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Evaluation of the feasibility of the electronic tongue as a rapid analytical tool for wine age prediction and quantification of the organic acids and phenolic compounds. The case-study of Madeira wine

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ABSTRACT

A set of fourteen Madeira wines comprising wines produced from four *Vitis vinifera* L. varieties (Bual, Malvasia, Verdelho and Tinta Negra Mole) that were 3, 6, 10 and 17 years old was analysed using HPLC and an electronic tongue (ET) multisensor system. Concentrations of 24 organic acids, phenolic and furanic compounds were determined by HPLC. The ET consisting of 26 potentiometric chemical sensors with plasticized PVC and chalcogenide glass membranes was used. Significance of the effects of age and variety on the ET response and wine composition with respect to the organic acids, phenolics and furanic derivatives were evaluated using ANOVA—Simultaneous Component Analysis (ASCA). Significance of the effects was estimated using a permutation test (1000 permutations). It was found that effects of age, grape variety and their interaction were significant for the HPLC data set and only the effect of age was significant for the ET data. Calibration models of the HPLC and ET data with respect to the wine age and of the ET data with respect to the concentration of the organic acids and phenolics were calculated using PLS1 regression. Models were validated using cross-validation. It was possible to predict wine age from HPLC and ET data with the accuracy in cross-validation of 2.6 and 1.8 years respectively. The ET was capable of detecting the following components (mean relative error in cross-validation is shown in the parentheses): tartaric (8%), citric (5%), formic (12%), protocatehuic (5%), vanillic (18%) and sinapic (14%) acids, catechin (6%), vanillin (12%) and *trans*-resveratrol (5%). The ET capability of predicting Madeira wine age with good accuracy (1.8 years) as well as quantify of some organic acids and phenolic compounds was demonstrated.

1. Introduction

Madeira is a type of fortified wine produced at the Madeira island of Portugal. Characteristic aroma and exceptional stability of these wines are results of the singular vinification methods employed in its production. It includes fortification for obtaining an ethanol content of 18–22%, after which wines undergo aging in wood casks at the temperatures up to 35 °C depending on the season and humidity levels of 70%. Some of the wines are previously submitted to baking or "estufagem" at 45–50 °C during at least 3 months. A "maderized" aroma typical for the Madeira and other sweet fortified wines aged under strong oxidation conditions is thus developed. An important role of oxygen in wine aging has been emphasised a long time ago. Moreover, oxidation was considered more important for flavour development than extraction of aroma compounds from oak wood for the wines undergoing oxidative aging to which Madeira wine belongs [1]. Another important contribution to the typical Madeira wine aroma comes from the products of Maillard reaction that are formed during heating [1,2].

Similarly to the other wines with controlled origin, Madeira wine is a subject to adulterations and reproductions, which makes necessary controlling authenticity and quality of these wines. Madeira wines similarly to the other fortified wines have very long lifetime and quality and price of these wines increases with the age. Therefore, there is a practical interest in development of the rapid methods for the age estimation of Madeira wines. Classification of the fortified wines including Madeira according to their age or variety using sets of volatile compounds has been reported [2–8]. Depending of particular set of samples used and analyses run, the importance of different components for discrimination between wines of different age has been pointed out. In particular, ethanal,

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ethyl acetate, total and volatile acidity [3], diethyl succinate and *cis*-oak-lactone [4], ethyl esters of fatty acids (C_6–C_{16}), ethyl lactate, diethyl succinate and furanic derivatives such as sotolon, furfural, 5-methylfurfural and 5-ethoximethylfurfural [2,5,6] were identified as compounds discriminating between Madeira wines of different ages. Volatile compounds including higher alcohols, fatty acids, ethyl esters [7] and terpenoids [8] were used for discriminating Madeira grape varieties. No reports on the differentiation of Madeira wines according to their age or variety based on the content of organic acids and phenolics have been published.

Concentration of total phenolics, or some of the phenolic fractions, in wine (i.e. non-flavonoid, flavonoid, anthocyanins, etc.) and total and volatile acidity can be measured using relatively simple techniques and minimal sample preparation, such as spectrophotometry [9,10] or FTIR spectroscopy [11]. However, currently used methods for the determination of individual organic acids and phenolic compounds, such as gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC) or liquid chromatography–mass spectrometry (LC–MS), are quite cumbersome and require skilled personal, expensive apparatus and very often laborious sample preparation procedures (e.g. [1–13]). Development of the analytical instrument that would allow rapid quality control, detection of components of interest and prediction of wine age avoiding at the same time use of expensive equipment and sample preparation is therefore of practical interest.

One of the analytical instruments meeting the requirements of simple instrumentation, minimal sample preparation and low cost is the electronic tongue multisensor system (ET) [14,15]. The electronic tongue is defined as an analytical instrument comprising a set of chemical sensors with partial selectivity to the compounds of interest and data processing tools. Such approach allows improving selectivity of the system compared to discrete sensors as well as performing tasks such as recognition, classification and taste assessment. Since the output of the ET is in most cases non-selective, chemometric methods are necessary for ET data processing. The ET sensor system enables conducting rapid measurements and requires little or no sample preparation, which makes the ET sensor systems a very attractive analytical tool for quality control, on-line analysis and process monitoring. Several applications of ETs to the analysis of foodstuffs and wines in particular have been reported. E-tongue based on an array of 23 potentiometric and chemical sensors with chalcogenide and plasticized PVC membranes was applied to the analysis of the 38 samples of red table wines from three different regions in Italy [16]. Recognition of wines according to their origin and quantification of total acidity (5%), tartaric acid (7%), glycerol (2%), total phenolics (6%), wine colour density (5%) and hue (6%), chemical age (8%), anthocyanins (7%), potassium (6%) and calcium (7%) have been performed [16,17]. Mean relative errors of prediction for the test data set for the PLS calibration models are shown in the parenthesis. Discrimination of wine tannins with different polymerisation degree using potentiometric ET and correlation of its response with perceived astringency of those compounds was reported [18]. Application of an ET comprising amperometric sensors based on the doped electropolymerised polypyrrols and carbon paste sensors modified by rare-earth bisphthalocyanines for wine analysis was reported [19–22]. This ET was successfully used for the discrimination of 12 samples of monovarietal wines according to the grape variety [19] and for the detection of common wine adulterants such as addition of sucrose, tannic, tartaric or acetic acid, acetaldehyde, among others [20]. Wines aged in different types of aging systems (e.g. stainless steel, oak, ships and staves) [21], during different periods of time and in barrels of oak of different origin [22] could be discriminated using a voltammetric ET. Quantification of eight wine parameters namely tannins, tartaric acid, glycerol, alcoholic grade, dry extract, total acidity, volatile acidity and reducing sugars was reported as well [20]. Application of the hybrid systems combining electronic nose and tongue was reported. Electronic nose based on the metal oxide sensors and ET based on two amperometric sensors was used for the discrimination of wines from different geographical areas [23] and prediction of sensory attributes [24]. Electronic nose and tongue both employing metalloporhyrins as active substances were used for prediction of chemical parameters such as alcohol content (2%), total acidity (7%), tartaric acid (8%) and glycerol (4%), and sensory attributes [25]. Mean relative errors of prediction for the validation data set for the PLS calibration models are shown in the parenthesis.

