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A novel method for classification of wine based on organic acids

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ABSTRACT

Bio-electronic tongue was linked to artificial intelligence processing unit and used for classification of wines based on carboxylic acids levels, which were indirectly related to malolactic fermentation. The system employed amperometric biosensors with lactate oxidase, sarcosine oxidase, and fumarase/sarcosine oxidase in the three sensing channels. The results were processed using two statistical methods – principal component analysis (PCA) and self-organized maps (SOM) in order to classify 31 wine samples from the South Moravia region in the Czech Republic. Reference assays were carried out using the capillary electrophoresis (CE). The PCA patterns for both CE and biosensor data provided good correspondence in the clusters of samples. The SOM treatment provided a better resolution of the generated patterns of samples compared to PCA, the SOM derived clusters corresponded with the PCA classification only partially. The biosensor/SOM combination offers a novel procedure of wine classification.

1. Introduction

From the ancient age, wine was produced and considered as drink from gods; hence, it was used by emperors and kings and later reached privileged classes. From the moment when beneficial properties of the wine were discovered, wine-making substantially increased and it became available to common people (Estreicher, 2006). The methods and techniques for production were further developed; these can be categorised as traditional and modern ones (Ribereau-Gayon, Dubourdieu, Don'eche, & Lonvaud, 2006). Fermentation processes of wine are divided to primary (classic) and secondary (malolactic fermentation, MLF). The classic fermentation is characteristic for the south regions of Europe (France, Italy, Spain and Portugal) and regarding carboxylic acids, tartaric acid is the major one present (Sablayrolles, 2009). MLF is very common to the north regions (Austria, Germany, Czech Republic and UK); climate is cooler and intensity of sunshine is lower, leading to higher levels of carboxylic acids, particularly an increased malic acid content (Lasik, 2013). The type of fermentation is determined by chemical composition of grapes and thus major differences of resulting wines between north and south region stem from different ratios among carboxylic acids (malate, tartrate, lactate and citrate) (Abrahamse & Bartowsky, 2011; Jackson, 2014; Ribereau-Gayon et al., 2006).

Regarding technological aspects, analytical methods for monitoring quality of wines have been developed using spectrophotometric, enzymatic, chromatographic and electrophoretic techniques (Mato, Suarez-Luque, & Huidobro, 2005). For determination of carboxylic acid in wine, enzymatic biosensors are widely used for individual principal compounds as lactate (Zeravik et al., 2015) and also for the whole MLF process (Gamella et al., 2010); the analytes typically serve as substrates for the immobilised specific enzyme. Alternatively, selected carboxylic acids can competitively inhibit the enzyme sarcosine oxidase (SOX), and consequently it will slow down its reaction with substrate and generation of H_2O_2; This principle was applied in an electrochemical biosensor (Zeravik, Lacina, Jilek, Vlcek, & Skladal, 2010); the produced H_2O_2 was measured amperometrically on the Pt working electrode protected against other potentially interfering species present in the sample matrix. The screen-printed format was employed as this seems best suited for portable applications (Dominguez Renedo, Alonso Lomillo, & Arcos Martinez, 2007; Turner, 2013). The instrumentation is easily miniaturized for convenient hand-held user formats (Rowe et al.,

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2011). Here, for a better characterisation of carboxylic acids, the basic SOX biosensor was used also with a variant with co-immobilised fumarase (FUM) in order to enhance response to tartrate, and another channel with lactate oxidase (LOX) served for L-lactate determination.

For complex food, beverage and waste water samples, the standard analytical methods as well as cheaper biosensor alternatives were linked to statistical procedures (e.g. principal component analysis, PCA) (Medina-Plaza, de Saja, & Rodriguez-Mendez, 2014) and bio-inspired learning algorithms called artificial neural networks (ANN) (Deisingh, Stone, & Thompson, 2004; Mato et al., 2005). Chemometric procedures as PCA and ANN can follow the measured experimental data and provide the results in a simplified form to the users. PCA is probably the most widely used multivariate statistical technique. It is based on a linear transformation of multidimensional data into different orthogonal coordinates using maximum variance and minimum correlation. PCA allows the researcher to reduce data multidimensionality, finding relationships among objects, estimate the correlation structure of the variables and investigate how many components (a linear combination of original features) are necessary to explain the greater part of variance with a minimum loss of information (Rodriguez-Delgado, Gonzalez-Hernandez, Conde-Gonzalez, & Perez-Trujillo, 2002). The 2D (or 3D) corresponding score plot can be used for groupings classification (Wei, Wang, & Ye, 2011). Recently in wine analysis, PCA was applied for characterization of phenolics using bioelectronic tongue (Ceto, Capdevila, Minguez, & del Valle, 2014). For assays of carboxylic acids, PCA was coupled to traditional methods as FTIR spectroscopy of wines (Zhang et al., 2010), fluorescent sensor for fruit juices (Tan, Li, & Jiang, 2014), as well as studying effects of wine geographical origin (Diaz, Conde, Estevez, Olivero, & Trujillo, 2003); no combination with biosensors was found so far.

ANN is a family of machine learning algorithms inspired by biological neuronal networks. It was used to estimate or approximate functions depending on a large number of inputs which are unknown. ANNs are generally presented as systems of interconnected "neurons" which send messages to each other. The connections have numeric weights that can be tuned based on experience, thus making neural nets adaptive to inputs and capable of learning. Self-organizing map (SOM) is a type of ANN that is trained using unsupervised learning to produce a low-dimensional (typically 2D), discretised representation of the input space of the training samples and called a map. SOMs are different from other ANNs in the sense that they use a neighbourhood function to preserve the topological properties of the input space (Feng & Xu, 2011; Rousi, Anagnostopoulou, Tolika, & Maheras, 2015). Although topology of a data set is well preserved locally, distances between points in original space are not preserved on a final map (Kohonen, 1982). In unsupervised nets analysis networks such as the SOM, the nodes compete to best represent the data. Therefore, the advantage of the unsupervised methods is that there are no assumptions made as to the final clustering, visualization, and abstraction of the data (Kiang, 2001). The final map evolves, or learns, from the input data. The classes become characteristic of the input data by being 'trained' by them (Cassano, Lynch, Cassano, & Koslow, 2006). For wine analysis, SOM helped to characterise polyphenol related effects (Cabrita, Aires-De-Sousa, Gomes Da Silva, Reiand, & Costa Freitas, 2012) and aroma, flavour, appearance and texture (Alves, de Araujo, Pessota, & dos Santos, 2013).

