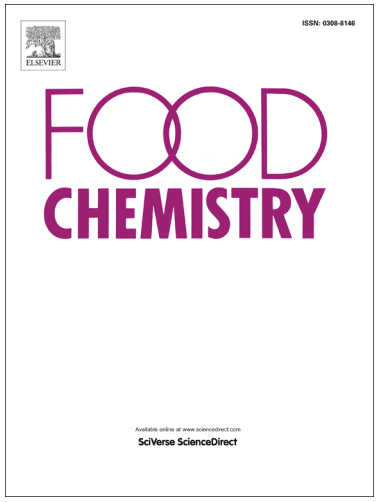

Various instrumental approaches for determination of organic acids in wines

Jiri Zeravik, Zdenka Fohlerova, Miodrag Milovanovic, Ondrej Kubesa, Marta Zeisbergerova, Karel Lacina, Aleksandar Petrovic, Zdenek Glatz, Petr Skladal

PII: S0308-8146(15)01196-6
DOI: http://dx.doi.org/10.1016/j.foodchem.2015.08.013
Reference: FOCH 17958

To appear in: *Food Chemistry*

Received Date: 6 January 2015
Revised Date: 3 August 2015
Accepted Date: 4 August 2015

Please cite this article as: Zeravik, J., Fohlerova, Z., Milovanovic, M., Kubesa, O., Zeisbergerova, M., Lacina, K., Petrovic, A., Glatz, Z., Skladal, P., Various instrumental approaches for determination of organic acids in wines, *Food Chemistry* (2015), doi: http://dx.doi.org/10.1016/j.foodchem.2015.08.013

Various instrumental approaches for determination of organic acids in wines

Jiri Zeravik[1]*, Zdenka Fohlerova[2], Miodrag Milovanovic[3], Ondrej Kubesa[3], Marta Zeisbergerova[1], Karel Lacina[1], Aleksandar Petrovic[4], Zdenek Glatz[1,3], Petr Skladal[1,3]

[1] *CEITEC - Central European Institute of Technology, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

[2] *CEITEC - Central European Institute of Technology, Brno University of Technology, Technicka 10, 616 00 Brno, Czech Republic*

[3] *Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

[4] *Department of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Serbia*

* Corresponding author

Tel.: +420549493463

Fax: +420549492690

E-mail address: zery@centrum.cz

Abstract

Biosensors based on lactate oxidase, sarcosine oxidase and mixture of fumarase and sarcosine oxidase were used for monitoring of organic acids in wine samples. Additionally, tartaric acid was determined by modified colorimetric method based on formation of the

vanadate-tartrate complex. The above mentioned methods were used for the analysis of 31 wine samples and obtained data were compared with the results from capillary electrophoresis as a basic standard method. This comparison showed a certain degree of correlation between biosensors and capillary electrophoresis. The provided information pointed to the potential uses of biosensors in the field of winemaking.

Keywords: biosensor, capillary electrophoresis, carboxylic acids, wine

1. Introduction

Organic acids belong to the most important components that complete the overall character and taste of wine. Ripe grapes contain major amounts of tartaric and malic acids, in a minor extent, citric acid is present, too. In addition to the above mentioned organic acids, the wines contain the products of yeast and malolactic fermentation such as acetic, lactic and succinic acids (Lasik, 2013; Mato, Suarez-Luque, Huidobro, 2005).

The organic acids content in wine is related to the region and it is also associated with climate during the growth and ripening of grapes. Generally, wines from warmer regions contain more tartaric acid, but malic acid predominates in products from colder regions. The excess of malic acid gives wine a distinctly more sour character, therefore winemakers removes it by the process of de-acidification. The most common process is malolactic fermentation; the malic acid becomes converted to softer lactic acid. On the other hand, tartaric acid does not affect the taste of wine so intensively, and excessive levels can be removed through cold stabilization. Furthermore, gluconic acid is considered as a very specific and important parameter of the health of grapes used for the production of wine. This compound serves as an indicator of the *Botrytis cinerea* disease which plays an important role

mostly in the organoleptic properties of wine (del Torno-de Roman, Alonso-Lomillo, Dominguez-Renedo, Arcos-Martinez, 2013). Although growth of *B. cinerea* generally decreases quality of grapes (Gray rot), infections are in some cases encouraged to produce high-quality dessert wines (so-called noble rot) (Fugelsand, Edwards, 2007). From these facts, it is evident that the analysis of organic acids is needed to allow the winemaker to control the ripening of grapes and the acidity development during the processes of fermentation and aging (Lasik, 2013).

The recommended methods for organic acid analysis were processed by the International Organization of Vine and Wine (OIV, Paris, France). The listed standard methods are reviewed annually and adjusted on the basis of the latest achievements in research. The widely employed analytical methods for determination of organic acids in wine are focused on the total quantity of acids in wine. To date, capillary electrophoresis (CE) with direct and indirect detection (Golubenko, Nikonorov, Nikitina, 2012; Gomez, Monasterio, Vargas, Silva, 2012), high performance liquid chromatography in various modes (Flores, Hellin, Fenoll, 2012; Lin, Liu, Shen, Yang, 2011), enzymatic flow injection analysis (Oliviera, Lopes, Toth, Rangel, 2010) and titrimetic techniques were performed. As a very interesting alternative, the gas chromatography with mass spectrometry detection without any derivatization step was used (Jurado-Sanchez, Ballesteros, Gallego, 2011). These techniques are characterized by a relatively simple and fast detection; however, certain associated restrictions including sophisticated operation, high cost of acquisition and operation of equipment, are at the end reflected in the price of analysis. Another limitation is some delay between sampling, analysis, and delivery of results to the producer. The winemakers working with wine need to get the relevant information about wine state and progress of the fermentation *in situ* in order to respond to them instantly during the technological processing of wine.

Therefore, there are efforts to simplify, accelerate and to make cheaper the process of analysis. For this purpose, several commercial kits based on thin layer and paper chromatography and enzymatic kits are available (e.g. Merck KGaA, Darmstadt, Germany; Accuvin LLC, Napa, CA, USA; Megazyme International Ireland, Wicklow, Ireland). These commercial kits serve for determination of malic, citric, acetic, lactic and succinic acids. These kits have a lot of limitations and provide only rough estimation of analyte levels. As another alternative, various types of biosensors can be considered. In this case, several individual organic acids, which more or less influence the quality of wine can be monitored, including malic, lactic, tartaric, citric and acetic acid.

Generally, the methods for enzymatic detection of organic acids are mostly based on the use of NAD^+-dependent dehydrogenases or utilize the coupled enzymatic systems where dehydrogenases are located at the end of the enzymatic cascade and serve as a final signaling element (Shapiro & Silanikove, 2011).

Utilization of these systems in the construction of electrochemical biosensors is quite problematic due to the complicated electrochemical determination of the NAD^+/NADH redox pair. In this case, diaphorase can serve as an additional supporting element and recycling of ferro/ferricyanide as an electroactive mediator between the enzyme and the working electrode is utilized. Alternatively, oxidase can be used as a final signaling element which is more readily usable for the construction of the electrochemical biosensors. Despite above mentioned variations in biosensor design, the use of single or two enzyme systems for biosensor construction is preferred (Zeravik, Hlavacek, Lacina, Skladal, 2009).