Potential of the ET sensor systems as an analytical tool for wine analysis was demonstrated. Though in many cases the ET systems cannot completely replace conventional analysis, they would allow a significant reduction in the number and frequency of such analysis therefore saving time and labor.

Application of the potentiometric ET to the prediction of Port wine age was reported [26]. The ET was calibrated in 160 samples of Port wine aged from 2 to 70 years and of different types (Tawny, Vintage, Late Bottled Vintage and Harvest). Wine age could be predicted by the ET with accuracy of 5 years for full sample set and with the accuracy of 1.8 years for the wines aged from 10 to 35. Capability of the ET to predict Port wine age was attributed to the sensor response to the changes in the phenolic compounds, which undergo oxidation and condensation in the course of aging. Madeira wines have lower initial content of phenolics compared to Port wines as they are produced from different grape varieties. Moreover, Madeira wines are aged at stronger oxidation conditions and consequently changes in the chemical composition including polyphenols, which take place during aging, are not identical for these two types of fortified wines. Therefore, results of age prediction of Port wines using ET system cannot be automatically transferred to the other types of fortified wines such as Madeira. The main purpose of this study was evaluation of the feasibility of the potentiometric ET to predict age of Madeira wines. Furthermore, the ET was applied to the quantification of the organic acids and phenolic compounds in Madeira wines. Effects of such factors as grape variety and age on the concentrations of organic acids, furanic and phenolic compounds were studied.

2. Materials and methods

2.1. Materials

Fourteen samples of Madeira monovarietal wines were used in this study. These included wines produced from four *Vitis vinifera* L. varieties, three white: Bual, Malvasia and Verdelho and one red: Tinta Negra Mole (TNM) aged for 3, 6, 10 and 17 years. Wines made of Tinta Negra Mole grapes with the ages 10 and 17 years were not available. Wines made from white grapes were not subjected to the baking process and underwent aging process in wood casks. Wines made from TNM were subjected to baking at up to 50 °C during 3 months and then transferred to the wood casks for further aging. This sample set constituted a full factorial design with two factors (variety and age) with four levels each that had two samples missing. Madeira wines were packed in the hermetically closed 250 mL glass bottles and opened before measurements.

2.2. Instrumentation

The chromatographic experiments were carried out on a Waters Alliance System (Milford, MA, USA) equipped with a separations module (Waters 2695) and a photodiode array detector (Waters 2996) and using an Atlantis dC18 column (250 mm × 4.6 mm, I.D., 5 μm; Milford, MA, USA). The chromatographic data system was

Table 1
List of organic acids, furanic and phenolic compounds measured in Madeira wines by HPLC together with respective working ranges and relative standard deviation (adapted from [25]).

#	Compound	Working range	Precision R.S.D.[a] (%)
Organic acids ($g\,L^{-1}$)			
1	Oxalic acid	0.012–0.307	1.2
2	Tartaric acid	0.060–1.512	1.2
3	Formic acid	0.001–0.030	1.4
4	Malic acid	0.122–3.045	1.0
5	Lactic acid	0.002–0.060	3.8
6	Acetic acid	0.002–0.060	1.0
7	Citric acid	0.090–2.252	1.0
Furanic compounds ($mg\,L^{-1}$)			
8	5-Hydroxymethylfurfural	1.50–30.00	0.2
9	Furfural	0.75–15.00	0.9
Phenolic compounds ($mg\,L^{-1}$)			
10	Gallic acid	0.003–0.054	0.2
11	Protocatechuic acid	0.80–15.90	0.5
12	*p*-Hydroxybenzoic acid	0.75–15.00	2.2
13	(−)-Epigallocatechin	0.75–15.00	0.8
14	(+)-Catechin	0.75–15.00	0.6
15	Vanillic acid	0.79–15.75	0.6
16	Caffeic acid	0.84–16.80	0.5
17	Syringic acid	0.75–15.00	0.5
18	Vanillin	0.75–15.00	0.9
19	Syringaldehyde	0.78–15.60	1.0
20	*p*-Coumaric acid	0.79–15.75	0.8
21	Ferulic acid	0.79–15.75	0.7
22	Sinapic acid	0.77–15.30	0.7
23	Ellagic acid	0.86–17.10	2.8
24	*trans*-Resveratrol	0.77–15.45	0.7

[a] R.S.D.—relative standard deviation.

controlled by the Empower Pro Software from Waters (Milford, MA, USA).

ET measurements were made using multisensor system comprising 26 potentiometric chemical sensors. It comprised the following sensors: plasticized PVC sensors displaying sensitivity to organic anions and phenols in particular (A1–A9) and to organic cations (C1–C5), chalcogenide glass sensors displaying redox response (G1–G11) and a conventional glass pH electrode. Responses of the sensor array were measured vs. conventional Ag/AgCl reference electrode. All sensors used in this study except the reference and pH electrodes were produced at the Laboratory of Chemical Sensors of St. Petersburg University [14].