In our report, the determination of carboxylic acid in wine by biosensor procedures is linked to evaluation using chemometric methods; PCA and SOM are compared as novel procedures for characterisation of carboxylic acids to develop a new classification of wines. The aim of this work is to evaluate the PCA and SOM for clustering of red, white and rose wines from the South Moravia region (part of the Czech Republic), in order to discriminate the consistency of classification methods.

2. Materials and methods

2.1. Reagents and samples

L-Lactic acid, L-malic acid, L-tartaric acid, succinic acid, citric acid, fumaric acid and all enzymes (SOX, FUM, LOX) were purchased from Sigma-Aldrich (www.sigmaaldrich.com). Wines (31 samples, complete list in Table S1 in Supplementary Material, SM) originated in the Moravian region were from producers Znovín (Znojmo, *P*1) and Winery Jaroslav Tichý (Rybníky, *P*2); the white wines included Chardonay (sample 28wCh), Chenin Blanc (26wChB), Moravian Muscat (17wMM), Müller Thurgau (18wMT), Pálava (8wP), Pinot Blanc (21wPB), Welschriesling (4wW), cuvée Sauvignon-Neuburger (11wSBN), Frühroter Veltliner (10wFV, 20wFV), Riesling (7wR, 15wR), Sauvignon Blanc (23wSB, 25wSB, 29wSB), Grüner Veltliner (1wGV, 13wGV, 14wGV, 22wGV, 24wGV), Pinot Gris (6wPG, 12wPG, 16wPG, 27wPG, 30wPG, 31wPG); the rose wine was Lemberger Rosé (2wrL) and the red wines included Saint Laurent (3rSL), Zweigeltrebe (19rZ) and Lemberger (5rL, 9rL).

2.2. Biosensor analysis

The screen-printed electrode AC1.W2.R1 (SPE, 1 mm Pt as working, Pt as counter and Ag as pseudo-reference electrodes) were obtained from BVT Technologies (Brno, Czech Republic) and modified with anti-interference and enzyme layers as described in SM to obtain the biosensors (LOX, SOX and FUM/SOX variants, details in SM). This was connected to a potentiostat PalmSens from Palm Instruments (Houten, The Netherlands) and placed in a flow-through cell constructed in a laboratory. The minielectronic 8-channel switching valve was from Vici Valco Instruments (Houston, TX, USA) and the peristaltic pump Minipuls 3 was purchased from Gilson (Middleton, WI, USA).

Inhibition measurements with the SOX and FUM/SOX biosensors were performed with 50 mM phosphate pH 7.4 containing 100 mM KCl – the carrier solution, flow rate was 50 µL min^{-1} and the working potential was 650 mV. The measuring cycle consisted of the following steps (10 min each): 1) carrier zone for baseline stabilization ($i_{baseline}$); 2) zone of 5 mM sarcosine ($i_{sarcosine}$); 3) zone of 10-times diluted sample containing carboxylic acids with added 5 mM sarcosine (i_{inhib}); 4) 5 mM sarcosine (to confirm recovery of enzyme activity after sample analysis). Relative inhibition value was calculated as follows:

$$RI = (i_{sarcosine} - i_{inhib})/(i_{sarcosine} - i_{baseline}) \quad (1)$$

The LOX biosensor was used at the same working conditions, the 10 min steps were: 1) carrier buffer for baseline; 2) either sample ($i_{lactate}$) or L-lactic acid standard.

2.3. Chemometric data processing with PCA and SOM

All computations related to PCA were carried out using procedures available in the software package Statistica ver. 20.0 from Statsoft (Tulsa, OK, USA). For SOM (description in SM), the custom C implementation of the Kohonen algorithm was used, details and parameters are in Table 1. The U-matrix approach served for visualization (Ultsch, 1992). For the composition maps, a gray shade gradient was used with lighter colours representing lower concentrations and darker colours representing higher concentrations, respectively.

3. Results and discussion

The biosensor analysis was focused on providing maximum amount of information about the content of carboxylic acids in individual wine samples, in comparison with CE as the chosen standard instrumental method (details on CE procedures are available in SM). Three electrochemical biosensor variants sensitive to carboxylic acids utilised SOX, FUM/SOX and LOX; the corresponding analytical parameters are

Table 1
Details on implementation of the SOM algorithm.

Distance metric	Squared Euclidean
Initial learning rate decrease	Exponential decay
Effected radius decrease	Exponential decay
Neighbourhood learning function	Gaussian
Map initialization	Random – in range of the lowest decile
Pattern drawing	Random
Parametrization:	
Maximal iterations	15,000
Stopping conditions	10^{-20} weight change over 1000 iterations
Map dimensions	35 × 35
Initial learning rate	0.1

summarised in Table 2. The LOX channel provides L-lactate contents with negligible interference from other carboxylates. As regards the SOX and FUM/SOX options, where enzyme inhibition is employed, these are sensitive to diverse carboxylates, and thus the exact concentrations of individual components cannot be easily obtained for complex samples. Consequently, such results are conveniently linked to subsequent chemometric methodologies as PCA and SOM in order to identify and analyse patterns representing the measured data (Rousi et al., 2015).

3.1. Analysis of wines using CE

CE provided a comprehensive information both on composition and quantity of individual carboxylic acids (Table S1). The content and the ratio of tartaric and malic acids corresponded to the regional expectations for the south Moravia where grapes ripen (Pavlousek & Kumsta, 2011). In several cases of white wines, the typical higher content of malic acid in wines was reduced by the malolactic fermentation partially or completely transforming malic to lactic acid. The analysis of red wines showed very low content of malic acid and high amount of lactic acid which indicated the completed malolactic fermentation. Total contents of carboxylic acids ranged from 5 to 9 $g\,L^{-1}$ of the equivalents of tartaric acid (Pavlousek & Kumsta, 2011), which is usually determined by the acid-base titration to pH 7 according to the official method OIV-MA-AS313-01:2009. Such values reflect only the sum of titratable carboxylic groups expressed for convenience as equivalent of tartaric acid.