Various biosensors for the determination of individual organic acids were developed and tested for the wine analysis. Biosensors for determination of acetic acid were based on the coupled three-enzymes system where acetate kinase (AK), pyruvate kinase (PK) and lactate dehydrogenase (LDH) were co-immobilized on the working electrodes (Mieliauskiene,

Nistor, Laurinavicius, Csoregi, 2006). Also, well known is the microbiological acetate biosensor (Wang, Tsujimura, Kano, 2008). Lactic acid is determined using the enzymatic biosensor having either lactate oxidase (LO) or LDH (Rassaei, Olthuis, Tsujimura, Sudholter, van den Berg, 2014; Monosik, Stredansky, Greif, Sturdik, 2012). Biosensors for malic acid employed enzymes including malate dehydrogenace (MDH) (Gamella, et al., 2010; Mazzei, Botre, Favero, 2007) and malate:quinone oxidoreductase (MQO) (Molinero-Abad, Alonso-Lomillo, Dominguez-Renedo, Acros-Martinez, 2014). The determination of citric acid requires co-immobilization of three enzymes - citrate lyase (CL), oxalacetate decarboxylase and pyruvate oxidase (PO) (Kim, 2006; Maines, Prodromidis, Tzouwara-Karayanni, Karayannis, Ashworth, Vadgama, 2000). Whereas the determination of tartaric acid using a biosensor has not been published yet. In this case, tartaric acid was determined by colorimetric reaction using vanadate which generates vanadate-tartrate complex with the absorption maximum at 495 nm (Bastos, Tafulo, Queiros, Matos & Sales, 2009; Rangel & Toth, 1998). The determination of gluconic acid is based on the utilization of either gluconate dehydrogenase (GADH) (del Torno-de Roman, Alonso-Lomillo, Dominguez-Renedo, Jaureguibeitia, Arcos-Martinez, 2014) or the bienzymatic system using gluconate kinase (GK) and 6-phospho-D-gluconate dehydrogenase (6PGDH) (del Torno-de Roman et al., 2013). The biosensing system based on GADH utilizes tetrathiafulvalene film as electrocatalytic layer which reduces the working potential of electrochemical determination of NADH from 800 mV to 100 mV vs. Ag/AgCl reference electrode. The bienzymatic biosensing system with GK and 6PGDH employed direct electrochemical oxidation of NADH at 800 mV vs. Ag/AgCl.

The current trends in design of biosensors are mostly based on unification of the mechanism of detection which should simplify the construction of biosensor-based devices. In this case, various types of oxidases and dehydrogenases are incorporated to the biosensing systems and serve as modules working in a similar manner. Biosensing systems based on

oxidases utilize oxygen in samples which serves as an acceptor of electrons providing either hydrogen peroxide or water. Enzymatic reactions based on oxidases can be monitored by measuring the consumption of oxygen or production of hydrogen peroxide. More often the biosensors monitor the production of hydrogen peroxide at 650 mV vs. Ag/AgCl while the decrease of the oxygen level monitored at -650 mV vs. Ag/AgCl is not so frequently used (Zeravik et al., 2009).

Unfortunately, wine contains many compounds interfering at the positive working potential, hence procedures reducing interference are required. The electrochemical interference can be reduced or completely removed using different approaches. Firstly, the permselective barrier e.g. poly-*o*-phenylenediamine and overoxidized non-conducting polypyrrole probably represents the simplest choice (Carelli, Centonze, De Giglio, Quinto, Zambonin, 2006; Zeravik, Lacina, Jilek, Vlcek, Skladal, 2010). The second approach reflects efforts to reduce the working potential using either artificial redox mediators or bi-enzymatic system based on the oxidase-peroxidase cascade (Gonzalo-Ruiz, Alonso-Lomillo, Munoz, 2007; de Prada, Pena, Parrado, Reviejo, Pingarron, 2004; Smutok, Ngounou, Pavlishko, Gayda, Gonchar, Schumann, 2006). Lowering of working potential inspired also the third approach - the direct electron transfer between heme of peroxidase and working electrode appears to be a promising alternative; this was observed in the presence of hydrogen peroxide at -50 mV vs. Ag/AgCl (Zeravik, Ruzsgas, Franek, 2003). Finally, various nanomaterials are able to improve the properties of the above mentioned systems through either electrocatalytic activity towards small molecules or the ability to mediate transfer of electrons between active redox centre of enzymes and working electrode (Chen, et al., 2013; Walcarius, Minteer, Wang, Lin and Merkoci, 2013).

The above mentioned biosensors for the determination of organic acids in wine converted the target analytes as substrates of the immobilized enzymes. Another techniques

for monitoring of content of organic acids in the sample is the use of enzyme inhibition by organic acids. The developed biosensor was based on a competitive inhibition of sarcosine oxidase (SO) by organic acids in the presence of sarcosine. SO was inhibited by malic, citric, succinic, acetic and formic acids while tartaric and lactic acid provided negligible inhibition effects (Zeravik et al., 2010).

The presented research partially builds on the previously designed SO biosensor; the improved biosensor is able to monitor the tartaric acid together with the above mentioned organic acids. We found that fumarase (FUM) is able to convert tartaric acid to a compound that strongly inhibits SO. This observation was utilized for the construction of biosensor with co-immobilized FUM and SO on the working electrode. Prepared biosensors were used for characterization of wine samples. In this case, the content of organic acids was monitored using SO, FUM + SO and LO based biosensors, providing very quickly the desired information about total acidity in real wine samples.

2. Experimental

2.1. Reagents

Sarcosine oxidase (from *Bacillus* sp., 25-50 $U.mg^{-1}$), lactate oxidase (from *Pediococcus* sp., ≥20 $U.mg^{-1}$), fumarase (from porcine heart, 300-500 $U.mg^{-1}$), sarcosine, bovine serum albumin (BSA), *o*-phenylenediamine, resorcinol, glutaraldehyde, L-lactic acid, L-malic acid, L-tartaric acid, succinic acid, citric acid, fumaric acid and sodium vanadate, ethylenediaminetetraacetic acid (EDTA), cetyltrimethylammonium bromide (CTAB) and phthalic acid were purchased from Sigma-Aldrich (www.sigmaaldrich.com). Sodium

hydrogenphosphate, sodium dihydrogenphosphate, potassium chloride, hydrogen peroxide and acetic acid were from Penta (www.pentachemicals.eu).

2.2. Apparatus

Three-electrode (AC1.W2.R1) screen-printed (SPE) sensors with platinum working and auxiliary electrode and Ag/AgCl as reference electrode were obtained from BVT Technologies (Brno, Czech Republic) and connected to the potentiostat PalmSens from Palm Instruments (Houten, The Netherlands). Flow-through cell was designed and constructed in-house. The minielectronic 8-channel switching valve was purchased from Valco Instruments (Houston, TX, USA) and the peristaltic pump Minipuls MP-3 was from Gilson (Middleton, WI, USA).

2.3. Electropolymerization and characterization of the copolymer permeability

The bare SPE sensor was immersed into acetone for 1 h in order to remove organic impurities, subsequently it was washed with distilled water and inserted into the flow-through cell. The flow-through system was built up from the 8-channel valve, the flow-through cell, the PalmSens potentiostat and the peristaltic pump (flow rate 50 μL min^{-1}); 100 mmol L^{-1} KCl was used as the carrier solution. After baseline stabilization, 10 mmol L^{-1} hydrogen peroxide, 1 mmol L^{-1} ascorbate in 100 mmol L^{-1} KCl were 3-times sequentially injected for 180 s to obtain responses at 650 mV vs. Ag/AgCl as reference electrode, respectively. After interferent measurements and baseline stabilization at the same potential, the electropolymerization was performed according to our previously published method (Zeravik et al., 2010). The electropolymerization process lasted for 90 minutes and mixture consisting of 2 mmol L^{-1} *o*-

phenylenediamine and 2 mmol L^{-1} resorcinol was used for this purpose. The process was self-terminated as indicated by the decrease of the current corresponding to the limited thickness of the non-conductive copolymer formed on the working electrode. Afterwards, 10 mmol L^{-1} hydrogen peroxide and 1 mmol L^{-1} ascorbate were injected again to evaluate the quality of the formed electrocopolymer. Permeability of the formed copolymer for testing compounds was calculated using the following formula: % permeability = i_{after} / i_{before} * 100. Thus modified sensor was further used for the enzyme immobilization.

