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Chip-based separation of organic and inorganic anions and multivariate analysis of wines according to grape varieties

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ABSTRACT

This report describes the use of electrophoresis microchips integrated with contactless conductivity detection for the determination of organic acids and inorganic anions in wine samples and the subsequent classification based on the grape varieties. The best separation was achieved using a buffer composed of 30 mmol L^{-1} 2-(N-morpholino)ethanesulfonic acid, 15 mmol L^{-1} L-histidine and 0.05 mmol L^{-1} cetyltrimethylammonium bromide (pH 5.8), allowing the determination of chloride, nitrate, sulfate, oxalate, tartrate, maleate, succinate, citrate, acetate, lactate, pyroglutamate and phosphate within ca. 100 s. The relative standard deviations obtained for the migration times were lower than 2%, while the obtained values for peak areas ranged from 2.5 to 8.4%. The limits of detection achieved for all compounds ranged between 3.0 and 12.6 μmol L^{-1}. A total of 18 wines from Brazil and Chile were successfully investigated, including red, white and rosé, and the anionic species were quantified with recovery values between 92 and 117%. A statistical difference has not been observed between the data obtained by using electrophoresis microchips integrated with contactless conductivity detection (ME-C^4D) and capillary electrophoresis with ultra-violet detection (CE-UV) and thus the results from newly developed method is validated. Finally, similarities among the anionic profile of wines were investigated by using a multivariate approach, and it was possible to discriminate samples mainly by grapes varieties. Furthermore, the proposed methodology has provided instrumental simplicity and good analytical performance, demonstrating to be useful for routine quality control of wines.

1. Introduction

Foodomics is one important field that has been growing since the detailed knowledge of the food composition is essential for processing, safety and quality control [1–3]. Methodologies using miniaturized analytical techniques are required to provide fast and low-cost food analysis. Microchip electrophoresis (ME) systems have been widely used for food science applications [4–6]. ME devices have emerged as a microscale separation platform with many advantages over conventional separation methods, such as short analysis time, low consumption of reagents and samples, reduced generation of waste and portability [7,8].

Wines are composed of a complex mixture of organic acids (OAs), carbohydrates, proteins, alcohol, minerals and polyphenols [9,10]. OAs are responsible for organoleptic properties, such as flavor, aroma, color and stability, and they are produced during the fermentation process depending on the grape growing region and the wine production process. The analysis of OAs is important to monitor fermentation process, pH and wines stability [10–12]. The determination of major constituents in wines, especially OAs, has been mostly reported using capillary electrophoresis [10,13–20], high performance liquid chromatography [21–26] and gas chromatography [27]. However, these techniques may require a long analysis time, a laborious sample preparation step or large reagent volumes. Some other unconventional approaches described in literature are the use of Langmuir–Blodgett films based on functionalized nanoparticles [28] and direct infusion electrospray ionization mass spectrometry [29].

Regarding to ME devices, a few studies demonstrating the

Abbreviations: ME, microchip electrophoresis; **C^4D**, capacivitely coupled contactless conductivity detection; **PCA**, principal component analysis; **PMMA**, poly (methyl methacrylate).

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determination of phenolic compounds, inorganic ions, aldehydes, sugars, alcohols and neuroactive amines detection in wines have been found in the literature [30]. In addition, examples of applications related to the determination of OAs have been also described using chip-based devices. Masár and coauthors described a methodology to determine 13 anions, including inorganic anions and OAs, by isotachophoresis using a poly(methyl metacrylate) (PMMA) microchip [31]. The same group have reported the determination of 23 species (inorganic anions and OAs) using chip-based electrophoresis [32]. In both studies, the anionic compounds were separated within 10–15 min and they were detected using a contact conductivity detector. Kubáň and Hauser demonstrated the use of an external capacitively coupled contactless conductivity detector (C^4D) integrated on a PMMA microchip to detect inorganic ions and OAs in beverages and they analyzed one white and one red wine samples [33]. As well described in the literature, conductivity detection in both contact and contactless modes has successfully demonstrated great potential for monitoring separations of inorganic and organic compounds as well as their determination in different real samples including alcoholic beverages. When compared to the contact mode, the contactless approach (known as C^4D) is advantageous since it makes use of electrically insulated electrodes, thus avoiding bubble formation due to electrolysis phenomenon or interference from the applied electric field for electrophoretic separations [34–37].