The content of volatile acids is predominantly related to acetic acid. Formic, propionic and butyric acids can be only found in minor levels. The determination of volatile acids in wines provides information about the degree of improper fermentation processes during winemaking (Gonzalez, Hierro, Poblet, Mas, & Guillamon, 2005). For the determination of total volatile acids OIV recommends the official method OIV-MA-AS313-02:2009 based on steam distillation and following acid-base titration to pH 7. Given the dominance of acetic acid, only this component was analysed by CE.

3.2. Analysis of carboxylic acids in wine using biosensor

The current trends in design of biosensors are based on unification of the detection mechanism to simplify the construction. Enzymatic reactions based on oxidases were adopted here; L-lactic acid served as a substrate for LOX, other carboxylic acids inhibited conversion of sarcosine with SOX. The latter option was further supplemented with co-immobilised FUM to extend the scope of inhibiting acids. The final step was always anodic oxidation of enzymatically generated hydrogen peroxide. Unfortunately, wine contains many compounds electrochemically interfering at positive working potentials, hence procedures reducing interference must be implemented. The perm-selective non-conductive copolymer barrier probably represents the simplest choice, it was electrodeposited from *o*-phenylenediamine and 1,3-resorcinol (1:1) on the Pt electrode surface (Zeravik et al., 2010). Although, the copolymer layer formed on the SPE surface reduced the efficiency of the transport of hydrogen peroxide to 70% compared to the bare electrode, it sufficiently shielded most of oxidizing interferents.

The LOX biosensor provided levels of L-lactic acid in wines; all numerical concentration values from different methods are summarised in Table S1. The concentrations were correlated to 1 mM lactate, this concentration served as a re-calibrator during long-term use of the biosensor. To obtain more precise data, the wine samples were measured alone and after the addition of 1 mM lactate and the difference was further used to determine the initial concentration. The comparison of the data provided by the LOX biosensor with the data obtained from CE (Table S1) showed good agreement. The LOX biosensor can be considered as an equivalent tool for convenient determination of lactic acid in comparison with the standard procedure as CE.

For other carboxylic acids relevant to wine, the enzymatic methods are generally based on multiple enzymes coupled to suitable terminal NAD^+-dependent dehydrogenases (Shapiro & Silanikove, 2011). Utilisation of these systems in biosensors seems problematic due to complicated electrochemical determination of NADH, and the use of a single or two enzymes only is preferred (Zeravik, Hlavacek, Lacina, & Skladal, 2009). Therefore, the choice of the assay based on enzyme inhibition is an attractive alternative. We previously found that SOX is reversibly inhibited by malic acid, citric acid, succinic acid, oxaloacetic acid, acetic acid and formic acid, while in the presence of lactic acid no inhibition was observed; tartaric acid inhibited SOX much less (Zeravik et al., 2010). The SOX based biosensor was used for the analysis of the acidity in wines. In case of wine analysis, SOX is inhibited mainly by malic acid and comparison of the biosensor and CE pointed to a certain correlation of the obtained data (Zeravik et al., 2010). In fact, the sum of carboxylates from CE improved the correlation with the biosensor inhibition data. The SOX biosensor seems more suitable for on-line monitoring of various processes such as malolactic or acetic fermentations. Generally, the biosensor can advantageously indicate when the content of carboxylic acids is changed. The combination of SOX and LOX biosensors for on-line monitoring of malolactic fermentation is preferable; here, the LOX biosensor serves as a control if winemaker prefers only partial degradation of malic acid (Zeravik et al., 2010).

The main limitation of the SOX biosensor was only weak inhibition by tartaric acid in comparison to the above mentioned carboxylic acids. In wine, tartaric acid together with malic acid represent the major component from the sum of carboxylic acids. The possibility of enzymatic conversion of tartaric acid into a compound which can be detected using SOX pointed to fumarase, which provides product with

Table 2
Analytical parameters of the developed biosensors.

Biosensor channel	LOD[1] (mM)	Working range (mM)	Analysis time (min)	Repeatability[2] (%)	Reproducibility[2] (%)	Durability[3] (week)
LOX	0.03	0.05–4.5	30	3.8	4.2	12
SOX	0.20	0.6–5.3	30	2.6	5.4	6
FUM/SOX	0.50	0.8–5.3	30	2.3	2.1	6

[1]LOD – limit of detection was calculated as average of blank measurements plus 3x standard deviation of the background signal. [2]The repeatability and reproducibility values were calculated using the software EffiValidation 3.0 (demoversion). [3]Durability was based on continual 24-h measurement. The calibrations and other parameters for the LOD, SOX and FUM/SOX biosensors were determined using 1 mM lactic acid (LOX) and 2 mM malic acid (the other biosensors).

Table 3
Analysis of the wine samples using biosensor and taste characteristics.