2.4. Enzyme biosensors

2.4.1. Enzyme biosensors

Enzyme layer was prepared by cross-linking of the following enzymes SO, LO or FUM + SO (each 10 U cm^{-2}) with 2 % glutaraldehyde in 50 mmol L^{-1} phosphate buffer (pH 7.4). The mixture was supplemented with 100 mg mL^{-1} BSA in order to reach the density of 2 mg cm^{-2}. The immobilization mixture (1 μL) was deposited on the polymer-coated working Pt electrode, dried at room temperature and stored at a water-saturated atmosphere at 4°C.

2.4.2. Comparative electrode

The working electrode was coated with the layer in which the enzyme was replaced by BSA and the signal reflected the influences of interference. The blank signal was subtracted from each of the specific signals – enzyme covered electrodes (SOX, SOX + FUM, LOX).

2.5. Single-channel electrode system

Inhibition measurements with the SO biosensor and the FUM + SO biosensor were performed in the flow-through cell, the carrier buffer was 50 mmol L^{-1} phosphate containing 100 mmol L^{-1} KCl pH 7.4, the flow rate was 50 μL.min^{-1} and the working potential was 650 mV vs. Ag/AgCl. The measuring cycle consisted of the following steps: 1) 10 min carrier buffer for baseline stabilization, 2) 10 min flow of 5 mmol L^{-1} sarcosine, 3) 10 min organic acid containing sample (inhibitor) and 5 mmol L^{-1} sarcosine, 4) 10 min flow of 5 mmol L^{-1} sarcosine, 5) 5 min carrier buffer. Relative inhibition value (*RI*) was calculated as follows: $RI = (i_{sarcosine} - i_{inhib}) / (i_{sarcosine} - i_{baseline})$.

Measurement with the LO biosensor was based on the same working condition as above and it varies in the steps: 1) 10 min carrier buffer for baseline establishment 2) 10 min flow of lactic acid 3) 10 min carrier buffer.

2.6. Analysis of wine samples

31 wines originating in the Moravian region from producers Znovín (Znojmo, Czech Republic) and Winery Jaroslav Tichý (Rybníky, Czech Republic) were chosen: the white wines included Chardonay, Chenin Blanc, Frühroter Veltliner (2 samples), Grüner Veltliner (5 samples), Moravian Muscat, Müller Thurgau, Riesling (2 samples), Pálava, Pinot Blanc, Pinot Gris (6 samples), Sauvignon blanc (3 samples), Welschriesling, cuveé Sauvignon-Neuburger and Lemberger rosé; the red wines included Lemberger (2 samples), Saint Laurent and Zweigeltrebe. Each electrochemical measurement of the sample of wine was repeated three times and average value of the signal was calculated.

2.7. Colorimetric determination of tartaric acid

The principle and procedure of determination of tartaric acid were adapted according to Fernandes et al. (2006) with minor modifications.

2.7.1. Reagent solution

The following standard stock solutions were prepared: 1 mol L^{-1} acetic acid solution, 50 mmol L^{-1} tartaric acid in 1 mol L^{-1} acetic acid, 20 mmol L^{-1} sodium vanadate in 0.5 mol L^{-1} NaOH.

2.7.2. Calibration curve

The calibration curve for colorimetric determination of tartaric acid was performed as follows: 500 µL of tartaric acid in 1 mol L^{-1} acetic acid were mixed with 500 µL of 20 mmol L^{-1} sodium vanadate in 0.5 mol L^{-1} NaOH. Finaly, 100 µL of the mixture were added into 96-wells microplate in triplicates and subsequently measured at 490 nm. Final concentrations of tartaric acid in calibration solutions were 0, 0.1, 0.5, 1, 2, 3, 5, 8, 10, 15 and 20 mmol L^{-1}.

2.7.3. Measurement of wine samples

80 µL of 50 mmol L^{-1} tartaric acid in 1 mol L^{-1} acetic acid and 320 µL of 1 mol L^{-1} acetic acid were added to 100 µL of wine sample. This mixture was added to 500 µL of 20 mmol L^{-1} sodium vanadate in 0.5 mol L^{-1} NaOH and mixed for 30 s. 100 µL of the resulting mixture was added to microplate wells and absorbance was read at 490 nm. The modified procedure was used for blank measurements to reduce matrix effect of wine samples. Only 500 µL of 0.5 mol L^{-1} NaOH without 20 mmol L^{-1} sodium vanadate was used as a blank. The final value of tartaric acid concentration was calculated as difference between the wine sample and blank measurements.

2.7.4. Tartaric acid content in wine samples

The amount of tartaric acid was calculated as a difference between wine sample measurement with addition of 2 mmol L^{-1} tartaric acid and the values obtained at 2 mmol L^{-1} tartaric acid measurement.

2.8. Capillary electrophoresis analysis

The analysis was performed using the Agilent 7100 capillary electrophoresis (Agilent Technologies, Waldbronn, Germany) operated under the ChemStation software. The instrument is equipped with built-in photometric diode array detector. A fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) with an inner diameter of 75 µm and total length of 83 cm (effective length 74.5 cm) was used. Prior to daily use, the capillary was conditioned with 0.1 M NaOH for 5 min, followed by Milli-Q water for 10 min and background electrolyte for 15 min. The capillary was washed with the background electrolyte for 3 min before each run. The background electrolyte consisted of 5 mmol L^{-1} phthalic acid, 0.5 mmol L^{-1} EDTA and 0.5 mmol L^{-1} CTAB, pH 5.65. The operating conditions for the system were as follows, separation voltage of 28 kV (negative polarity) and capillary temperature 15 °C. Hydrodynamic injection at 5 kPa (50 mbar) 3 s was applied. Analytes were monitored using indirect UV detection at 254 nm. Peak identification was accomplished by comparing migration times of suspected peaks with that of authentic standard. Wine samples were diluted 1:20 with deionized water. All samples and standards were injected in triplicate.

3. Results and discussion

Our effort was to provide a maximum amount of information about the content of organic acids in individual analyzed wine samples obtained from the various suppliers and compare them with the standard analytical method (CE). In this case we collected data from: 1) certificates of analysis done by certified laboratories allowing wine trading; these certificates of analysis were provided by winemakers, 2) CE as the standard method, 3) colorimetric determination of tartaric acid, and 4) various biosensors based on SO, FUM + SO and LO developed in our laboratory.

3.1. Wine analysis by CE

Analysis of 31 wine samples using CE provided information both on composition and quantity of organic acids as described in Table 1. The content and the ratio of tartaric and malic acid correspond well to the regions of the South Moravia where grapes were grown (Pavlousek & Kumsta, 2011). In several cases, higher content of malic acid in wines was reduced by malolactic fermentation which partially or completely transformed malic acid to lactic acid (see Table 1). In the case of white wines, higher values of malic acid were determined while several samples of white wines showed the decrease of content of malic acid accompanied by the increase of lactic acid which corresponded to partial malolactic fermentation. The analysis of red wines showed a very low content of malic acid and high levels of lactic acid, which pointed to complete malolactic fermentation.