2.3. Procedures

2.3.1. Determination of organic acids and phenolic compounds

Wines were characterized with respect to the content of the organic acids furanic and phenolic compounds using HPLC. The following mobile phases were used: A—10 mM of phosphate solution buffered at pH 2.70 with concentrated sulphuric acid and B—100% acetonitrile. Organic acids separation was achieved using an isocratic elution: 100% A. The separation of polyphenols required a gradient elution: 0–30 min, 20% B, linear; 30–50 min, 50% B, linear; followed by 10 min washing and re-equilibration of the column. The compounds under study were determined at different wavelengths (210, 254, 280, 315 and 360 nm) according to the corresponding UV absorption maxima. The wines samples were analysed after filtration (0.45 μm) and dilution, when necessary. Overall, 24 compounds were determined including seven organic acids, two furanic derivatives and fifteen phenolic compounds. All determinations were done in triplicates. A list of the compounds detected in Madeira wine by HPLC, together with the respective working ranges and relative standard deviation (R.S.D.—%) are shown in Table 2 [2].

2.3.2. Analysis using ET

Potentiometric measurements were carried out using a custom made high input impedance multichannel voltmeter connected to a PC. Madeira wines were diluted 2 times with distilled water prior to measurements to decrease the load of lipophilic wine compounds on the sensor membranes and therefore to speed up washing of the sensors between samples. Sensors were conditioned for 10 min in red table wine before each measuring session and then washed with distilled water until stable potential readings were reached. Between samples, sensors were also washed with distilled water. Measurement time in wine was 8 min. Two replicated measurements were run on each sample.

2.4. Data processing

Data processing consisted in recognition of Madeira wine samples, assessment of effect of the grape variety and age on the organic acids, phenolics and furanic derivatives composition of wine and ET response, and prediction of the concentrations of wine compounds using the ET. Both data sets were mean centred and standardized prior to all calculations to equalize importance of different variables. Sample recognition was performed using principal component analysis (PCA). Calibration models of HPLC and ET data with respect to the wine age and of the ET data with respect to the concentrations of the organic acids and phenolic compounds in wine were calculated using PLS1 regression. All calibration models were validated using cross-validation due to the limited number of samples. All replicated measurements in the same sample were always included in the same cross-validation segment. HPLC data set i.e. concentrations of organic acids, phenolic and furanic compounds, averaged over three replicates were used as a reference for the ET calibration. A separate calibration model was calculated for each parameter. Predictive power of the calibration models was evaluated using parameters of the predicted vs. measured curve for the validation data (slope, offset, correlation), root mean square error of cross-validation (RMSECV) [27], mean relative error and ratio of prediction to deviation (RPD) [28]. RPD is calculated as the ratio of the standard deviation of the data set to RMSECV value and characterize prediction accuracy. Five levels of prediction accuracy were suggested in [28] based on the RPD value. RPD below 1.5 means that calibration is not usable, between 1.5 and 2 means that there is a possibility to distinguish between high and low levels, between 2.0 and 2.5 – approximate quantitative prediction is possible, between 2.5 and 3.0 – good and above 3.0 excellent prediction. Detailed description of PCA and PLS regression can be found elsewhere [27].

Analysis of the influence of wine age and variety on the acidic, phenolic and furanic composition of Madeira wine and ET response was carried using ANOVA—Simultaneous Component Analysis (ASCA). ASCA is a combination of analysis of variance (ANOVA) and PCA and was proposed for the analysis of multivariate data sets from designed experiments [29,30]. It consists in partitioning of original data matrix into set of matrices corresponding to design factors and their interactions and running PCA on each of these matrices. In the present study ASCA model comprising main effects and grape variety and age and their interaction was calculated for both HPLC and ET data. Partitioning of original data matrix in accordance to the design factors led to the following equation:

$$X = X_a + X_b + X_{ab} + E \qquad (1)$$

where X_a is a matrix containing variation related to the effect of age of wine, X_b is a matrix containing variation related to the grape variety, X_{ab} is a matrix containing the variation relates to the interaction between factors age and grape variety, and E is a residual error. After running PCA on each of those matrices the following

ASCA model is obtained:

$$X = T_a P_a' + T_b P_b' + T_{ab} P_{ab}' + E \qquad (2)$$

where T and P are scores and loadings respectively for each of the sub-models, and E is a residual error. Detailed description and algorithm of ASCA method can be found in [29,30]. Number of significant principal components for each of the sub-models was determined using cross-validation. Samples for the cross-validation were split in a way to use one or two replicates for calibrations and the third one for validation. I.e. three calibration models using replicates 1 and 2, 1 and 3, and 2 and 3 were built using HPLC data and validated using replicates 3, 2 and 1, respectively. In the case of ET data only two replicates were available therefore two calibration models were built using replicates 1 and 2. Explained variance was calculated for each sub-model and number of significant PCs was chosen. Explained variance was calculated according to the formula:

$$E_a^i\ (\%) = \left(1 - \frac{\sum_m \sum_n (X_a - T_a^i P_a^{i'})^2}{\sum_m \sum_n X_a^2}\right) \times 100 \qquad (3)$$

where E_a^i is an explained variance for the sub-model a and i principal components, T_a and P_a are score and loadings for the sub-model a for i PCs, X_a is a data matrix with variance related to the factor a (Eq. (1)), and m and n are the number of variables and samples respectively in the data set. Significance of each effect was assessed using a permutation test [31]. Data were permuted 1000 times and the percentage of the variance explained by each sub-model in the total model was used as quality-of-fit criterion for the permutation test. Variance explained by each sub-model in the total model was calculated for each sub-model, which was optimised as described above, using the formula:

$$E_{a,tot}\ (\%) = \left(1 - \frac{\sum_m \sum_n (X - T_a P_a')^2}{\sum_m \sum_n X^2}\right) \times 100 \qquad (4)$$

where $E_{a,tot}$ is the percentage of explained variance of the sub-model a in the total model, T_a and P_a are score and loadings for the sub-model a, X is an original data matrix, and m and n are number of variables and samples in the data set.