Wine sample		Code	Biosensor (mM)			PCA Group	Taste characteristics	
Grape varieties	Year-Prod.		LOX Lactic	SOX Sum	FUM/SOX Sum		Expected characteristics[1]	Our description (MM)
Gruner Veltliner	10-P1	1wGV	0.9	120	139	B_4	flavours of citrus and peach; spicy notes of pepper and sometimes tobacco	little bit above of average on sour and bitterness with a good fresh note
Lemberger (rose)	10-P2	2wrL	2.0	151	170	B_6	aroma of dark ripe cherries and dark berries; spicy, medium tannins; very good acidity	red variety of grapes produced as type of white wine; too much acid character (sour and tartness); rich on fresh fruity flavour
Saint Laurent	09-P2	3rSL	28.6	67.0	68.5	B_1	highly aromatic dark-skinned grape; too much tannins; flavour bitterness; good quality as old wine	acidity characteristic rounded up; flavour tartness; good aroma
Welschriesling	09-P2	4wW	1.5	94.3	91.9	B_2	aromatic grapes; flowery taste; high acidity	high tartness with expressed flowery bouquet
Lemberger	08-P1	5rL	27.8	68.0	70.7	B_1	aroma of dark ripe cherries and dark berries; spicy, medium tannins; very good acidity;	medium tannins; medium sourness and oak bouquet
Pinot Gris	10-P2	6wPG	1.6	126	151	B_4	low acidity; higher alcohol levels; full-body wine	higher acidity; aroma close to botrytis bouquet
Riesling	09-P2	7wR	1.2	88.1	95.7	B_2	aromatic white grapes; flowery taste; high acidity	high tartness with expressed apple bouquet
Palava	10-P2	8wP	1.0	133	155	B_5	aromatic white grape variety; flowery taste; high acidity; sweetness flour	higher acidity; sour effect; aroma spicy; residual sugar
Lemberger	09-P2	9rL	26.8	50.4	43.3	B_1	aroma of dark ripe cherries and dark berries; spicy, medium tannins; very good acidity	medium tannins; low sourness and vanilla bouquet
Fruhroter Veltliner	10-P2	10wFV	1.5	106	120	B_3	red-skinned white grape; neutral bouquet; high alcohol; low acidity	apple flavour; average dessert wine
Savignon Blanc Neuburger	07-P2	11wSBN	1.1	82.3	84.4	B_2	middle aroma flavour; low acidity	tartness above average; dry wine with average aroma effect
Pinot Gris	09-P2	12wPG	2.1	76.6	74.8	B_2	low acidity; higher alcohol levels; full-body wine	similar to Riesling; dry wine with flavour of mango
Gruner Veltliner	10-P1	13wGV	3.3	112	133	B_4	flavours of citrus and peach; spicy notes of pepper and sometimes tobacco	highly sour and bitterness; dominant of acidity flavour
Gruner Veltliner	09-P2	14wGV	1.1	118	170	B_4	flavours of citrus and peach; spicy notes of pepper and sometimes tobacco	highly sour; aroma of acetic acid; without fresh smell
Riesling	10-P1	15wR	2.6	121.2	104.5	B_4	aromatic white grape variety; flowery taste; high acidity	too shape acidity; not present aroma in first 3 s
Pinot Gris	10-P1	16wPG	4.6	126.2	135.5	B_5	low acidity; higher alcohol levels; full-body wine	high acidity; tonality of botrytis bouquet
Moravian Muscat	10-P1	17wMM	6.3	111.8	116.5	B_4	low content of acids; aroma muscat	late harvest with higher content of acids; first tonality of muscat then botrytis
Müller Thurgau	10-P1	18wMT	3.4	111.0	114.7	B_3	aroma muscat; fresh taste	average content of acids; in the background tonality of fruits flavor; light summer wine
Zweigeltrebe	09-P1	19rZ	48.1	71.8	55.2	B_1	crossing of two varieties St. Laurent and Blaufrankisch	oak bouquet; tannin and spicy character rounded up with ageing
Fruhroter Veltliner	09-P1	20wFV	13.7	100.8	94.2	B_2	red-skinned white grape; neutral bouquet; high alcohol; low acidity	acidity round up; average desert wine; sour under average;
Pinot Blanc	09-P1	21wPB	3.9	101.4	97.3	B_3	full-bodied dry or sweat white wine; fruity aromas (apple, citrus fruit); floral character; high in acidity	recommended summer wine; apple taste of acidity; with fish food
Grüner Veltliner	10-P1	22wGV	2.5	130.0	125.3	B_3	flavours of citrus and peach; spicy notes of pepper and sometimes tobacco	tartness high with flavour of apple; specific for red fish meat
Sauvignon Blanc	08-P1	23wSB	10.9	98.1	117.0	B_3	"green flavours" of grass; green bell peppers and nettles with some tropical fruit; floral (such as elderflower) notes	elegant wine with tonality of tartness; neutralized aroma
Grüner Veltliner	09-P1	24wGV	9.7	82.3	92.5	B_2	flavours of citrus and peach; spicy notes of pepper and sometimes tobacco	rounded up acidity; low acidity; semi dry; lost aroma because of ageing
Sauvignon Blanc	10-P1	25wSB	0.9	129.7	126.2	B_5	"green flavors" of grass; green bell peppers and nettles with some tropical fruit; floral (such as elderflower) notes	highly sour, tartness and bitterness wine; so high acidity character; in background after 3 s aroma of apple presented
Chenin Blanc	09-P1	26wChB	1.7	143.4	170.0	B_5	high acidity; recommended to producing from sparkling to well-balanced dessert wines	dry wine; flavour with apple; higher content of acid; full-body; recommended with grilled red fish meat
Pinot Gris	07-P1	27wPG	12.8	91.3	76.6	B_3	low acidity; higher alcohol levels; full-body wine	dull wine on taste; absence of aroma note
Chardonnay	06-P1	28wCh	5.5	111.6	128.3	B_3	flavours commonly associated; higher influents of terroir and oak	shape peak of apple flavour;
Sauvignon Blanc	09-P1	29wSB	20.9	108.3	131.9	B_3	"green flavours" of grass; green bell peppers and nettles with some tropical fruit; floral (such as elderflower) notes	aroma of acetic acid; higher content of acids
Pinot Gris	06-P1	30wPG	0.7	85.1	117.7	B_3	low acidity; higher alcohol levels; full-body wine	rounded up of acidity; dry wine; recommended with white fish meats
Pinot Gris	10-P1	31wPG	10.9	148.6	152.9	B_5	low acidity; higher alcohol levels; full-body wine	too high acids; sharp sour of apple tonality

[1] According to wikipedia.com and www.vinoadestilaty.cz.

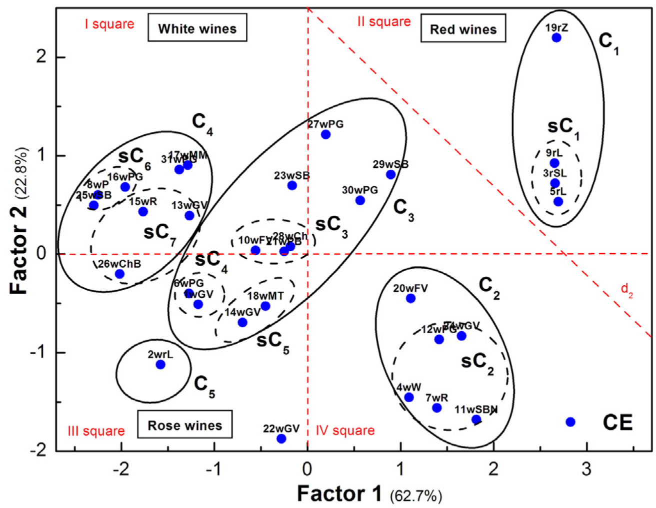

Fig. 1. Principal component analysis evaluation of wine samples based on the concentration of carboxylic acids (acetic, citric, lactic, malic, oxaloacetic, succinic and tartaric) and determined using capillary electrophoresis. Original data are in the SM Table S1. Groups (full lines) and subgroups (dashed lines) are highlighted.