3.1.1 Total content of titratable acids

The total content of titratable acids ranged from 5 to 9 g L^{-1} tartaric acid equivalents (Pavlousek & Kumsta, 2011) and it was determined using the acid-base titration to pH 7

according to the official method OIV-MA-AS313-01:2009 (OIV, 2014). In our case, related data are depicted in Table 1 in the part Certificates of analysis. These values do not reflect the content of individual acids but only the sum of titratable carboxylic groups which are converted to the equivalent of tartaric acid. The comparison of the data obtained from certificates of analysis and sum of amounts of individual organic acids provided by CE do not show any degree of relevance. The found discrepancies are related with a higher content of malic acid compared to tartaric acid. The comparison of weight of equimolar amount of malic and tartaric acids differ by 11% of total weight, therefore the higher amount of malic acid in wine samples in comparison to tartaric acid provides higher discrepancies and it is difficult to use it as relevant method for qualitative determination of composition of organic acids in wines. The total content of titratable acids as the equivalent of tartaric acid can be considered as a relevant method in given regions (e.g. southern Europe) where tartaric acid is dominant. Today, there is a general effort to replace this traditional expression of total titratable acids content by miliequivalent units related to sulphuric acid (OIV, 2014).

3.1.2 Content of volatile acids

The content of volatile acids is predominantly due to acetic acid. Formic, propionic and butyric acids can also be found in minor level. The determination of volatile acids in wines provides information about the degree of improper fermentation processes occurring during winemaking (Mateo, Torija, Mas, Bartowsky, 2014; Gonzalez, Hierro, Poblet, Mas, Guillamon, 2005). In our case, the total content of volatile acids from Certificates of analysis and the content of acetic acid determined by CE were compared (see Fig. 1).

For the determination of total volatile acids, OIV recommended official method OIV-MA-AS313-02:2009 (OIV, 2014) which is based on steam distillation and the following acid-base titration to pH 7. Given the dominance of acetic acid in samples, the total content of

volatile acid was in good agreement with the results of analysis by CE where only acetic acid for comparison was chosen (Fig. 1). Differences in some cases may be given by long-term storage of bottles, by processing of wine samples or by higher content of other volatile acids in the tested wine samples (Bartowsky & Henschke, 2008).

3.2. Colorimetric determination of tartaric acid

The analysis using vanadate was designed as a very simple and rapid method utilizing microwell plates. The advantage of this system is reliability of measurement and simple elimination of non-specific responses of the wine samples in comparison with flow-injection analysis (Rangel & Toth, 1998). Regarding the content of tartaric acid in analyzed wine samples (from 4 mmol L^{-1} to 20 mmol L^{-1}, see Table 1) and subsequent 10-fold dilution for elimination of non-specific responses, 10 mmol L^{-1} vanadate was chosen as an optimal concentration of the reagent for the construction of a calibration curve and the subsequent wine sample analysis. The resulting calibration dependence showed a linear response within 0.1 - 5 mmol L^{-1} tartaric acid (Fig. 2A). The obtained data also show that the molar ratio of formation of a complex of tartaric acid and vanadate was 1:2 in the steady-state system. In contrast, a molar ratio necessary for the formation of tartaric acid-vanadate complex in flow-through system was determined as 1:3 (tartaric acid : vanadate) (Bastos et al., 2009). The increase of molar ratio was associated with the arrangement of the flow-through system, where a significant diffusion of reactants can be expected, and hence increased their molar ratio of the complex formation.

It follows that the extent of the linear relationship also depends on the concentration of vanadate. In our case, the value of tartaric acid in the diluted wine samples reached values from 0.4 to 2.0 mmol L^{-1}. These values lie at the lower end of the calibration curve. To

improve and highlight the responses for tartaric acid, it was decided to add a constant addition of 2 mmol L^{-1} tartaric acid into the reaction mixture. The resulting predicted concentrations ranged from 2.4 to 4 mmol L^{-1} tartaric acid.

The comparison of the vanadate based method with CE for determination of tartaric acid provides a good agreement (see Fig. 2B.). Unfortunately, vanadate is a toxic compound which is considered as a significant disadvantage of this method. However, this method can be considered as reliable due to its simplicity. As an alternative to this method, the electrochemical sensor based on the formation of calcium vanadate nanorods on the surface of glassy carbon electrode was used (Pei, Pei, Xie, Fan, Li, Zhang, 2012). Tartaric acid was monitored through its electrochemical behavior where one pair of semireversible electrochemical peaks was observed. The detection limit of this sensor was 2.4 μmol L^{-1} with a linear range of 0.005 - 2 mmol L^{-1} of tartaric acid.

3.3 Biosensor design

3.3.1 Formation of copolymer sieve and its characterization

The non-conductive copolymer layer above the working electrode surface can create sufficiently efficient barrier for the interfering compounds. Efficiency related with compactness of the barrier can be studied as a permeability of various electroactive compounds (Zeravik et al., 2010). In our case, the copolymer layer formed on the surface of SPE reduced the efficiency of the transport of hydrogen peroxide to 70% compared to the bare electrode. The response of current for ascorbic acid was reduced to 0.8% compared to the bare electrode. The shielding effect for ascorbic acid as an interferent does not reach the effectivity of the copolymer layer published previously (Zeravik et al., 2010). It is evident that copolymer layer formed on the screen printed electrode surface is massive but is not compact

and subsequently higher amount of interfering compounds can penetrate. Despite this finding, the formed copolymer layer can be regarded as sufficiently effective to eliminate interfering compounds and can serve as a base for the future biosensor design.

3.3.2 Lactate oxidase based biosensor

The biosensor was based on the commercially available platform produced by our laboratory that differed only in the addition of permeable copolymer membrane for hydrogen peroxide. In this case, the real response was correlated to 1 mmol L^{-1} L-lactic acid concentration which served as a calibrator. To obtain more precise data information the real biosensor response was related to 1 mmol L^{-1} concentration of L-lactic acid. This principle of measurement with addition 1 mmol L^{-1} L-lactic acid as calibrator was used for wine sample measurement. The final concentration values were calculated according to the Michaelis-Menten equation describing non-linear behavior of the biosensor. The comparison of the data provided by the LO biosensor with the data obtained from CE showed good agreement (see Fig. 4A). The developed biosensor can be considered as an equivalent device for the measurement of lactic acid in comparison with standard analytical method such as CE.

3.3.3 Sarcosine oxidase based biosensor

The SO based biosensor was used for the analysis of acidity in wines (see Fig. 4B). Due to the fact that SO is inhibited mainly by malic acid, the rate of the inhibition of organic acids in the wine was recalculated to the equivalent of malic acid. The data obtained by the biosensor correlate to a certain degree with the data from CE. The bias in the correlation is given by the different principle of the used analytical method; CE provides sum of absolute concentrations of individual acids, the biosensor provides sum of inhibitions by acidic content. In the case of CE, a sum of the concentrations of acids which inhibited SO was used

for more correct evaluation. Inhibition rate of the SO based biosensor by mixture of organic acids strongly depends on the content of the mono- and di-carboxylic acids (Zeravik et al., 2010).

Possible use of the SO biosensor is related to its on-line applications where this biosensor is suitable for monitoring of various fermentation processes e.g. malolactic fermentation or acetic fermentation. Generally, the biosensor can be advantageously used if the content of organic acids is changed within the monitored process. The combination of SO and LO biosensors for on-line monitoring of the malolactic fermentation is preferable. LO biosensor serves as a control of the L-lactic acid level if winemaker prefers only partial malic acid degradation.

3.3.4 Fumarase and sarcosine oxidase based biosensor

The biosensor based on SO showed several limitations for determination of the total content of organic acids. The main complication was only partial inhibition by tartaric acid in comparison with the aforementioned organic acids. Tartaric acid together with malic acid represents the major portion from the sum of organic acids in wines. Another limitation of this biosensor is the inability to detect lactic acid. This shortage can be simply solved by help of the second biosensor specific to lactic acid. Thus, the only component that can not be identified enzymatically is tartaric acid.