In this context, the current study describes a fast, accurate and green methodology based on ME chips integrated with C^4D to quantitatively investigate the anionic profile of wines, including inorganic anions and organic acids. Afterwards, the wines were classified in accordance with the grape features based on their anionic profiles.

2. Materials and methods

2.1. Chemicals and samples

Sodium hydroxide, 2-(N-morpholino)ethanesulfonic acid (MES), L-histidine (His), cetyltrimethylammonium bromide (CTAB), tris (hydroxymethyl)aminomethane (TRIS), phthalic acid, sodium chloride, sodium sulfate, sodium acetate, sodium lactate, monosodium phosphate, sodium citrate dehydrate, oxalic acid, succinic acid, tartaric acid, malic acid and pyroglutamic acid were purchased from Sigma Aldrich Co. (Saint Louis, MO, USA). Sodium nitrate was purchased from Neon Comercial LTDA (São Paulo, SP, Brazil). Buffer stock solutions were prepared at 100 mmol L^{-1} each and the standard solutions of anionic compounds were prepared at 10 mmol L^{-1} each. The solutions were prepared using ultrapure water (resistivity equals to 18.2 MΩ cm) processed through a purification system (Direct-Q®3, Millipore, Darmstadt, Germany). All solutions were filtered through nylon filters with 0.22 μm pore diameter prior analysis. Running buffer composed of MES and His was prepared in different pH and ionic strength (IS) values. As EOF modifier, CTAB was tested at different concentrations. All experiments were performed at 23 ± 1 °C.

2.2. Wine samples

A total of eighteen wine samples of different brands was acquired at a local store (Goiania, GO, Brazil). For this current study, three samples of the brands named red Cabernet from Chile (RC_CH), red Cabernet from Brazil (RC_BR), red Merlot from Chile (RM_CH), red Merlot from Brazil (RC_BR), white Chardonnay wines from Chile (W_CH) and rosé Syrah wines from Chile (not 100% Syrah) (RS_CH) were used to investigate their organic and inorganic profiles. Prior to analysis, all the samples were filtered through nylon filters with 0.22 μm pore diameter and diluted in ultrapure water.

2.3. Instrumentation

The Quad HV microchip electrophoresis system (model ER455) supplied by eDAQ (Denistone East, NSW, Australia) containing a high-voltage sequencer, a platform to settle the electrophoresis microchip and a C^4D detector was used in this study. Glass electrophoresis chips with integrated sensing electrodes (model ET190 from Micronit Microfluidics, Enschede, Netherlands) and microchannels (100 μm wide and 10 μm deep) designed in a double-T format were employed to perform electrophoresis experiments, as detailed elsewhere [38]. In summary, the total and effective separation channel lengths were 85 mm and 77 mm, respectively. The detection cell for C^4D measurements was composed of two pairs of sensing electrodes (200 μm wide, 500 μm long and 200 nm thick). In each pair, the electrodes were spaced by a 250 μm gap.

2.4. Electrophoresis procedures

Prior to analysis, microchannels were sequentially conditioned with 0.1 mol L^{-1} NaOH for 10 min, ultrapure water for 5 min, and running buffer for 30 min. Sample injection was performed through a gated protocol under the application of negative polarity. The voltages required for sample injection and separation as well as the operational parameters (frequency and amplitude) for C^4D measurements were optimized to achieve the best analytical performance.

2.5. Comparative analysis

Capillary electrophoresis (CE) experiments were performed in a CE 7100 System (Agilent Technologies, Palo Alto, USA) with diode array detector set at 240 nm. For experiments, a fused-silica capillary with 49 cm length (40.5 cm effective length), 50 μm inner diameter and 375 μm outer diameter was used.

Prior to analysis, capillary was flushed with NaOH 0.1 mol L^{-1} for 20 min followed by water and the BGE for 5 and 30 min, respectively. The BGE was composed of 20 mmol L^{-1} phthalic acid, 15 mmol L^{-1} TRIS and 0.8 mmol L^{-1} CTAB (pH 3.4). Standards and samples were hydrodynamically injected by applying 25 mbar for 2 s. Separations were performed under negative polarity applying a constant voltage of −15 kV.