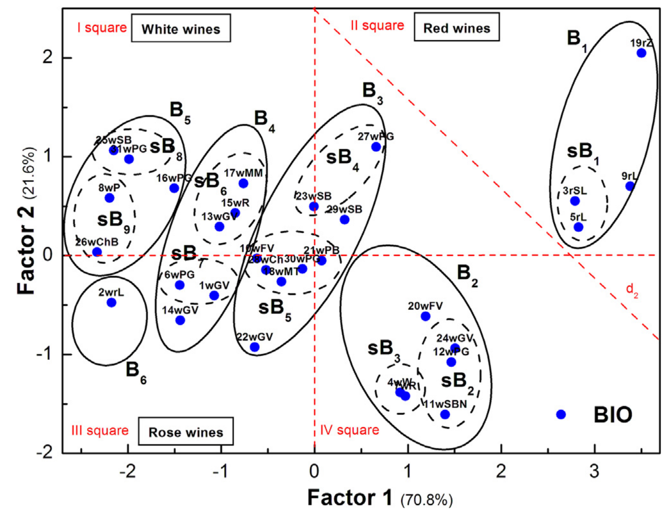

Fig. 2. Principal component analysis evaluation of wine samples based on the biosensor analysis. Levels of lactate determined using biosensor with lactate oxidase, sums of other carboxylic acids measured as inhibition effects on biosensors with sarcosine oxidase and fumarase/sarcosine oxidase channels. Original data are in the SM Table S1. Groups (full lines) and subgroups (dashed lines) are highlighted.

inhibition properties corresponding to malic acid. Thus, the FUM/SOX bienzyme sensor supplements the LOX and SOX data with information on the level of tartaric acid. All three biosensors were used for the analysis of 31 wine samples (Table 3, together with other wine quality characteristics, and Table S1 providing biosensor and CE data comparison). A good agreement between biosensors and CE is apparent and especially the FUM/SOX version provides wine characteristics better correlating with CE levels than the SOX biosensor alone (Table S1), as both main components responsible for the acidity of wine – malic and tartaric acid are addressed.

3.3. PCA evaluation of the CE and biosensor analysis of wines

The values obtained by the CE and biosensor methods were initially subjected to PCA analysis. Samples are projected on to new uncorrelated dimensions (principal components, factors) and the largest variation or difference between objects is extracted. The classification of South Moravian wines as grouping distribution of carboxylic acids (acidic character of wine) is chosen as the experimental object. The PCA score plots of both sets of values are shown in Fig. 1 (CE data) and 2 (biosensor data), respectively.

In case of the CE data in Fig. 1, the PC2 (factor 2) vs. PC1 (factor 1, related to the malic acids contents) together explained 85.5% of the variance; the influence of tartaric acid on distribution of wines is reflected as fine-tuning of the individual positions. All samples were reliably classified in the form of five clusters C_1 to C_5 and six subclusters sC_1 to sC_6. (Fig. 1).

The formed cluster C_1 consisted of samples 19rZ and the subcluster sC_1 representing samples 9rL, 3rSL and 5rL. Similarly, all (sub)cluster members are assigned in Table S2. The red, white and rose wine samples are distributed on three sections in Fig. 1. To the red wines, the cluster C_1 belonged. The section of white wines consisted of clusters C_2, C_3 and C_4 and the rose wine was cluster C_5; unfortunately, C_5 represented only one sample 2wrL, thus being insignificant. The PCA method has shown the sections of white and red wines samples, from left and right sides of the d_2 diagonal, respectively.

The subclusters are formed according to the difference of 10 BioCarboxylic Units (BCU, for relationship to other concentration units see Table S3) represented by organic acids of wine. Thus, the sC_1 consists of wines 9rL, 3rSL and 5rL. The differences in values of malic and lactic acids between two wines can not be more than 10 BCU in order to belong to the subcluster, the assigned wines share similar characteristics of wine acidity (CWA). Therefore, the subcluster is noticed only when the difference between both of these values of two wines is less than 10 BCU (Table S3). The continuity and long sequence (string) of the subcluster is lost if the sequence of wines samples does not follow the 10 BCU rule (Table S4). The least number of wines samples in subcluster is 2, the maximum is not limited.

For the biosensor data (Fig. 2), PC1 vs. PC2 together explained 89.41% of the variance and again, all samples were reliably classified. The variations in the PC1 dimension were related to the level of malic acids group, PC2 represented lactic acid, similarly as in the previous case of CE. The presented sample positions were in the form of six clusters B_1 to B_6, the 9 subclusters were sB_1 to sB_9, the distribution of individual samples into (sub)clusters is provided in Table S6.

Wine samples were distributed on three sections as red (cluster B_1, 3rSL, 5rL, 9rL, 19rZ), white (clusters B_2, B_3, B_4 and B_5) and rose wines (assigned as the cluster B_6 containing only 2wrL). The red section of wines took the position from the right side of the diagonal d_2. Based on the positions on the PCA figure and analysis of the BCU levels (Table S1), the prediction factor 1 can be presented as increased values of malic acid and decreased values of the lactic acid, respectively from negative to positive scale on the x-axis. The prediction factor 2 presented as increased values of lactic acid and decreased values of malic acid, respectively from positive to negative scale on the y-axis. These two factors and their combination can serve as an indication of the finished malolactic fermentation of wines.

The subclusters were again formed according to the rule of 10 BCU that are presented by organic acids of wine. Thus, the formed subcluster sB_1 has wines 3rSL, 5rL. The differences in malic and lactic values between two wines cannot be more than 10 BCU for the given cluster. The continuity and long sequence (string) of the subcluster become lost if the sequence of wines samples do not fill out of the rule 10 BCU (Tables S7 and S8 corresponding to B_1 and B_3, respectively), quite similarly as in the above case of CE analysis (Fig. 1).