In this case, we sought the possibility of enzymatic conversion of tartaric acid into a compound which could be detected using SO. FUM has been recognized as an enzyme that fully meets these requirements (Fig. 5A). Tartaric acid was transformed by FUM to oxaloacetate (Flint, 1994) which exhibited similar inhibition parameters as malic acid (Fig. 5A, 5B). The comparison of the determination of malic acid using SO and FUM + SO biosensors provides similar responses, which means that FUM does not affect the inhibition

effect of malic acid (Fig. 5B). Finally, FUM + SO biosensor was prepared to improve the ability to detect total content of organic acids through the increase of the inhibition properties of tartaric acid.

The developed FUM + SO biosensor was used for the analysis of 31 wine samples and the obtained results were compared with CE analysis providing the sum of organic acids including malic, tartaric, citric, succinic and acetic acids. As can be seen from Fig. 4C, a good agreement between biosensor and CE is apparent. The response of this biosensor to various wine samples corresponds significantly better to the CE determined organic acids in comparison with the simple SO biosensor (Fig. 4B).

Coupling of FUM and SO provided an interesting improvement of the method for the determination of organic acids using biosensor. This combination of enzymes is able to monitor two major components which are responsible for the acidity of wine, malic and tartaric acid. Partial disadvantage of this system of detection is inability to recognize individual acids as in the case of SO biosensor.

3.3.5 Analytical parameters of biosensors

Three biosensors were tested for the repeatability and reproducibility measurements. Data used for evaluation were provided from 6 measurements for each biosensor. The parameters of repeatability and reproducibility (Table S-1) were calculated using software EffiValidation 3.0 DEMO version. Non-linear equations based on the Michaelis-Menten kinetics were used for the evaluation of the real samples. Repeatability and reproducibility of the biosensors were determined as relative values to the actual responses of the repeatedly measured calibrator. The calibrators for LOX and SOX (FUM + SOX) biosensors were 1 mmol L^{-1} lactic acid and 2 mmol L^{-1} malic acid, respectively.

4. Conclusions

The comparison of the method recommended by OIV with the developed biosensors was presented. The data obtained by CE as basal parameters for the comparison with other methods and biosensors were used. In addition, the data obtained from the certificates of analysis of individual wine samples were also used in order to provide comprehensive information about the analyzed wines. These information were used for the interpretation of obtained results and explanation of some discrepancies incurred in the comparison of various biosensors with CE.

The results obtained by CE provided extensive information about the content of individual acids present in wines and simultaneously reflect both the climatic conditions prevailing in the South Moravia region (Czech Republic), as well as information about fermentation processes that form the character of wine. The comparison of total acidity obtained from certificates of analysis with CE showed that the recalculation of the total organic acids content to the equivalent of tartaric acid are not completely satisfactory because of the high levels of malic acid. In contrast to these findings, the volatile acids content obtained from certificates of analysis mostly correspond to the level of acetic acid determined by CE.

The main task of this work was comparison of CE as a standard method with the developed biosensors. In this case, individual enzymes LO and SO and mixture of FUM and SO were used for this purpose. The LO based biosensor for lactic acid monitoring provided satisfactory correlation with CE data. The other two biosensors based on the inhibitory measurement were less straightforward because various levels of inhibition effect in the case of individual organic acids were observed. The SO based biosensor provided lower degree of correlation with CE since the tartaric acid only weakly inhibited SO. An improvement in the

inhibition efficiency was observed using mixture FUM + SO. Tartaric acid was converted by FUM to the compound which inhibited SO in a similar extent as malic acid. Increasing inhibition efficiency for tartaric acid was accompanied with the improvement of correlation with CE.

To provide a comprehensive overview in the field of organic acid analysis, the colorimetric vanadate based method for determination of tartaric acid was utilized. Slightly modified method provided good correlation with CE.

Finally, the analysis of the samples of wine using biosensors and CE showed certain correlation between the results obtained by above mentioned methods. It is evident that the biosensors can not fully replace the standardized methods, but on the other hand, their mobility and simplicity of usage allow their application in the field during on-line *in-situ* monitoring of fermentation processes. In this case, their function is not to determine the absolute values of the analytes, but continuous monitoring of change of the signal in the given time frame which can be a major improvement in the production of quality wines. Additionally, single biosensors can be integrated into multichannel flow-through systems where the monitoring of the group of selected analytes in single step is possible (Lacina, Vondal, Skladal, 2012) and subsequently evaluated with advanced statistical methods. The bioelectronic tongue for characterization of wine is thus obtained (Zeravik et al., 2009). This arrangement allows the approximation of biosensors to the standard methods.

5. Acknowledgements

This work has been supported by the Ministry of Education, Youth and Sports of Czech Republic, programme NPV II, project no. 2B08035, by grant no. GAP206/11/0009 **from the Grant Agency of the Czech Republic**, by the project "EXCELLENT TEAMS"

(CZ.1.07/2.3.00/30.0005) from European Social Fund and by CEITEC - Central European Institute of Technology (CZ.1.05/1.1.00/02.0068) from European Regional Development Fund. The authors are also very grateful to wine producers Znovín Znojmo and Winery Tichý Rybníky (Czech Republic) for providing the wine samples and additional information including Certificates of Analysis.

References

Bartowsky, E.J., Henschke, P.A. (2008). Acetic acid bacteria spoilage of bottled red wine – A review. *Internaional Journal of Food Microbiology, 125*, 60 – 70.

Bastos, S.S.T., Tafulo, P.A.R., Queiros, R.B., Matos, C.D., Sales, M.G.F. (2009). Rapid Determination of Tartaric Acid in Wines. *Combinatorial Chemistry & High Throiughput Screening, 12*, 712 – 722.

Carelli, D., Centonze, D., De Giglio, A., Quinto, M., Zambonin, P.G. (2006). An interference-free first generation alcohol biosensor based on a gold electrode modified by an overoxidised non-conducting polypyrrole film. *Analytica Chimica Acta, 565*, 27 – 35.

Chen, C., Xie, Q., Yang, D., Xiao, H., Fu, Y, Tan, Y., and Zao, S. (2013). Recent advances in electrochemical glucose biosensors: a review. *RSC Advances, 3*, 4473 – 4491.

Fugelsang, K.C., Edwards, C.G. (2007) *Wine Microbiology: Practical Applications and Procedures. 2nd ed.* New York: Springer, XX, 393.

Gamella, M., Campuzano, S., Conzuelo, F., Curiel, J.A., Munoz, R., Reviejo, A.J., Pingarron, J.M. (2010). Integrated multienzyme electrochemical biosensors for monitoring malolactic fermentation in wines. *Talanta, 81*, 925 – 933.

Golubenko, A.M., Nikonorov, V.V., Nikitina, T.G. (2012). Determination of Hydroxycarboxylic Acids in Food Products by Capillary Electrophoresis. *Journal of Analytical Chemistry, 67*, 778 – 782.

Gomez, F.J.V., Monasterio, R.P., Vargas, V.C.S., Silva, M.F. (2012). Analytical characterization of wine and its precursors by capillary electrophoresis. *Electrophoresis, 33*, 2240 – 2252.

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.

Gonzalo-Ruiz, J., Alonso-Lomillo, M.A., Munoz, F.J. (2007). Screen-printed biosensors for glucose determination in grape juice. *Biosensors & Bioelectronics*, *22*, 1517 - 1521.

Flint, D.H. (1994). Initial kinetic and mechanistic characterization of Escherichia-coli fumarase-A. *Archives of Biochemistry and Biophysics, 11*, 509 – 516.

Flores, P., Hellin, P., Fenoll, J. (2012). Determination of organic acids in fruits and vegetables by liquid chromatography with tandem-mass spectrometry. *Food Chemistry, 132*, 1049 – 1054.

Jurado-Sanchez, B., Ballesteros, E., Gallego, M. (2011). Gas chromatographic determination of 29 organic acids in foodstuffs after continuous solid – phase extraction. *Talanta, 84*, 924 – 930.

