	4069	212	118	5.9	nq		8349
19	TraQBa	300	30	711	135	3008	83	8537	1305	188	4.8	12	2.9	12757
20	TraQFa	445	47	977	124	3414	395	3920	371	183	8.3	7.4	0.66	8947

Results are expressed as mean of three determinations and standard deviation. nd = not detected (concentration sample < detection limit). nq = not quantified (detection limit < concentration sample < quantification limit).

3.3.2. Organic acids

The identified organic acids were oxalic, citric, tartaric, malic, shikimic and fumaric acids (Table 5). ANOVA showed significant differences between the organic acids profile among samples of different varieties, in terms of shikimic acid ($p < 0.05$). Welch test showed the influence of geographical origin on shikimic and fumaric acids ($p < 0.05$).

Malic acid was the most abundant compound in most of the samples, followed by tartaric acid. The sum of these two compounds constituted 79–92% of the total amount of the organic acids analysed. The highest percentage of oxalic or citric acids was 11% and shikimic acid was found in the range 0.18–2.0%. The fumaric acid concentration was so low that it could not be quantified in most samples.

3.3.2.1. Principal component analysis. PCA for organic acids yields three principal components with eigenvalues greater than 1 accounting for 79.4% of the total variance in the data (Table 4). Considering that the first two components already account for 61.6% of the total variance, the third one was not considered to simplify the analysis of the results. The first PC, which account for 35.3% of the variance, was correlated positively with all of the compounds, and the second one, which accounts for 26.4%, was correlated positively with malic, shikimic and fumaric acids and negatively with citric, oxalic and tartaric acids (Fig. 6a).

The scores of most of the samples fall along the second PC (Fig. 6b), due to their similar content in malic and tartaric acids. Some samples appeared more separated, for example, Alvarinho grapes appeared in the negative quadrant (observations 1, 2 and 3) due to a higher content in oxalic acid, while Trajadura grapes appeared in the positive quadrant (observations 17, 19 and 20) due to their higher content in citric, fumaric and shikimic acids. A strong mix of the samples from different varieties could also be observed due to the influence of the origin. So, Asal, Avesso, Batoca, Douradinha and Esganoso grapes (observations 5, 6, 7, 8 and 9) all of them from Felgueiras, appeared very close in the plot.

3.3.2.2. Organic acids profile of varieties with the same origin. If only the data of grapes from Felgueiras are considered, the organic acid profiles of grapes of nine different varieties with the same origin can be compared. The highest content in organic acids was found in the varieties Esganoso de Castelo Paiva and Alvarinho, followed by Batoca, Douradinha and Pedernã, while the lowest content was found in Rabigato, Asal Branco, Aveso and Trajadura. Malic acid was the most abundant compound in all the varieties (47–57%), with the sum of tartaric and malic acids being very similar for all of them (79–90%). Fumaric acid was the organic acid found in lowest amount and could only be quantified in Batoca grapes.

The highest percentage of oxalic acid was found in Alvarinho (11%), followed by Asal branco (6.8%) and Trajadura (6%) while Avesso, Batoca, Douradinha and Esganoso Castelo Paiva, Pedernã and Rabigato showed levels between 2.2 and 5.3%. The highest level of citric acid was found in Alvarinho (9.4%) and Esganoso Castelo Paiva (8%) while the rest of the samples showed levels around 5.7–7.4%.