2.6. Multivariate study

The anionic profile of each wine, considering the concentration of inorganic anions and OAs, was used in the multivariate study. The concentration of each analyte determined by the optimized ME-C^4D method was considered as a specific variable. Sulfate, tartrate, maleate, succinate, acetate, lactate and phosphate were found in the wine samples. Zero was assigned to the variable when the analyte was not found in a specific wine. A matrix containing eighteen lines (samples) and seven variables (analytes concentration) was built to perform a principal component analysis (PCA) and no additional data preprocessing was employed. The calculations were performed on Origin® 2016 software (Massachusetts, USA). Biplot scores graph with loadings projected on it was used to discuss the samples similarities and variables importance.

3. Results and discussion

3.1. Optimization of the running buffer

Separation of anionic species was performed under negative polarity by using a running buffer composed of MES, His and CTAB. Composition of the running buffer constituents (MES and His) was varied to achieve the best separation conditions using a mixture containing 100 μmol L^{-1} of chloride, nitrate, sulfate, tartrate, maleate, succinate, 200 μmol L^{-1} of oxalate acetate, lactate, pyroglutamate, phosphate and 500 μmol L^{-1} of citrate.

First, MES concentration was ranged from 10 to 40 mmol L^{-1} (10 mmol L^{-1} increments) while His and CTAB concentrations were kept constant at 10 mmol L^{-1} and 0.05 mmol L^{-1}, respectively. The pH values of these buffer solutions varied between 5.4 and 6.1 due to the increase of MES concentration. As can be seen in Fig. 1A, the best separation condition enabled to identify all the analytes was obtained using a running buffer containing 20 mmol L^{-1} MES at pH 5.8.

After choosing the best buffer composition, considering the pH value, different MES and His proportions were evaluated by keeping the pH value constant at 5.8 and ranging IS from 5.2 to 10.2 mmol L^{-1}. For this, CTAB was also kept constant at 0.05 mmol L^{-1}. Electropherograms displayed in Fig. 1B demonstrated that the best separation and peak definition was achieved using a buffer composed by 30 mmol L^{-1} MES and 15 mmol L^{-1} His (pH 5.8 and IS 10.2 mmol L^{-1}).

Since CTAB was used as an anionic EOF modifier, its concentration was evaluated to properly reverse the EOF and to achieve the shortest analysis time. For this reason, MES and His concentrations were kept constant at 30 and 15 mmol L^{-1}, respectively, while CTAB concentration was ranged from 0 to 0.09 mmol L^{-1}. Based on the data displayed in Fig. 2, the buffer composed of 30 mmol L^{-1} MES, 15 mmol L^{-1} His and 0.05 mmol L^{-1} CTAB provided the best performance allowing the separation of twelve compounds, including inorganic anions and organic acids. It was not possible to observe all peaks in concentrations lower than 0.05 mmol L^{-1}, indicating that the EOF was not completely reversed. This can be confirmed by visualizing the electropherogram b in Fig. 2, since only the three inorganic anions (Cl^-, NO_3^- and SO_4^{2-}) with higher electrophoretic mobilities under negative polarity were identified. In addition, for concentrations higher than 0.05 mmol L^{-1} CTAB, the anionic species started to comigrate.

To improve the analytical response based on C^4D measurements, the operating frequency and the excitation voltage were optimized and the best response was achieved by applying a sinusoidal wave with 1200-kHz frequency with excitation voltage of 20 $V_{peak\text{-}to\text{-}peak}$ (data not shown).

Besides that, the potentials applied to control the fluidic transport in the gated injection mode were also optimized. For this purpose, the potential applied in the sample loading stage was kept constant at −1.2 kV, while the separation potential was ranged from −1.6 to −2.2 kV by −0.2 kV increments. Additionally, the potential applied in the sample loading stage was kept constant at −1.4 kV and the separation potential was varied from −1.8 to −2.4 kV by −0.2 kV increments. These results are displayed in Figure S1 (available in the supplementary material). The best results were observed applying potentials of −1.4 and −2.0 kV at the sample and buffer reservoirs, respectively. The optimized conditions enabled the rapid separation (within 100 s) of twelve organic acids and inorganic anions present in wines and, consequently, these conditions were kept constant for the subsequent experiments.