The biosensor-based groups B_1 and B_2 very closely corresponded to the C_1 and C_2 groups resulting from CE (Figs. 1 and 2); the members of groups were the same as well as quite similar positions within the PCA plots. Interestingly, samples from the CE groups C_3 and C_4 were transformed to three groups B_3, B_4 and B_5; C_4 more or less corresponds to B_5, C_3 separated to B_3 and B_4. It seems that here the biosensor analysis provides some additional information; the data correspond not only to concentrations of individual carboxylic acids, but the bio-effect – the inhibitory properties on the SOX enzyme - can play some role, too. This effect is not obviously apparent when looking on the raw data, though the chemometric treatment using PCA emphasized importance of this biosensing contribution. Overall, both CE and biosensor data provided similar PCA score plots; this confirms the usefulness of the simpler biosensor analysis for rapid, possibly on-site, characterization of wines.

The PCA main clusters derived from the biosensor data (B_1 to B_6) were also linked to qualitative wine evaluations. For this purpose, Table 3 contains a column characterizing “Expected characteristics” combining general wikipedia.org characteristics derived from the wine grape brands and local producers. These descriptions appeared rather general and not reflecting modifications within the production of wines. The last column entitled “Our description (MM)” provided taste and flavour descriptions by one of the authors (M. Milovanovich) who is for some 15 years actively involved in small-scale wine production. The evaluation of wine taste and aroma on the real analysed samples (Table 3, the last column) provided some quality characteristics always shared by several members of the clusters. Thus, the B_1 wines were typical for medium tannins and oak/vanilla/flovery bouquets, B_2 samples shared high tartness and appeared (semi)dry, B_3 exhibited apple taste and flavour, B_4 members were mostly with higher acidity, high sourness and some with botrytis bouquet, B_5 wines eventually combined high acidity and apple flavour. These correlations highlight the usefulness of the chosen approach for providing information relevant to the wine quality.

3.4. SOM maps for CE and biosensor analysis of wines

The implementation of the SOM method utilised the architecture of a trained map containing *n* cells (or neurons). This can be explained as a superposition of as many grids of *n* neurons as the number of descriptors (here, four evaluated acids). All descriptors were used to characterize each of the objects to be computed (here, 31 samples of wine). Our matrix was neurons dimensioned in the format 35 × 35. In that map, results of classification of wines were projected. We used our custom C implementation of the SOM algorithm. It was trained on the data set and then projected whole data set on the trained rectangular grid. U-matrix values were calculated for every trained node and results were normalized to fit in 8-bit number. A gray scale was then used in order to identify clusters in our data set obtained from CE (Fig. 3) and biosensor (Fig. 4) assays, respectively.

The individual sample points were spread over the SOM area without immediately obvious group formations. However, the red wine samples SOM_1 (3rSL, 5rL, 9rL, 19rZ, green dots) in both cases stayed together within the same black area (high levels) close to the corner of the maps - bottom right at Fig. 3, and top left at Fig, similarly as C_1 and B_1 groups derived from PCA. Another defined group SOM_2 consisted of five samples 4wW, 7wR, 11wSBN, 12wPG and 24wGV (bottom left, Fig. 3, top middle, Fig. 4), exactly corresponding to both sC_2 and B_2. In case of the biosensor data, the sample 20wFV was in the same area (Fig. 4), but completely away in CE SOM, together with 29wSB (Fig. 3, top right area). The cluster SOM_3 (2wL), 8wP, 16wPG, 25wSB, 26wCB and 31wPG (Fig. 3, top left) overlaps with a similar group from biosensor data (Fig. 4, bottom right), where 16wPG and 25wSB are separated on a side. The corresponding groups from PCA are C_4 (larger) and B_5 (exactly the same members). For the rest of samples, several smaller groups of corresponding samples can be identified, e.g. (13wGV, 15wR, 17wMM) ~ sC_7 and = sB_6, (1wGV, 6wPG) = sC_4 and = sB_7.

The visualization of the output data based on the non-linear SOM mostly corresponded to the linear PCA, the discrimination of wines to red and white types was achieved, only the rose wine was not distinguished from white wines in the case of SOM; more rose samples will be required to clarify this situation.

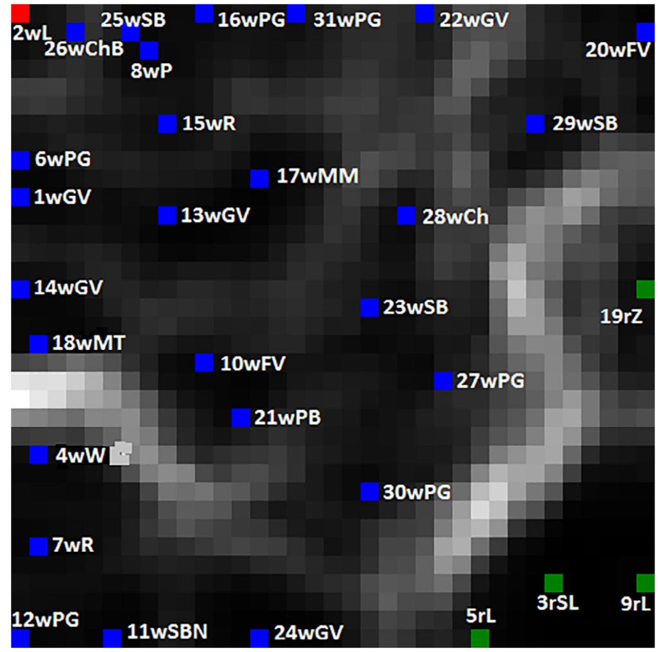

Fig. 3. Self-organizing map presenting wine samples according to concentrations of carboxylic acids determined using capillary electrophoresis; other details same as in Fig. 1.