Kim, M. (2006). Determining citrate in fruit juices using a biosensor with citrate lyase and oxaloacetate decarboxylase in a flow injection analysis system. *Food Chemistry, 99*, 851 – 857.

Lacina, K., Vondal, J., Skladal, P. (2012). A novel approach to the uniform distribution of liquid in multi-channel (electrochemical) flow-through cells. *Analytica Chimica Acta, 727,* 41 – 46.

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.

Lin, J.-T., Liu, S.-C., Shen, Y.-C., Yang, D.-J. (2011). Comparison of Various Preparation Methods for Determination of Organic Acids in Fruit Vinegars with a Simple Ion-Exclusion Liquid Chromatography. *Food Analytical Methods, 4*, 531 – 539.

Maines, A., Prodromidis, M.I., Tzouwara-Karayanni, S.M., Karayannis, M.I., Ashworth, D., Vadgama, P. (2000). An enzyme electrode for extended linearity citrate measurements based on modified polymeric membranes. *Electroanalysis, 12*, 1118 – 1123.

Mateo, E., Torija, M.J., Mas, A., Bartowsky, E.J. (2014). Acetic acid bacteria isolated from grapes of South Australian vineyards. *International Journal of Food Microbiology, 178*, 98 – 106.

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.

Mazzei, F., Botre, F., Favero, G. (2007). Peroxidase based biosensors for the selective determination of D,L-lactic acid and L-malic acid in wines. *Microchemical Journal, 87*, 81 – 86.

Mieliauskiene, R., Nistor, M., Laurinavicius, V., Csoregi, E. (2006). Amperometric determination of acetate with a tri-enzyme based sensor. *Sensors and Actuators B, 113*, 671 – 676.

Molinero-Abad, B., Alonso-Lomillo, M.A., Dominguez-Renedo, O., Arcos-Martinez, M.J., 2014). Malate quinone oxidoreductase biosesors based on tetrathiafulvalene and gold nanoparticles modified screen-printed carbon electrodes for malic acid determination in wine. *Sensors and Actuators B, 2014*, 971-975.

Monosik, R., Stredansky, M., Greif, G., Sturdik, E. (2012). A rapid method for determination of L-lactic acid in real samples by amperometric biosensor utilizing nanocomposite. *Food Control, 23*, 238 – 244.

OIV (2014). *Compendium of international methods of wine and must analysis.* Paris: Organisation Internationale de la Vigne et du Vin.

Oliviera, S.M., Lopes, T.I.M.S., Toth, I.V., Rangel, A.O.S.S. (2010), *Talanta, 81*, 1735 – 1741.

Pavlousek, P., Kumsta, M. (2011). Profiling of primary metabolites in grapes of interspecific grapevine varieties: sugars and organic acids. *Czech Journal of Food Science, 29*, 361 – 372.

de Prada, A.G.V., Pena, N., Parrado, C., Reviejo, A.J., Pingarron, J.M. (2004). Amperometric multidetection with composite enzyme electrodes. *Talanta*, *62*, 896 – 903.

Pei, L., Pei, Y., Xie, Y., Fan, C., Li, D., Zhang, Q. (2012). Formation process of calcium vanadate nanorods and their electrochemical sensing properties. *Journal of Materials Research, 27*, 2391 – 2400.

Rangel, A.O.S.S., Toth, I.V. (1998). Sequential determination of titrable acidity and tartaric acid in wines by flow injection spectrophotometry. *Analyst, 123*, 661 – 664.

Rassaei, L., Olthuis, W., Tsujimura, S., Sudholter, E.J.R., van den Berg, A. (2014). Lactate biosensors: current status and outlook. *Analytical and Bioanalytical Chemistry, 406*, 123 – 137.

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 (yougurt, wine) analysis. *Food Chemistry, 129*, 608 – 613.

Smutok, O., Ngounou, B., Pavlishko, H., Gayda, G., Gonchar, M., Schuhmann, W. (2006). A reagentless bienzyme amperometric biosensor based on alcohol oxidase/peroxidase and an Os-complex modified electrodeposition paint. *Sensors and Actuators B - Chemical*, *113*, 590 - 598.

del Torno-de Román, L., Alonso-Lomillo, M.A., Domínguez-Renedo, O., Arcos-Martínez, M.J. (2013). Gluconic acid determination in wine by electrochemical biosensing. *Sensors and Actuators B, 176*, 858 – 862.

del Torno-de Román, L., Alonso-Lomillo, M.A., Domínguez-Renedo, O., Jaureguibeitia, A., Arcos-Martínez, M.J. (2014). GADH screen-printed biosensor for gluconic acid determination in wine samples. *Sensors and Actuators B, 192*, 56 – 59.

Walcarius, A., Minteer, S.D., Wang, J., Lin, Y., Merkoci, A. (2013). Nanomaterials for bio-functionalized electrodes: recent trends. *Journal of Materials Chemictry B, 1*, 4878 – 4908.

Wang, Y.F., Tsujimura, S., Kano, K. (2008). Amperometric detection of acetate based on mediated bioelectrocatalysis using Escherichia coli cells cultivated with acetate. *Electrochemistry, 76*, 631 – 633.

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.

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.

Zeravik, J. Ruzgas, T., Franek, M. (2003). A highly sensitive flow-through amperometric immunosensor based on the peroxidase chip and enzyme-channeling principle. *Biosensors & Bioelectronics, 18*, 1321 – 1327.

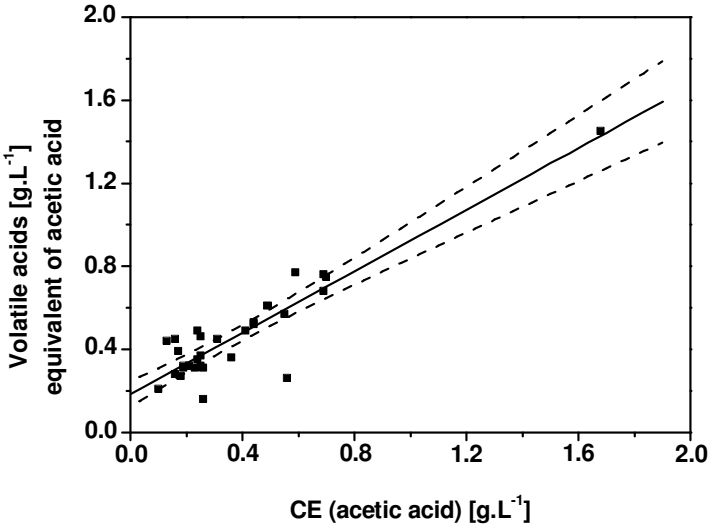

Fig. 1. The comparison of the content of volatile acids from Certificates of analysis and acetic acid amount determined by CE. Plotted linear regression: Y = A + B*X; A = 0.18 ± 0.03; B = 0.74 ± 0.06; R = 0.9125. Dash lines delimit the lower and the upper 95% confidence limits.