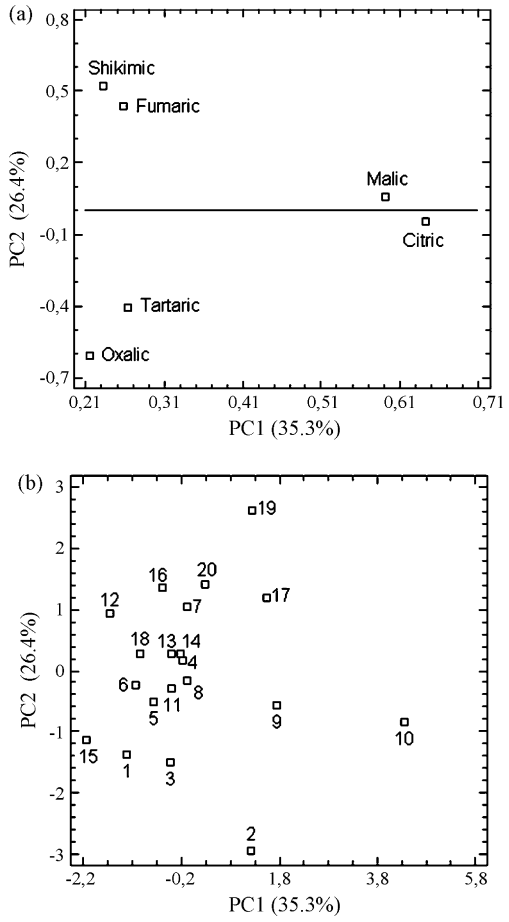

Fig. 6. PC1 vs. PC2 scatter plot of the main sources of variability between the "*Vinho Verde*" white grapes (a) relation between the organic acids (loadings); (b) distinction between the samples (scores). Observations identities are listed in Table 1.

The highest level of shikimic acid was found in Rabigato (1.7%), followed by Douradinha, Esganoso and Trajadura (1.4%) while the rest of the varieties showed levels equal or lower than 1%.

3.3.3. Global analysis

3.3.3.1. Principal component analysis. Fig. 7 presents the PCs of grape composition (phenolic compounds and organic acids). Although the analysis yields four PCs with eigenvalues greater than 1, the scree plot suggested involving two PCs into the model. So, only two PCs were retained to simplify the analysis of the results [31]. The two retained PCs account for 51.4% of the total variance: PC1 31.4% and PC2 19.9%.

PC1 was correlated positively with epicatechin and all of the organic acids except oxalic, and negatively with oxalic acid and flavonols. PC2 was correlated positively with all the compounds, except shikimic acid (Fig. 7a).

Grapes appeared again strongly mixed due to the influence of both origin and variety (Fig. 7b). Samples belonging to the same variety appear similar (Alvarinho, observations 1, 2, 3 and 4; Pedernã, observations 12, 13, 14 and 15; Trajadura, observations 17, 18, 19 and 20) but show important variations due to the dif-

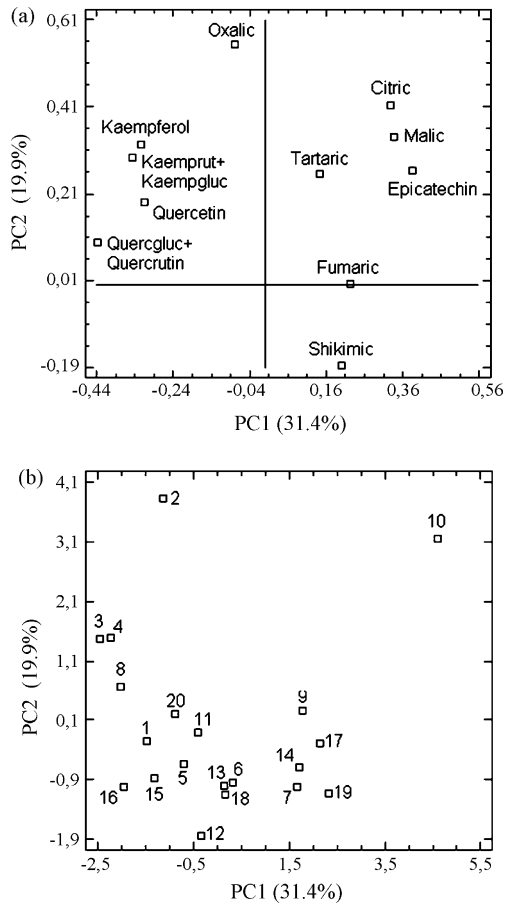

Fig. 7. PC1 vs. PC2 scatter plot of the main sources of variability between the "*Vinho Verde*" white grapes (a) relation between the phenolic and organic acids (loadings); (b) distinction between the samples (scores). Observations identities are listed in Table 1.

ferent origin. Samples of different variety but of same origin also look similar (different varieties from Felgueiras, observations 5, 6, 7, 8, 9, 13, 16 and 18).