PCA and PLS were run using Unscrambler v. 9.7 by CAMO, Norway. ASCA was implemented in MATLAB, v. 5.3, using the algorithm described in [29].

3. Results and discussion

3.1. Characterization of Madeira wine samples

3.1.1. Characterization based on HPLC data

Capability of the HPLC and ET data to differentiate Madeira wine samples and which variables are contributing most to this differentiation were studied using PCA. A PCA model of HPLC data had 9 significant PCs. A PCA score and loading bi-plot along the first 2 components is shown in Fig. 1. Variables on this plot are numbered according to the list from Table 1. Madeira wine samples are aligned on the score plot according to their age from the right to the left. Differences between varieties are not evident on the PCA plot except distinction between wines produced from the red TNM and white varieties along the second PC. Differences between different varieties with respect to the organic acids, phenolic and furanic compounds may be levelled off to some extent during ageing. Contribution of the variables to the model and their importance to the sample discrimination can be assessed by considering their loadings. It was observed that younger and older wines differed with respect to the concentrations of caffeic (16) and synapic (22) acids, which were higher in younger wines, and lactic (5), acetic (6), formic (3), protocatechuic (11) and vanillic (15) acids, syringaldehyde (19), furfural (9), vanillin (18) and *trans*-resveratrol (24), which were higher in the older ones. Numbers in the parenthesises denote number of compound in Table 1 and on the plots. Higher contents of furfural and acetic acid in aged wines were reported earlier [1]. As the main source of furfural, protocatechuic and vanillic acids and vanillin in wine is the extraction from the oak wood barrel, higher content of these compounds in the older wines may be related to the fact that they stayed longer time in the contact with wood. Differentiation between the Madeira wines produced from the red TNM and white grapes was due to the higher concentration of malic (4), caffeic (16), *p*-coumaric (20) and sinapic (22) acids and lower concentration of lactic (4), protocatechuic (11) and *p*-hydrobenzoic (12) acids, 5-hydroxymethylfurfural (8), furfural (9), epigallocatechin (13) and catechin (14) in the Tinta Negra Mole wines. When red grapes such as Tinta Negra Mole are used for the production of Madeira wines, grape skin and seeds are separated from the must immediately after crushing the grapes. This limits extraction of phenolic compounds and resulting wine has light colour and phenolic content similar to the wines made from white grape varieties. The same technique is used e.g. in the production of champagne from red grapes such as Pinot Noir. This explains that fact that differences in phenolic compositions between Madeira wines produced from white and red grape varieties were quite small.

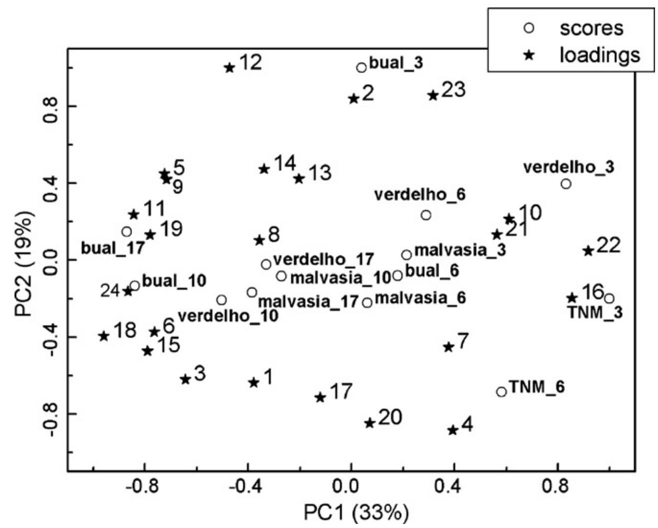

Fig. 1. PCA score and loading bi-plot of Madeira wine samples using HPLC data (concentrations of organic acids, furanic and phenolic compounds). Variables are numbered according to Table 1.

3.1.2. Characterization based on electronic tongue

Optimization of the sensor array composition was performed prior to the data analysis. The criterion used for choosing sensors was their response reproducibility in wine samples, estimated using R.S.D. in the replicated samples averaged over the sample set for each sensor. Only sensors that had average R.S.D. below 1.5% were retained for further data processing. Therefore, sensor array was reduced from 26 to 11 sensors.

PCA model of the ET data had 8 significant PCs. PCA score and loadings bi-plot along the first 2 components is shown in Fig. 2. Similarly to the results obtained using HPLC data set, the samples are aligned on the plot according to their age along the first PC and differences between grape varieties are not very pronounced. All 11 sensors left after optimization contributed to the discrimination of the wine samples. Some of the anion-sensitive sensors in particular A4 and A5, and A2 and A3 appeared to be highly correlated on the

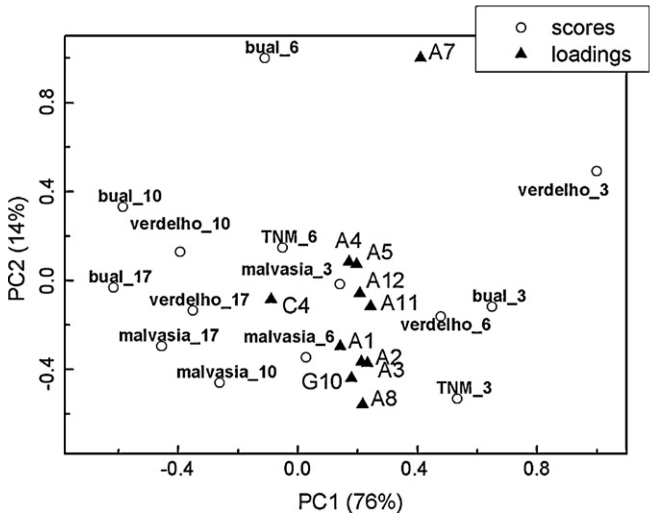

Fig. 2. PCA score and loading bi-plot of Madeira wine samples using ET data (responses of 11 sensors).

Table 2
Results of the prediction of Madeira wine age using chemical analysis and ET data.