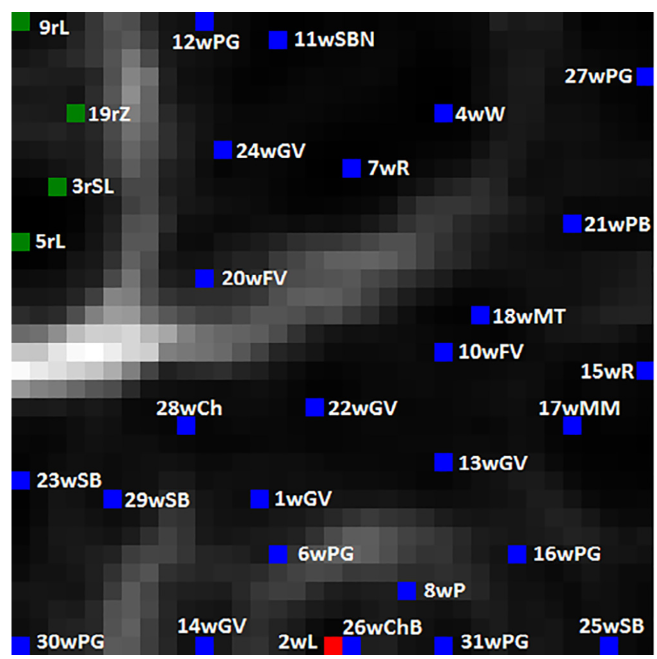

Fig. 4. Self-organizing map presenting wine samples according to biosensor analysis; other details same as in Fig. 2.

4. Conclusions

The levels of selected carboxylic acids in wine samples were determined using capillary electrophoresis and compared with results from enzyme-based biosensors. The latter approach utilised screen-printed amperometric sensors modified with lactate oxidase for direct assay; alternatively, inhibition of sarcosine oxidase was implemented for a sum of carboxylates. The SOX biosensor was also used in the variant with co-immobilised fumarase, which helped to enhance inhibitory properties of some target acids. Afterwards, the classification of the results was successfully carried out using pattern recognition techniques based on carboxylic acids contents. The traditional approach – principal component analysis – separated the samples to group patterns, which appeared quite similar for both CE and biosensor data; lactic and malic acids played the key roles, suggesting influence of the malolactic fermentation in wines. PCA derived clusters of samples typically shared several taste and flavour related characteristics resulting from wine tasting.

Alternatively, self-organizing map as a type of artificial neural networks was for the first time tested for wine classification using the same CE and biosensor sets of data. The classification patterns for both data were at the first sight rather different; however, closer inspection and separation to groups and comparison of the member samples again corresponded for both CE and biosensor types of analysis. Moreover, several SOM-derived groups were nearly identical to previous PCA classifications. On the other hand, several samples were not obviously belonging to any group. After some training period, the SOM approach provides immediately visible and separated patterns for the classification of wine samples. The classification of wines based on carboxylic acids is a complex procedure, as a level of the components depends on several factors (grapes type, region, weather, processing).

In summary, the biosensor measurement of carboxylic acids was successfully realised and it provides a novel method for classification of wine. Further processing of experimental data using both common (PCA) and novel (SOM) methods helps to obtain characteristic patterns correlating with wine tasting results. The simple construction of biosensor and the portable instrumentation make this approach suitable for field use. The on-site measurement is simply feasible, the following chemometric classification can be implemented in a portable computer/tablet, or realized in the central unit using the data/results transfers based on available "internet of things" concepts.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.01.113.

References

Abrahamse, C. E., & Bartowsky, E. J. (2011). Timing of malolactic fermentation inoculation in Shiraz grape must and wine: Influence on chemical composition. *World Journal of Microbiology and Biotechnology, 28*, 255–265. https://doi.org/10.1007/s11274-011-0814-3.

Alves, W. A. L., de Araujo, S. A., Pessota, J. H., & dos Santos, R. A. B. O. (2013). A methodology for sensory evaluation of food products using Self-Organizing Mapsand K-means algorithm. *Applied Mechanics and Materials* 263-266, 2191-2194. https://doi.org/ 10.4028/www.scientific.net/AMM.263-266.2191.

Cabrita, M. J., Aires-De-Sousa, J., Gomes Da Silva, M. D. R., Reiand, F., & Costa Freitas, A. M. (2012). Multivariate statistical approaches for wine classification based on low molecular weight phenolic compounds. *Australian Journal of Grape and Wine Research, 18*, 138–146. https://doi.org/10.1111/j.1755-0238.2012.00182.x.

Cassano, E. N., Lynch, A. H., Cassano, J. J., & Koslow, M. R. (2006). Classification of synoptic patterns in the western Arctic associated with extreme events at Barrow, Alaska, USA. *Climatic Research, 30*, 83–97. https://doi.org/10.3354/cr030083.

Ceto, X., Capdevila, J., Minguez, S., & del Valle, M. (2014). Voltammetric BioElectronic Tongue for the analysis of phenolic compounds in rose cava wines. *Food Research International, 55*, 455–461. https://doi.org/10.1016/j.foodres.2013.11.025.

Deisingh, A. K., Stone, D. C., & Thompson, M. (2004). Review: Applications of electronic noses and tongues in food analysis. *International Journal of Food Science and Technology, 39*, 587–604. https://doi.org/10.1111/j.1365-2621.2004.00821.x.

Diaz, C., Conde, J. E., Estevez, D., Olivero, S. J. P., & Trujillo, J. P. P. (2003). Application of multivariate analysis and artificial neural networks for the differentiation of red wines from the Canary Islands according to the island of origin. *Journal of Agricultural and Food Chemistry, 51*, 4303–4307. https://doi.org/10.1021/jf0343581.

Dominguez Renedo, O., Alonso Lomillo, M. A., & Arcos Martinez, M. J. (2007). Recent developments in the field of screen-printed electrodes and their related applications. *Talanta, 73*, 202–219. https://doi.org/10.1016/j.talanta.2007.03.050.

Estreicher, S. K. (2006). *WINE: From Neolithic Times of the 21st Century.* New York: Algora Publishing.

Feng, Z. G., & Xu, T. (2011). Comparison of SOM and PCA-SOM in fault diagnosis of ground-testing bed. *Procedia Engineering, 15*, 1271–1276. https://doi.org/10.1016/j.proeng.2011.08.235.