3.3.3.2. Linear discriminant analysis. After PCA, a stepwise linear discriminant analysis was applied in order to obtain the most useful variables in the differentiation between white "*Vinho Verde*" grapes using the same data set as for PCA. Forward selection method was employed using F to enter and to remove 4 (default value used in the Statgraphics software). A 5% significance level was predefined. Those variables which surpassed the predefined significance limit were excluded.

For differentiation of grapes according the variety, two variables had the highest discrimination power at the 5% significance level: quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside and shikimic acid. The percentage of correctly classified grapes with these variables was 70% (Table 6). Samples of the Alvarinho, Pedernã, Loureiro and Trajadura varieties were incorrectly classified by LDA as being of the Avesso, Asal Branco or Esganoso varieties. As only one sample of each of the Avesso, Asal Branco or Esganoso varieties was studied, this clearly shows that more samples need to be studied to improve the classification.

Table 6
Stepwise discriminant analysis of phenolics and organic acids-classification[a] according to variety

Observed group	Number of samples	Correct classification (%)
Alvarinho	4	75
Asal Branco	1	100
Avesso	1	100
Batoca	1	100
Douradinha	1	100
Esganoso Castelo de Paiva	1	100
Loureiro	2	50
Pedernã	4	75
Rabigato	1	100
Trajadura	4	25
Total	20	70

[a] Variables: Quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside and shikimic acid.

Table 7
Stepwise discriminant analysis of phenolics and organic acids-classification[a] according to geographical origin

	Number of samples	Correct classification (%)
Famalicão	2	100
Felgueiras	9	78
Monçao	3	67
Ponte da Lima	6	17
Total	20	60

[a] Variables: kaempferol and fumaric acid.

For differentiation of grapes according to geographic origin (Famalicão, Felgueiras, Monção, Ponte da Lima) two variables had the highest discrimination power at the 5% significance level: kaempferol and fumaric acid. The percentage of correctly classified grapes with these variables was 60% (Table 7). But when a more detailed origin was introduced, Menanços, Moreira, Ceivães for the samples from Monção and Quinta de Barreiros and Quinta da Facha for the samples from Ponte da Lima, the correctly classified grapes were 100%. This result shows the importance of the origin in the composition of the grapes.

4. Conclusions

In summary, comparing MSPD and SL-SPE for extracting organic acids and phenolic compounds from white grapes, MSPD showed to be a simpler and faster technique but a complete extraction of all the compounds studied could not be achieved, especially for organic acids. So, although MSPD could be a useful tool to identify the phenolic and organic acids composition of white grapes, SL-SPE was preferred to get quantitative recoveries of these compounds.

Phenolic compounds and organic acids profiles were obtained for white "*Vinho Verde*" grapes of 10 different varieties grown in four different geographical locations. The most abundant compounds found were quercetin-3-*O*-glucoside/quercetin-3-*O*-rutinoside and epicatechin, and tartaric and malic acids.

Analysis of the results by using multivariate techniques showed the high influence of both variety and origin in their composition. PCA of phenolic compounds accounted for the highest variability (77.9%) with two PCs, enabling characterization of the varieties of samples according their higher content in epicatechin or flavonols. One hundred and 70% of recognition ability was obtained by the application of stepwise linear discriminant analysis (SLDA) using origin or variety as classification variable, respectively. Considering the strong effect of origin on the results, another study with a higher number of samples of each variety and origin would be interesting to improve the differentiation of grapes according to their variety.

The study of phenolic compounds and organic acids profiles using PCA accounted for the percentages of variance in the data set similar to those obtained by other authors that compared grapes employing variables such as physicochemical measurements (70.1%) [32] or carotenoids (85%) [33], or wines, employing variables such as amines and organic acids (77–84%) [34], volatile compounds (67–91%) [35] and free amino acids and biogenic amines (77%) [36]. Therefore, phenolic compounds and organic acids have been shown to be useful variables to characterize white "*Vinho Verde*" grapes.

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