	ET		HPLC	
	Calibration	Cross-validation	Calibration	Cross-validation
Slope	0.88	0.87	0.84	0.73
Offset	0.92	1.00	1.40	2.60
Corr.	0.94	0.93	0.91	0.91
RMSE[a]	1.7	1.8	2.1	2.3
MRE[b]	15	20	21	27
RPD[c]	–	2.9	–	2.3
LVs[d]	2		1	

[a] RMSE stand for root mean square error. RMSE was calculated for both calibration and cross-validation data sets and it is expressed in the original units of the variable.
[b] MRE is a mean relative error i.e. averaged absolute deviation of the predicted values from the measured ones, which is expressed in percents.
[c] RPD—ratio of prediction to deviation.
[d] Number of latent variables in the PLS model.

scores and loading bi-plot. This is due to the similar sensitivities and selectivity toward organic acids and phenolics of those sensors.

Furthermore, effect of age and variety on the acidic, phenolic and furanic composition of wine and on ET response was studied in more details.

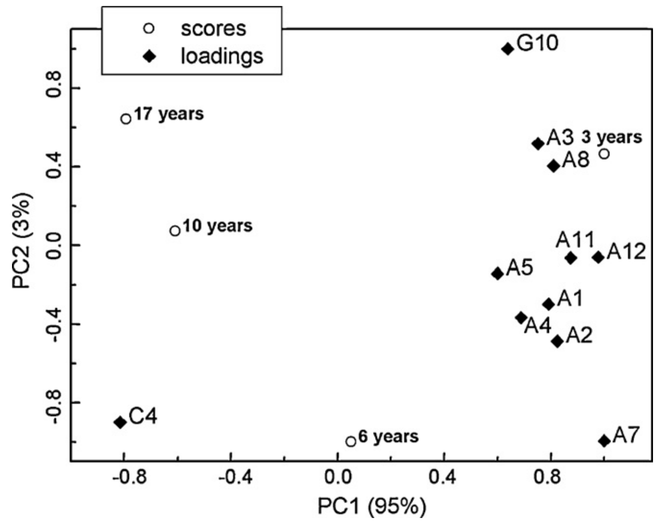

Fig. 4. Scores and loadings bi-plots of the ASCA sub-model age of the ET data.

3.2. *Effects of age and variety on the content of organic acids, phenolic and furanic compounds and on ET response*

The method of choice for analysis of data with underlying experimental design is ANOVA or its extension for the case of multivariate data—MANOVA. Being based on the linear regression, this method has some limitations in the case of multivariate data when e.g. variables are collinear or the number of variables exceeds the number of experiments. Another problem of ANOVA is that the number of model parameters increases drastically with the number of factors and especially higher order interactions. Alternative approaches usually consist in preliminarily decomposition of the data by e.g. PCA or related methods followed by the analysis of effects. As a consequence, the number of variables and therefore the number of the parameters to estimate is effectively reduced, which leads to the simpler, more parsimonious and more interpretable models. In this study both HPLC and ET data sets comprised more variables than samples. Therefore, ASCA was considered to be more adequate method for the analysis of effects compared to ANOVA. ASCA consists in the partitioning of the original data into sub-matrices related to the design factors and their interactions, running PCA on each of those sub-matrices and construction of

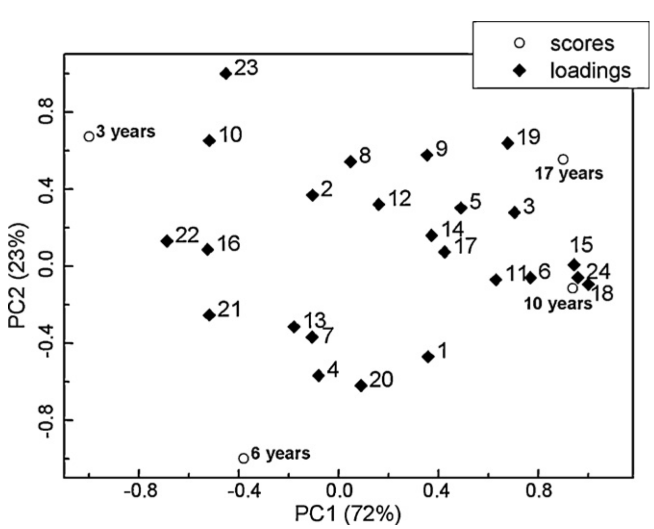

Fig. 3. Scores and loadings bi-plots of the ASCA sub-model age of the chemical data.

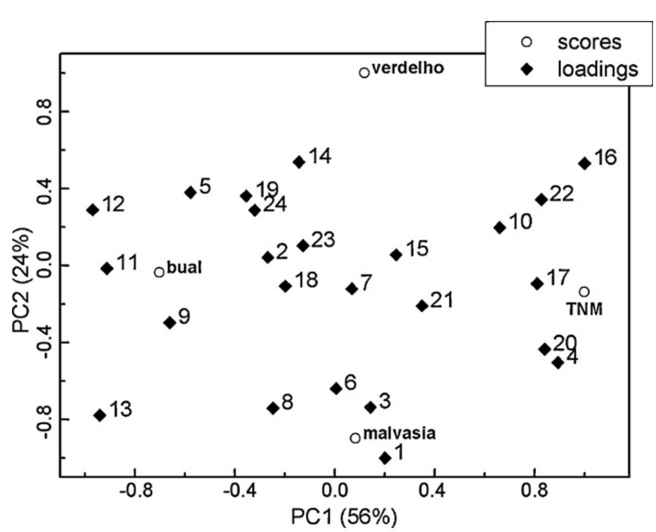

Fig. 5. Scores and loadings bi-plots of the ASCA sub-model grape of the chemical data.

Table 3
Results of prediction of Madeira wine age in validation samples using ET data and a set of concentration of organic acid, furanic and phenolic compounds. Means of three measurements with relative standard deviation (%) in the parentheses are shown.