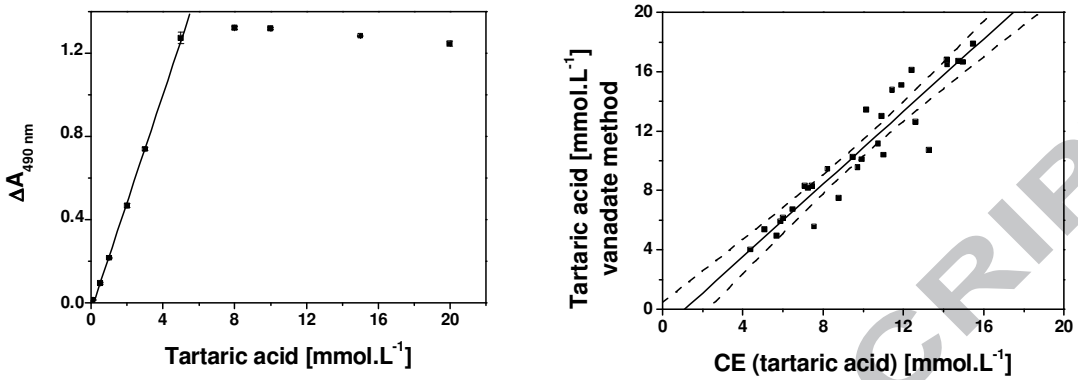

Fig. 2. A) Calibration curve for the determination of tartaric acid using the vanadate based colorimetric method. The linear response (straight line) was observed from 0.1 to 5 mmol.L^{-1}, with the following parameters: Y = A + B * X; A = -0.04 ± 0.01 and B = 0.259 ± 0.004, R = 0.99954. **B)** The comparison of the colorimetric vanadate method and CE as a standard method. Linear regression parameters: A = -1.35 ± 0.88 and B = 1.22 ± 0.08; R = 0.9409. Dash lines delimit the lower and the upper 95% confidence limits.

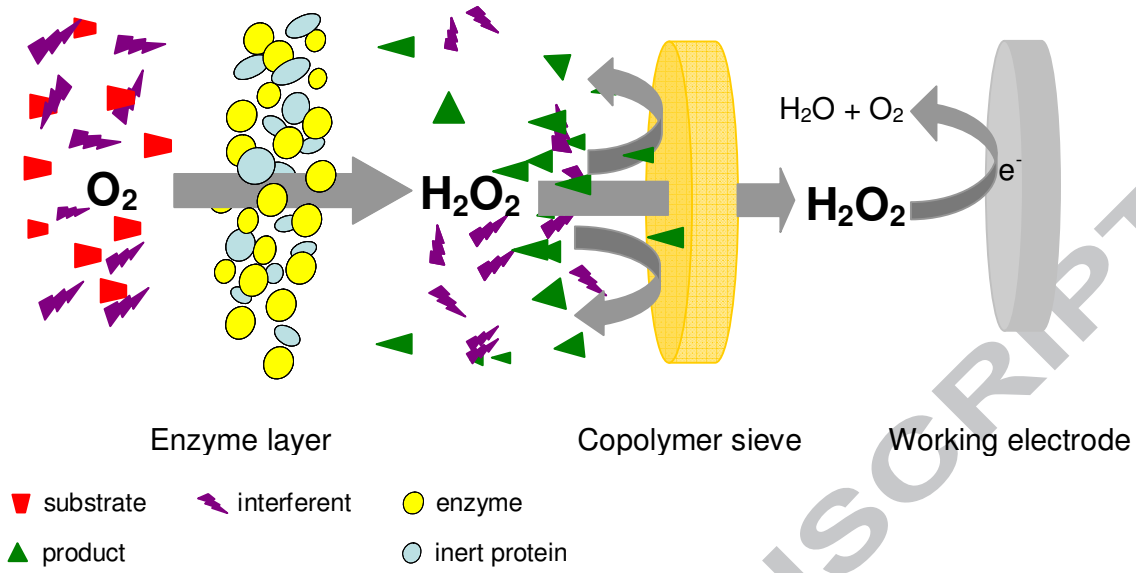

Fig. 3. Design and principle of the developed biosensors based on hydrogen peroxide detection. Hydrogen peroxide is electrochemically determined on the platinum working electrode at + 650 mV vs. Ag/AgCl as reference.

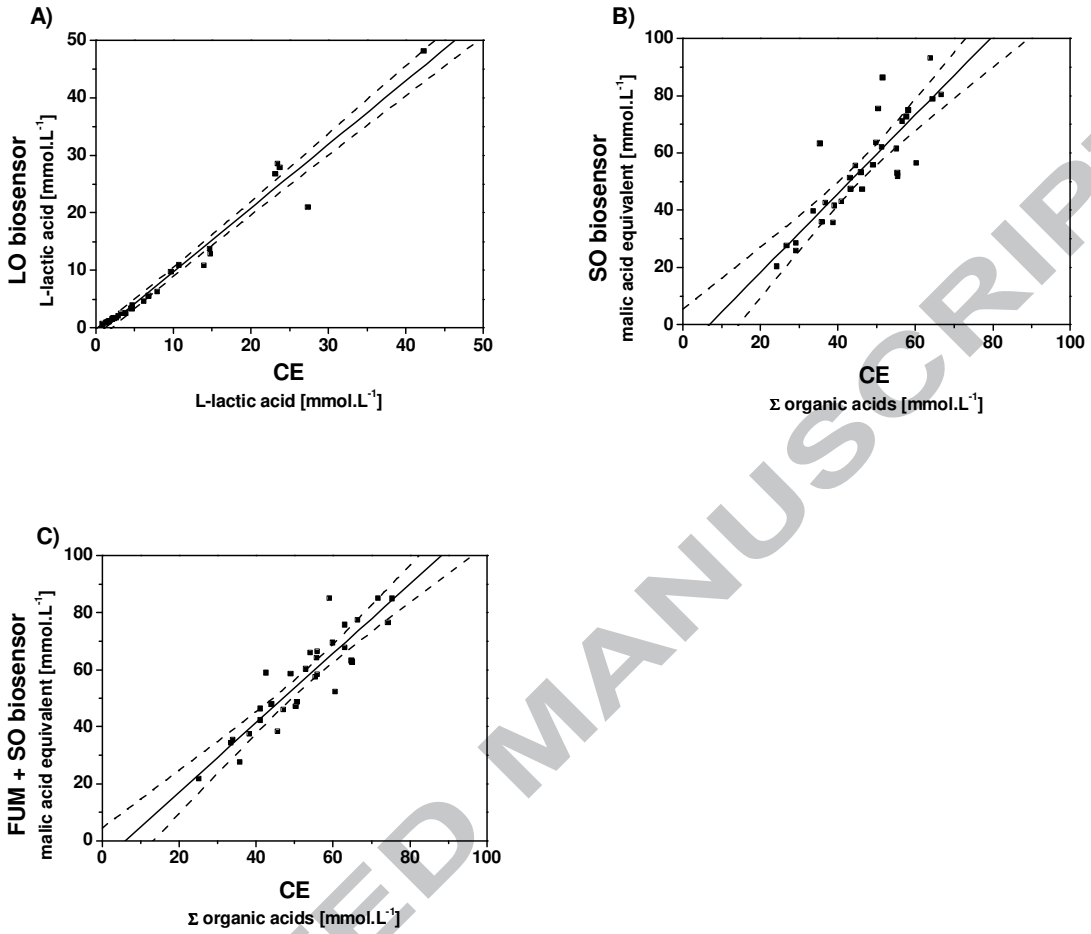

Fig. 4. The comparison of analysis of 31 wine samples by the developed biosensors and CE as a standard reference method. **A)** Comparison of the LO based biosensor and CE. Linear regression: Y = A + B*X; A = 1.48 ± 0.43 and B = 0.87 ± 0.03; R = 0.9829. **B)** Comparison of responses of the SO based biosensor and CE. In the former case, the content of organic acids was expressed as equivalent of malic acid. Linear regression parameters: A = -1.3 ± 7.2 and B = 1.38 ± 0.15; R = 0.8615). **C)** Comparison of the response of the co-immobilized FUM and SO based biosensor and CE. In the case of the FUM + SO biosensor, the content of organic acids was expressed as equivalent of malic acid. Linear regression parameters: A = -7.3 ± 5.8 and B = 1.22 ± 0.11; R = 0.9042. In all cases, dash lines delimit the lower and the

upper 95% confidence limits. In the case of B) and C), the sum of organic acids included malic, tartaric, citric, succinic and acetic acids (negligible inhibitory effect of lactic acid was observed).