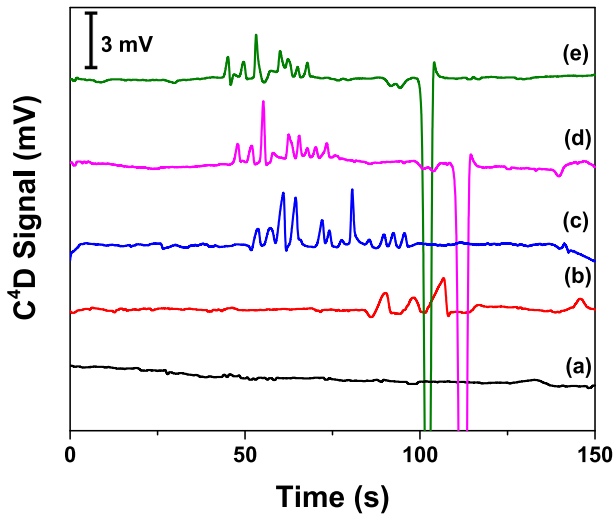

Fig. 2. Electropherograms showing the effect of the CTAB concentration on the separation performance. Running buffer: 30 mmol L^{-1} MES, 15 mmol L^{-1} His and CTAB at (a) 0, (b) 0.03, (c) 0.05, and (d) 0.07 and (d) 0.09 mmol L^{-1}. Other conditions: See Fig. 1.

3.2. Analytical performance

After optimizing operational and running conditions, the analytical performance was investigated aiming the quantitative determination of major organic acids and inorganic anions in wines.

To evaluate the separation parameters, three consecutive injections of a mixture containing chloride, nitrate, sulfate, tartrate, maleate, succinate (100 μmol L^{-1} each) plus oxalate, citrate, acetate, lactate, pyroglutamate and phosphate (200 μmol L^{-1} each) were performed. Based on these results (data not shown), acceptable separations were