Gamella, M., Campuzano, S., Conzuelo, F., Curiel, J. A., Muñoz, R., Reviejo, A. J., & Pingarrón, J. M. (2010). Integrated multienzyme electrochemical biosensors for monitoring malolactic fermentation in wines. *Talanta, 81*, 925–933. https://doi.org/10.1016/j.talanta.2010.01.038.

Gonzalez, A., Hierro, N., Poblet, M., Mas, A., & Guillamon, J. M. (2005). Application of molecular methods to demonstrate species and strain evolution of acetic acid bacteria population during wine production. *International Journal of Food Microbiology, 102*, 295–304. https://doi.org/10.1016/j.ijfoodmicro.2004.11.020.

Jackson, R. S. (2014). *Wine science: Principles and applications.* London: Academic Press.

Kiang, M. Y. (2001). Extending the Kohonen self-organizing map networks for clustering analysis. *Computational Statistics and Data Analysis, 38*, 161–180. https://doi.org/10.1016/S0167-9473(01)00040-8.

Kohonen, T. (1982). Self-organized formation of topologically correct feature maps. *Biological Cybernetics, 43*, 59–69. https://doi.org/10.1007/BF00337288.

Lasik, M. (2013). The application of malolactic fermentation process to create good-quality grape wine produced in cool-climate countries: A review. *European Food Research and Technology, 237*, 843–850. https://doi.org/10.1007/s00217-013-2083-x.

Mato, I., Suarez-Luque, S., & Huidobro, J. F. (2005). A review of the analytical methods to determine organic acids in grape juices and wines. *Food Research International, 38*, 1175–1188. https://doi.org/10.1016/j.foodres.2005.04.007.

Medina-Plaza, C., de Saja, J. A., & Rodriguez-Mendez, M. L. (2014). Bioelectronic tongue based on lipidic nanostructured layers containing phenol oxidases and lutetium bisphthalocyanine for the analysis of grapes. *Biosensors and Bioelectronics, 57*, 6–283. https://doi.org/10.1016/j.bios.2014.02.023.

Pavlousek, P., & Kumsta, M. (2011). Profiling of primary metabolites in grapes of interspecific grapevine varieties: Sugars and organic acids. *Czech Journal of Food Sciences, 29*, 361–372. https://www.cabdirect.org/cabdirect/abstract/20113334195.

Ribereau-Gayon, P., Dubourdieu, D., Don'eche, B., & Lonvaud, A. (2006). *Handbook of enology: The microbiology of wine and vinifications* (2nd ed.). Chichester: John Wiley & Sons.

Rodriguez-Delgado, M. A., Gonzalez-Hernandez, G., Conde-Gonzalez, J. E., & Perez-Trujillo, J. P. (2002). Principal component analysis of the polyphenol content in young red wines. *Food Chemistry, 78*, 523–532. https://doi.org/10.1016/S0308-8146(02)00206-6.

Rousi, E., Anagnostopoulou, C., Tolika, K., & Maheras, P. (2015). Representing teleconnection patterns over Europe: A comparison of SOM and PCA methods. *Atmospheric Research, 152*, 123–137. https://doi.org/10.1016/j.atmosres.2013.11.010.

Rowe, A. A., Bonham, A. J., White, R. J., Zimmer, M. P., Yadgar, R. J., Hobza, T. M., et al. (2011). CheapStat: an open-source, "do-it-yourself" potentiostat for analytical and educational applications. *PLoS ONE, 6*, e23783. https://doi.org/10.1371/journal.pone.0023783.

Sablayrolles, J. M. (2009). Control of alcoholic fermentation in winemaking: Current situation and prospect. *Food Research International, 42*, 418–424. https://doi.org/10.1016/j.foodres.2008.12.016.

Shapiro, F., & Silanikove, N. (2011). Rapid and accurate determination of malate, citrate, pyruvate and oxaloacetate by enzymatic reactions coupled to formation of a fluorochromophore: Application in colorful juices and fermentable food (yogurt, wine) analysis. *Food Chemistry, 129*, 608–613. https://doi.org/10.1016/j.foodchem.2011.04.074.

Tan, J., Li, R., & Jiang, Z. T. (2014). Discrimination of fresh fruit juices by a fluorescent sensor array for carboxylic acids based on molecularly imprinted titania. *Food Chemistry, 165*, 35–41. https://doi.org/10.1016/j.foodchem.2014.05.104.

Turner, A. P. F. (2013). Biosensors: Sense and sensibility. *Chemical Society Reviews, 42*, 3184–3196. https://doi.org/10.1039/c3cs35528d.

Ultsch, A. (1992). Self-organizing neural networks for visualization and classification. In O. Opitz, B. Lausen, & R. Klar (Eds.). *Information and classification. Studies in classification, data analysis and knowledge organization*Berlin: Springer. https://doi.org/10.1007/978-3-642-50974-2_31.

Wei, Z., Wang, J., & Ye, L. S. (2011). Classification and prediction of rice wines with different marked ages by using a voltammetric electronic tongue. *Biosensors and Bioelectronics, 26*, 4767–4773. https://doi.org/10.1016/j.bios.2011.05.046.

Zeravik, J., Fohlerova, Z., Milovanovic, M., Kubesa, O., Zeisbergerova, M., Lacina, K., et al. (2015). Various instrumental approaches for determination of organic acids in wines. *Food Chemistry, 194*, 432–440. https://doi.org/10.1016/j.foodchem.2015.08.013.

Zeravik, J., Hlavacek, A., Lacina, K., & Skladal, P. (2009). State of the art in the field of electronic and bioelectronic tongues – Towards the analysis of wines. *Electroanalysis, 21*, 2509–2520. https://doi.org/10.1002/elan.200900285.

Zeravik, J., Lacina, K., Jilek, M., Vlcek, J., & Skladal, P. (2010). Biosensor for determination of carboxylic acids in wines based on the inhibition of sarcosine oxidase. *Microchimica Acta, 170*, 251–256. https://doi.org/10.1007/s00604-010-0315-3.

Zhang, Y. L., Chen, J. B., Lei, Y., Zhou, Q., Sun, S. Q., & Noda, I. (2010). Discrimination of different red wine by Fourier-transform infrared and two-dimensional infrared correlation spectroscopy. *Journal of Molecular Structure, 974*, 144–150. https://doi.org/10.1016/j.molstruc.2010.03.021.