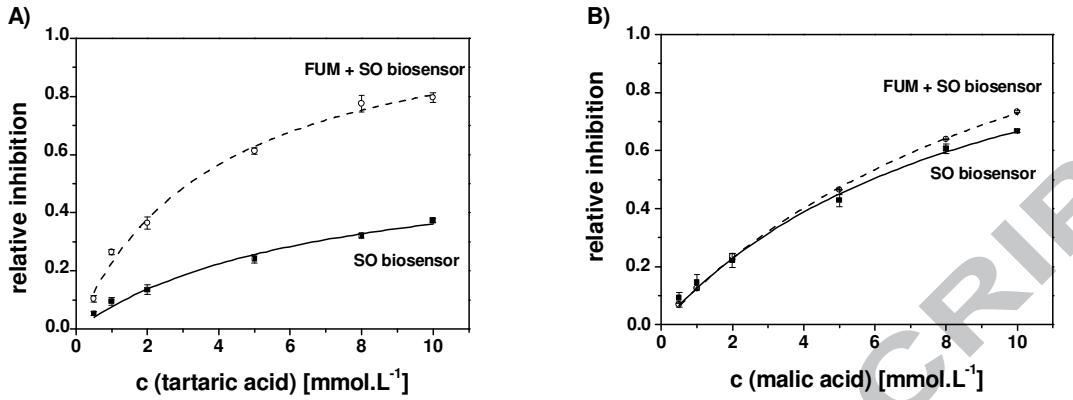

Fig. 5. Comparison of inhibition behavior of tartaric acid (**A**) and malic acid (**B**) using the SO (full line) and FUM + SO (dash line) biosensors. Parameters of the Michaelis-Menten equation: **A)** for SO biosensor V_{max} = 0.61 ± 0.07, K_M = 6.9 ± 1.6, R^2 = 0.989; FUM + SO biosensor V_{max} = 1.12 ± 0.07, K_M = 3.9 ± 0.6, R^2 = 0.993; **B)** for SO biosensor V_{max} = 1.28 ± 0.14, K_M = 9.2 ± 1.8, R^2 = 0.994; FUM + SO biosensor V_{max} = 1.60 ± 0.06, K_M = 11.9 ± 0.7, R^2 = 0.999.

Table 1 An overview of obtained data of 31 wine samples from CE and from Certificates of Analysis provided by producers. Using CE analysis, the following organic acids were found: TA – tartaric acid, MA – malic acid, CA – citric acid, SA – succinic acid, AA – acetic acid, LA – lactic acid.

Wine sample		**CE analysis**							**Certificate of analysis**	
Grape varieties	Year-Producer-Sample No.	**TA**	**MA**	**CA**	**SA**	**AA**	**LA**	**Total acids**	**Total acids**	**Volatile acids**
		[mmol.L^{-1}]						[g.L^{-1}]	[g.L^{-1}]	
Chardonnay	06-P1-28	9.5	26.1	1.7	1.9	9.9	8.8	***7.5***	**6.8**	**0.77**
Chenin Blanc	09-P1-26	8.8	41.6	1.9	3.4	8.2	2.6	***9.5***	**8.2**	**0.61**
Frühroter Veltliner	10-P2-10	7.3	31.0	1.0	2.8	4.2	2.2	***8.3***	**6.6**	**0.32**
Frühroter Veltliner	09-P1-20	14.2	15.5	0.9	5.4	7.3	14.7	***7.6***	**6.3**	**0.53**
Grüner Veltliner	10-P1-1	9.9	37.4	2.0	1.8	4.4	1.5	***8.1***	**5.1**	**0.31**
Grüner Veltliner	10-P1-13	6.5	38.2	1.7	1.5	3.5	4.7	***7.8***	**6.2**	**0.32**
Grüner Veltliner	10-P1-22	17.4	31.7	1.9	4.0	1.7	3.3	***9.3***	**8.3**	**0.21**
Grüner Veltliner	09-P1-24	14.2	13.9	1.1	2.6	4.1	9.7	***6.4***	**6.0**	**0.46**
Grüner Veltliner	09-P2-14	10.8	34.6	1.4	2.2	2.6	1.7	***8.2***	**7.5**	**0.45**
Moravian Muscat	10-P1-17	5.1	35.9	1.1	5.0	2.7	7.9	***8.1***	**6.8**	**0.28**
Müller Thurgau	10-P1-18	11.0	30.8	1.2	3.9	3.5	4.6	***7.8***	**6.4**	**0.32**
Riesling	09-P2-7	15.0	14.4	0.9	4.6	3.9	1.8	***6.1***	**6.6**	**0.31**
Riesling	10-P1-15	6.0	42.2	1.2	3.0	3.0	3.8	***8.5***	**6.4**	**0.27**
Palava	10-P2-8	4.4	43.3	1.9	1.3	9.4	1.7	***8.7***	**7.2**	**0.26**
Pinot Blanc	09-P1-21	8.2	27.7	1.5	3.1	4.0	4.7	***7.0***	**6.5**	**0.49**
Pinot Gris	10-P2-6	9.7	39.0	1.7	2.6	2.1	2.4	***8.7***	**8.6**	**0.44**
Pinot Gris	09-P2-12	11.9	12.4	1.1	5.1	3.2	2.9	***5.4***	**5.8**	**0.31**
Pinot Gris	10-P1-16	5.7	43.5	2.1	2.3	4.1	6.2	***8.9***	**6.9**	**0.37**
Pinot Gris	07-P1-27	5.9	18.7	2.2	5.5	6.8	14.8	***7.1***	**6.0**	**0.49**
Pinot Gris	06-P1-30	7.4	17.2	2.2	4.0	6.0	9.9	***6.4***	**6.6**	**0.36**
Pinot Gris	07-P1-31	7.6	27.2	2.9	1.1	27.9	14.0	***9.4***	**8.1**	**1.45**
Sauvignon Blanc	08-P1-23	7.1	27.5	1.6	1.9	5.1	10.8	***7.4***	**6.2**	**0.45**
Sauvignon Blanc	10-P1-25	4.9	46.4	1.7	2.1	3.1	1.4	***8.7***	**6.6**	**0.32**
Sauvignon Blanc	09-P1-29	12.6	16.7	1.9	3.3	11.5	27.4	***9.2***	**7.7**	**0.76**
Welschriesling	09-P2-4	14.7	14.9	0.8	7.6	2.9	2.2	***6.5***	**6.5**	**0.39**
Savignon Blanc – Neuburger (cuveé)	07-P2-11	15.5	12.4	1.1	2.4	4.0	1.6	***5.6***	**5.8**	**0.35**
Lemberger (rosé)	10-P2-2	13.3	42.2	1.4	3.3	4.3	2.1	***10.3***	**8.0**	**0.16**
Lemberger	08-P1-5	12.4	0.7	n.d.	4.6	11.5	23.8	***6.0***	**4.4**	**0.68**
Lemberger	09-P2-9	10.2	0.9	n.d.	5.8	7.4	23.2	***4.9***	**5.8**	**0.52**
Saint Laurent	09-P2-3	11.4	0.9	n.d.	5.4	9.1	23.4	***5.9***	**5.8**	**0.57**
Zweigeltrebe	09-P1-19	10.9	n.d.	n.d.	6.7	11.6	42.3	***7.8***	**4.5**	**0.75**

P1 – producer 1, P2 – producer 2

RSD were bellow 10%.

Highlights

- Various biosensors were used for monitoring of organic acids in wine samples.
- Capillary electrophoresis served as reference standard method.
- Biosensor for lactic acid monitoring provided satisfactory correlation with CE data.
- Biosensors based on sarcosine oxidase were used for monitoring of total content of organic acids.
- The developed biosensors can be used for on-line monitoring of fermentation processes.