Sample	Real age	Predicted age	
		ET	HPLC
1	3	3.4 (9)	3.1 (10)
2	6	4.5 (16)	4.3 (5)
3	10	11 (18)	12.5 (2)
4	17	15 (13)	14.2 (1)
5	3	4 (3)	4 (3)
6	6	7 (14)	6.8 (3)
7	10	10 (20)	12.2 (1)
8	17	13.5 (1)	13.7 (2)
9	3	1.8 (6)	3.8 (5)
10	6	5 (14)	8.1 (1)
11	10	11 (18)	13.1 (2)
12	17	17 (12)	13.3 (1)
13	3	3.5 (17)	4.5 (2)
14	6	8 (25)	8.4 (4)

ANOVA-like model using resulting PCA scores. Detailed description of the method, its properties and related algorithms can be found in [29,30].

ASCA models of the HPLC and ET data were calculated according to the Eqs. (2) and (3). Each ASCA model comprised sub-models describing effects of age and variety and their interaction. Optimization of the number of PCs for each of the sub-models was done using segmented cross-validation. Cross-validation segments comprised one of the replicates. Explained variance for each sub-model for the calibration and cross-validation sets was calculated according to Eq. (4). The following number of PC was chosen for the ASCA sub-models of HPLC data: 3 components for the sub-models of age and variety and 7 components for the mixed effects. This ASCA model described 97% of total variance in the data with age sub-model accounting for 36%, variety sub-model for 31% and interaction effect sub-model for 30% of the variance. The number of PC used in the ASCA sub-models of ET data was 2 components for the age, 3 for the grape variety and 6 for the mixed effect. ASCA model of the ET data described 83% of the total variance with age sub-model accounting for 46%, variety sub-model for 9% and interaction effect sub-model 28% of the variance.

Statistical significance of the factors and their interactions is the main question in the analysis of the designed data. Significance testing of the effects was done using permutation testing as was recently described in [31]. The permutation test consists in changing randomly design settings in the data set and calculating the ASCA models. After permutations were executed and respective models calculated big enough number of times (1000 in this case), the distribution of the explained variance for the ASCA sub-models is obtained. Variance explained by the corresponding sub-model after permutation can be equal or bigger than the variance explained by the model calculated with original data in no more than 50 out of 1000 random permutations for the effect to be significant on 0.05 probability level. According to the permutation test main effects of age and grape variety and their interaction were significant for the HPLC data and only main effect of age was significant for the ET data, all with the probability of 0.000.

Scores and loadings bi-plots of the ASCA sub-model age of the HPLC and ET data are shown in Figs. 3 and 4 respectively. There were certain similarities between bi-plots of ASCA age sub-model and PCA as the age was the main effect visible on the PCA score and loading bi-plots for both data sets (Fig. 1). However, as all the variance on the age sub-model plot is related only to the effect of the age, more detailed information is provided by the ASCA sub-model plots. The larger difference was observed between younger wines compared to the older ones, with 10 and 17 years old wines lying quite close to each other on both plots. Compounds mainly contributing to the discrimination between young and aged Madeira wines were sinapic (22) and caffeic (16) acids, whose content was higher in the young wines, and acetic (6), protocatechuic (11) and vanillic (15) acids, vanillin (18) and *trans*-resveratrol (24), whose content was higher in the aged wines. The possible reasons for it were discussed above. Moreover, 3 years old wines had higher content of gallic (10) and ellagic (23) acids whilst 6 years old wines had higher content of malic (4), oxalic (1), citric (7) and *p*-coumaric (20) acids, and epigallocatechin (13). The main difference between 10 and 17 years old wines was due to the concentrations of formic acid (3) and syringaldehyde (19).

The main input to the discrimination of Madeira wines according to their age was from the anion-sensitive electrodes of the ET that are denoted by the letter A on the scores and loadings bi-plot (Fig. 4). These sensors display cross-sensitivity to a wide range of phenolic compounds many of which are acids or have acidic character hence the capability of the ET to discriminate Madeira wines with respect to their age similarly to the HPLC data set.

Scores and loadings bi-plot of ASCA sub-model variety of the HPLC is shown in Fig. 5. As it follows from the plot, wines made from Tinta Negra Mole differed due to the higher concentration of malic (4), syringic (17) and *p*-coumaric (20) acids. Oxalic (1), formic (3) and acetic (6) acids and 5-hydroxymethylfurfural (8) differentiated between Malvasia and Verdelho wines, being present in higher concentration in the former and in lower concentrations in the lat-

Table 4
Parameters of the predicted vs. measured curves and error measures of the PLS calibration models for the determination of organic acids and phenolics in Madeira wine using ET.

Compound	Calibration					Cross-validation						LV[a]
	Slope	Offset	Corr.	RMSEC[b]	MRE[c]	Slope	Offset	Corr.	RMSECV[b]	MRE[c]	RPD[d]	
Organic acids												
Tartaric acid	0.70	0.58	0.84	0.16	7	0.60	0.76	0.77	0.18	8	1.8	3
Citric acid	1.0	0.0	1.0	0.01	1	0.85	0.07	0.95	0.06	4	3.2	6
Formic acid	0.86	0.00	0.93	$7*10^{-4}$	10	0.82	0.00	0.88	$8*10^{-4}$	12	2.3	3
Phenolics												
Protocatehuic acid	0.89	0.43	0.93	0.21	4	0.84	0.61	0.88	0.28	5	2.2	6
Catechin	0.94	0.04	0.97	0.03	4	0.88	0.08	0.93	0.05	6	2.8	4
Vanillic acid	0.91	0.18	0.95	0.24	12	0.85	0.31	0.90	0.35	18	2.3	2
Vanillin	0.96	0.04	0.98	0.12	10	0.94	0.08	0.96	0.16	12	3.6	3
Sinapic acid	0.96	0.05	0.98	0.10	8	0.86	0.20	0.88	0.24	14	2.1	5
trans-Resveratrol	0.92	0.02	0.96	0.01	4	0.85	0.05	0.91	0.02	5	2.5	2

[a] Number of latent variables in the PLS model.
[b] MSEC and RMSECV stand for root mean square error in calibration and cross-validation respectively. RMSE is expressed in the original units of the variable.
[c] RE is a mean relative error i.e. averaged absolute deviation of the predicted values from the measured ones. MRE is expressed in percents.
[d] PD—ratio of prediction to deviation.

ter. Wines made from the Bual grapes had higher content of furfural (9), protocatechuic (11) and *p*-hydrobenzoic acids (12).