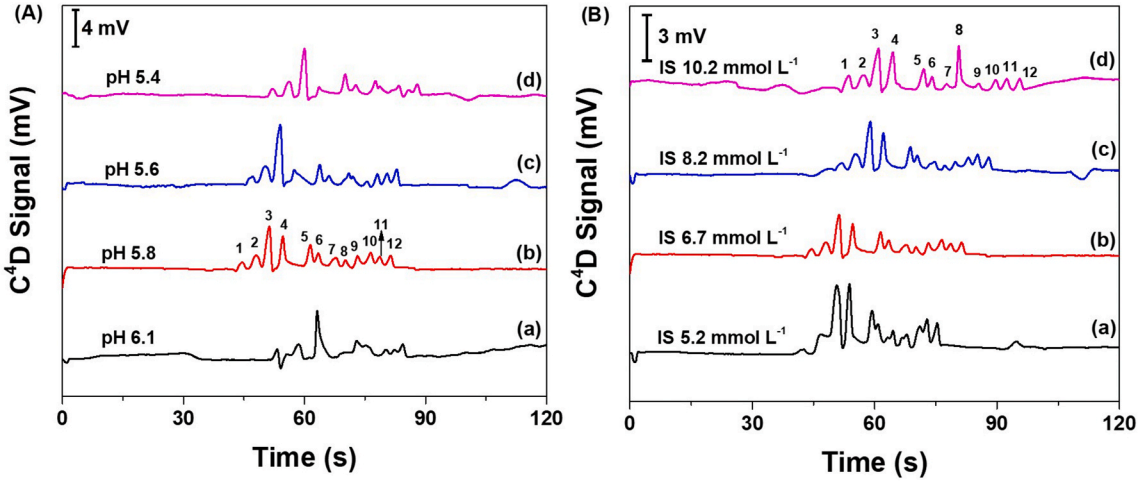

Fig. 1. Electropherograms showing the effect of (A) MES concentration in a running buffer composed of 10 mmol L^{-1} His, 0.05 mmol L^{-1} CTAB and MES at (a) 10, (b) 20, (c) 30, and (d) 40 mmol L^{-1}, and the effect of (B) IS in running buffer with pH approximately 5.8, composed of 0.05 mmol L^{-1} CTAB and (a) 15 mmol L^{-1} MES and 8 mmol L^{-1} His, (b) 20 mmol L^{-1} MES and 10 mmol L^{-1} His, (c) 25 mmol L^{-1} MES and 12 mmol L^{-1} His, and (d) 30 mmol L^{-1} MES and 15 mmol L^{-1} His. Electrokinetic control: voltages applied to sample and buffer reservoirs were −1.4 kV and −2.0 kV, respectively. Injection time: 1 s. Detection parameters: 700-kHz sinusoidal wave with 20 $V_{peak\text{-}to\text{-}peak}$ amplitude. Anions were tested at 100 μmol L^{-1} of (1) chloride, (2) nitrate, (3) sulfate, (5) tartrate, (6) maleate, (7) succinate, 200 μmol L^{-1} of (4) oxalate, (9) acetate, (10) lactate, (11) pyroglutamate, (12) phosphate and 500 μmol L^{-1} of (8) citrate.

achieved within 100 s with baseline resolution greater than 1. The achieved separation efficiencies ranged from 1.2×10^5 to 3.3×10^5 plates m^{-1}, which are comparable with other studies involving ME devices for food applications [39,40].

The linear range was investigated for sulfate, tartrate, maleate, succinate, acetate, lactate and phosphate. A linear behavior for all the species was observed with determination coefficients higher than 0.99 for the concentration ranges between 25 and 150 μmol L^{-1} for sulfate, tartrate, maleate and succinate, and between 100 and 250 μmol L^{-1} for acetate, lactate and phosphate. The analytical curves obtained are displayed in supplemental Figure S2. The limits of detection (LOD) were estimated based on the signal-to-noise ratio equal to 3 and the values found were 3.6, 4.4, 8.8, 3.0, 8.1, 8.9 and 12.6 μmol L^{-1} for sulfate, tartrate, maleate, succinate, acetate, lactate and phosphate, respectively. The LOD values obtained in this study were compared to other studies found in the literature for organic acids using ME-C^4D. The achieved LODs were lower than those reported by Law and colleagues [41] and similar to the values obtained by Kubáň and Hauser [33]. In addition, the calibration sensitivity of the developed methodology was extracted from calibration curves and the achieved values ranged from 4.1 to 31.5 (μV)/(μmol L^{-1}). The analytical parameters obtained for sulfate, tartrate, maleate, succinate, acetate, lactate and phosphate are displayed in supplemental Table S1.

In addition, a Cabernet Brazilian wine was chosen to extract information about the run-to-run repeatability. A sequence of six injections of the wine sample diluted 10 times in water was performed. Based on the typical electropherograms displayed in Fig. 3, it can be noted the presence of sulfate, tartrate, succinate, acetate, and lactate in the analyzed wine. The relative standard deviation (RSD) values for the migration times were lower than 2% and the RSD values for peak areas were between 2.5 and 8.4%. Also, the interday precision was evaluated. For this purpose, a sequence of three consecutive injections was performed during four days (non-consecutive days) and the resulting electropherograms are shown in Fig. 3. The interday precision was calculated based on the peak areas and migration times. While the peak areas integrated based on all recorded electropherograms varied between 8.2 and 15.3%, the RSD calculated for the migration times was lower than 3.2%, thus demonstrating great performance for the separation of