The main effect of the age-variety interaction of the HPLC data ASCA sub-model is the diminution of the differences in the acidic, phenolic and furanic composition of wines, which are related to the variety and age, in the course of the aging. For the wines produced from the same variety, difference in composition was significant for the younger wines but decreased for the wines that were 10 and 17 years old. Differences in the composition between wines produced from different varieties also had tendency to decrease with the age e.g. Malvasia and especially Bual and Verdelho wines became quite similar to each other after 10 years.

3.3. Prediction of the Madeira wine age using HPLC and ET data

Based on the PCA and ASCA results described above it was assumed that age of the Madeira wines could be predicted using either of the HPLC and ET data sets. Therefore, respective calibration models were calculated using PLS regression. Parameters of the predicted vs. measured curves together with error measures for these calibration models are shown in Table 2. Results of the prediction of Madeira wine age in validation samples using HPLC and ET data sets are shown in Table 3. It was found that wine age could be predicted with accuracy of 2.7 and 1.8 years in the validation for the HPLC and ET data respectively. Parameter RPD, describing predictive power of the calibration model, was 2.3 and 2.9 for the models calculated using HPLC and ET, respectively. Therefore, good quantitative prediction of the age of Madeira wine was possible using ET data whilst only approximate quantitative prediction was possible using HPLC data for organic acids, phenolic and furanic compounds [28]. Accuracy of age prediction using ET data is in agreement with published results of the determination of the Port wine age, which was done with the accuracy of 1.8 years [26].

Lower prediction accuracy of the HPLC data model is presumably a result of Madeira wines getting more similar with respect to the content of organic acids, phenolic and furanic compounds with the age as it was discussed above. In particular, very little difference was observed between 10 and 17 years old wines (Table 3). New calibration model that excluded 17 years old wines was calculated to confirm this supposition. Indeed, RMECV value of 1.4 year and RPD of 3.8 were obtained using new calibration model, which means excellent quantitative prediction. Lesser improvement in predictive ability was observed for the ET calibration model after removing 17 years wines: RMECV of 1.6 and RPD of 3.3. The reason for it could be that information in HPLC and ET data sets coincided only partly, i.e. sensors of the ET responded not only to some of the compounds determined in this study but also to other wine components and redox potential.

3.4. Prediction of concentrations of the organic acids and phenolic compounds using ET

Based on the PCA and ASCA results it was expected that ET response could be correlated with concentrations of at least some of the organic acids and phenolic compounds measured in wine. Respective calibration models using ET data were calculated by PLS regression for each compound individually. Satisfactory models were obtained for 9 out of 24 compounds determined in the Madeira wine. Parameters of the predicted vs. measured curves and error measures for these calibration models are shown in Table 4. Results of the concentration prediction in the validation samples are shown in Table 5. According to RPD values, ET was capable of excellent quantitative prediction of vanillin and citric acid concentration (RPD value above 3.0) good prediction of catechin and *trans*-resveratrol concentrations (RPD in the range from 2.5 to 3.0), approximate quantitative determination of formic, protocatechuic,

Table 5
Results of the prediction of the concentrations of the organic acids and phenolic compounds using ET in the validation samples. Mean values of 3 replicas together with relative standard deviation (%) in the parentheses are shown. Meas—measured, Pred—prediction.

Sample	Tartaric acid ($g L^{-1}$)		Citric acid ($g L^{-1}$)		Formic acid ($g L^{-1}$)		Protocatechuic acid ($mg L^{-1}$)		Catechin ($mg L^{-1}$)		Vanillic acid ($mg L^{-1}$)		Vanillin ($mg L^{-1}$)		Sinapic acid ($mg L^{-1}$)		*trans*-Resveratrol ($mg L^{-1}$)	
	Meas	Pred	Meas	Pred	Meas	Pred	Meas	Pred	Meas	Pred	Meas	Pred	Meas	Pred	Meas	Pred	Meas	Pred
1	2.733 (0.3)	2.32 (0.4)	ND[a]	ND	0.003 (0.0)	0.0026 (12)	4.66 (2.9)	4.1 (5)	0.76 (7.9)	0.73 (7)	ND	ND	ND	ND	1.32 (1.5)	1.3 (8)	ND	ND
2	1.506 (0.4)	1.65 (3)	0.555 (3.2)	0.563 (1)	0.003 (0.0)	0.0028 (4)	4.28 (0.3)	4.0 (5)	ND	ND	ND	ND	0.81 (3.4)	0.9 (11)	0.57 (1.6)	0.8 (13)	0.23 (1.7)	0.23 (9)
3	1.851 (0.2)	2.0 (10)	ND	ND	0.006 (0.0)	0.0063 (2)	5.14 (0.4)	4.9 (2)	0.50 (1.2)	0.49 (6)	2.65 (2.7)	2.7 (15)	1.69 (5.2)	1.7 (12)	ND	ND	0.33 (1.2)	0.35 (6)
4	1.845 (0.2)	1.9 (16)	ND	ND	0.006 (0.0)	0.0069 (1)	4.56 (0.9)	4.9 (12)	0.76 (1.4)	ND	3.23 (3.3)	2.9 (10)	1.45 (5.3)	1.7 (6)	0.42 (6.0)	0.45 (2)	0.39 (3.9)	0.36 (3)
5	1.614 (0.4)	1.6 (6)	ND	ND	0.006 (0.0)	ND	3.46 (0.2)	3.7 (8)	ND	ND	0.76 (0.7)	1.2 (8)	0.72 (1.7)	0.62 (5)	1.16 (2.8)	1.15 (3)	ND	ND
6	2.052 (3.1)	2.0 (10)	ND	ND	0.006 (0.0)	0.0053 (9)	4.19 (0.3)	4.3 (5)	0.61 (1.2)	0.63 (3)	1.24 (4.4)	1.7 (10)	0.65 (3.9)	0.7 (29)	0.95 (3.5)	1.21 (4)	ND	ND
7	2.139 (0.4)	2.1 (10)	ND	ND	0.006 (0.0)	0.0058 (5)	4.17 (1.2)	4.0 (8)	0.69 (2.6)	0.7 (14)	2.21 (1.7)	2.5 (4)	1.38 (1.2)	1.32 (1)	1.12 (0.5)	1.2 (8)	0.33 (3.9)	0.33 (3)
8	2.217 (1.4)	1.93 (1)	0.507 (13)	0.51 (8)	0.009 (0.0)	0.0067 (3)	4.16 (0.8)	4.0 (8)	0.67 (1.6)	0.65 (2)	2.65 (3.5)	2.7 (7)	1.31 (1.5)	1.4 (7)	0.92 (1.5)	1.00 (3)	0.28 (3.2)	ND
9	2.193 (0.0)	2.16 (1)	0.318 (1.9)	0.32 (6)	0.003 (0.0)	0.0026 (4)	3.80 (0.8)	3.7 (5)	0.90 (5.8)	0.88 (2)	0.76 (4.9)	0.2 (10)	ND	ND	2.27 (0.4)	2.22 (2)	ND	ND
10	2.168 (1.0)	2.23 (3)	0.141 (0.0)	0.180 (3)	0.003 (0.0)	0.0039 (15)	3.43 (0.3)	3.5 (6)	0.71 (3.0)	0.75 (1)	1.39 (1.8)	1.6 (6)	0.74 (1.6)	0.74 (9)	1.36 (0.9)	1.28 (2)	0.25 (0.8)	0.251 (0.4)
11	1.536 (0.2)	1.81 (4)	ND	ND	0.006 (0.0)	0.0065 (9)	4.33 (1.1)	4.14 (0.5)	0.48 (4.3)	0.48 (2)	2.46 (1.7)	2.3 (13)	1.53 (1.6)	1.33 (2)	0.69 (2.0)	0.6 (13)	0.33 (2.6)	0.33 (6)
12	2.130 (0.4)	2.01 (1)	ND	ND	0.006 (0.0)	0.0066 (2)	4.35 (1.0)	4.3 (7)	0.77 (1.1)	0.71 (4)	2.63 (2.8)	2.28 (2)	1.35 (2.2)	1.16 (7)	1.09 (0.9)	0.8 (4)	0.29 (3.4)	0.32 (6)
13	1.725 (0.2)	1.8 (6)	ND	ND	0.003 (0.0)	0.0028 (4)	2.67 (2.5)	2.76 (0.4)	ND	ND	ND	ND	ND	ND	1.76 (2.2)	1.55 (2)	ND	ND
14	1.821 (0.5)	1.7 (6)	0.707 (0.4)	0.70 (5)	0.006 (0.0)	0.0043 (2)	3.84 (2.4)	4.1 (2)	0.49 (0.8)	0.56 (5)	2.19 (3.5)	2.2 (14)	0.39 (9.3)	ND	2.10 (1.2)	ND	ND	ND

ND—not detected.

vanillic and sinapic acids concentrations (RPD in the range from 2.0 to 2.5) and only recognition of high and low levels of tartaric acid (RPD in the range of 1.5–2.0) [28] (Table 4).

Sensitivity of the sensors used in the ET to a wide range of organic acids and phenolic compounds was reported earlier [32,33]. Evidently, ability of the sensor system to quantify compounds in the mixture will depend not only on the sensors sensitivity to these compounds but also on their selectivity and relative concentration levels of the compounds in question. Study on the range of organic acids that are most often present in the foodstuffs has shown that selectivity of the plasticized sensors was defined by the organic acid lipophylicity, higher lipophylicity of the compound resulting in higher selectivity of the sensors toward it [33]. The same dependence between selectivity of the sensors and lipophylicity of the compounds can be expected for the phenolic compound. This explains the fact that though sensors displayed sensitivity to all acids determined in the Madeira wines in the individual solutions, only three of them—tartaric, citric and formic could be detected in wine by ET. Similarly, such compounds as i.e. gallic and caffeic acid could be measured in the individual solutions but were not detected in wine due to their low concentrations and insufficient sensors selectivity to them.

Capability of the ET to detect a range of compounds in wine is of practical interest in particular its capability to determine the content of *trans*-resveratrol with good precision. *Trans*-resveratrol is a stilbene-type aromatic phytoalexin that is predominantly found in grapes, peanuts, berries, turmeric, and other foods. Recently, *trans*-resveratrol attracted attention of the researchers as the antioxidant, anti-inflammatory and anticancer activity of this compound was demonstrated in numerous *in vitro* and animal assays [34,35]. The development of methodologies for the determination of this compound in food products has presented a growing interest in the last decade. Various methodologies were suggested reported in the literature in the recent years, most of which were based on chromatography and require extensive sample preparation (extraction and derivatization, among others). Therefore, a rapid and simple to use instrument such as ET that can be applied to the express analysis appears very attractive. Further studies using the ET system and sample sets containing *trans*-resveratrol in larger concentrations intervals are required for the development of the methodology applicable in practice.

4. Conclusions

A set of Madeira wines from four *V. vinifera* L. varieties (three white varieties: Bual, Malvasia and Verdelho and one red variety Tinta Negra Mole), that were 3, 6, 10 and 17 years old were analysed with respect to the content of organic acids, phenolic and furanic compounds by HPLC and measured by the electronic tongue based on potentiometric chemical sensors. Influence of age and variety on the acidic, phenolic and furanic composition of Madeira wine as well as on the ET response was evaluated using ASCA. Significance of the effects age and variety and their interaction was assessed using permutation test. Effects of age, grape variety and their interaction were found to be significant for the HPLC data whilst only effect of wine age was significant for the ET data. Madeira wine age could be predicted both using HPLC and ET data with RMSECV of 2.6 and 1.8 years respectively. The ET was capable of predicting concentrations of the following components (mean relative error in cross-validation is shown in the parentheses): tartaric (8%), citric (5%), formic (12%), protocatehuic (5%), vanillic (18%) and sinapic (14%) acids, catechin (6%), vanillin (12%) and *trans*-resveratrol (5%). The ET was demonstrated to be a promising technique for rapid assessment of the age of Madeira wine as well as simultaneous quantification of some organic acids and phenolic compounds.

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