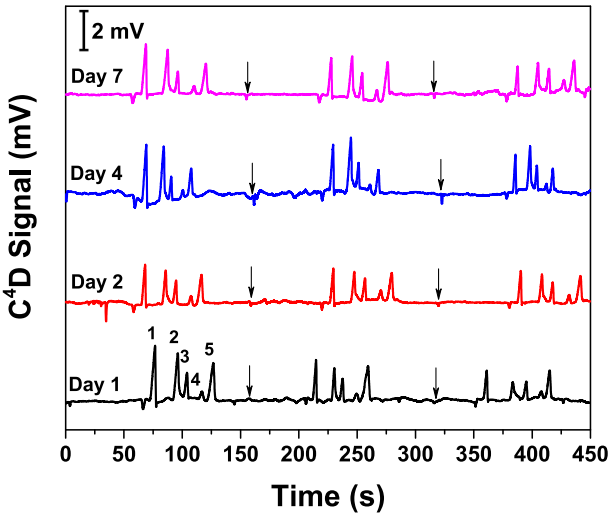

Fig. 3. Electropherograms showing the reproducibility of the detection of major organic acids and inorganic anions in a wine sample diluted 10 times during four days (n = 3). (1) Sulfate, (2) tartrate, (3) succinate, (4) acetate and (5) lactate. Running buffer: 30 mmol L^{-1} MES, 15 mmol L^{-1} HIS and 0.05 mmol L^{-1} CTAB. Electrokinetic control: voltages applied to S and B reservoirs were −1.4 kV and −2.0 kV, respectively. Injection time: 1 s. Detection parameters: 1200-kHz sinusoidal wave with 20 $V_{peak\text{-}to\text{-}peak}$ amplitude. The arrows mean the sample injection.

negative charged species, including organic acids and inorganic anions.

To further verify the reliability of ME based analysis, a comparison with CE was performed using a procedure reported by Vaz and coworkers [42]. The reliability was investigated using three wine types (rosé, red and white). The results achieved through ME-C^4D devices and CE-UV system are summarized in Table 1.

The results obtained using both methods were not significantly different at 95% confidence level and therefore, two methods are in good agreement. In this way, it can be inferred that ME-C^4D devices provided reliable results allowing their use for quantitative analysis of organic and inorganic compounds in wines.

The performance of the proposed method was compared to other publications related to OAs determination in wines (Table 2). It is important to note that the proposed approach based on ME-C^4D platform has provided faster analysis in comparison with conventional techniques including HPLC and CE as well as performance similar to other ME-C^4D devices. On the other hand, this current study is the only one using ME system with multivariate analysis, which successfully enabled the sample discrimination.

3.3. Wine analysis

The feasibility of the proposed methodology was evaluated through the analysis of wine samples to obtain their anionic profiles. For this purpose, all samples were diluted 10 times in water. As can be seen in Fig. 4, it was possible to detect sulfate, tartrate, maleate, succinate, acetate, lactate and phosphate in samples including Cabernet and Merlot red wines from Brazil and Chile, white and rosé wines from Chile.

The migration time for the analytes in wine samples was slightly longer than in standard solutions. This may be associated to the alcoholic content in the wine sample. This behavior was confirmed by adding ethanol in increasing concentrations (0, 5 and 10%) to the standard solutions of organic acids and inorganic anions, as displayed in supplemental Figure S3. The presence of organic solvents affects the viscosity of the solution, and consequently, the microchannel surface and the EOF mobility, providing higher separation efficiency [43,44]. For quantification, the wines were diluted in water in different proportions to estimate the concentration levels of anionic species. The achieved results are summarized in Table 3.

In addition to the good agreement with CE-UV, the accuracy of the analytical methodology was also evaluated based on recovery experiments performed with a red wine in three concentration levels (also in triplicates), and the achieved values ranged from 92 to 118% (supplemental Table S1).

Table 1
Comparison of concentration levels (in mmol L-1) of anions found in wines by ME-C4D and CE-UV (n = 3).

	RM6_CH		W3_CH		RS3_CH	
	ME	CE	ME	CE	ME	CE
Sulfate	5.2 ± 0.2	5.1 ± 0.4	4.4 ± 0.3	4.1 ± 0.0	3.6 ± 0.3	3.5 ± 0.1
Tartrate	13.8 ± 0.9	12.2 ± 0.7	19.5 ± 1.3	20.4 ± 0.5	15.4 ± 0.6	15.4 ± 1.2
Maleate	n.d.	n.d.	30.1 ± 1.7	27.7 ± 3.4	5.4 ± 0.2	5.2 ± 0.2
Succinate	8.7 ± 0.5	7.6 ± 0.5	2.9 ± 0.3	2.4 ± 0.2	4.2 ± 0.3	4.2 ± 0.1
Acetate	8.5 ± 0.3	8,2 ± 0.1	11.9 ± 1.0	11.2 ± 0.7	5.5 ± 0.3	5.7 ± 0.1
Lactate	10.5 ± 0.8	10.5 ± 0.1	n.d.	n.d.	3.2 ± 0.2	3.3 ± 0.3
Phosphate	8.2 ± 0.4	8.4 ± 0.2	5.2 ± 0.4	5.0 ± 0.3	3.5 ± 0.3	3.4 ± 0.1

n.d. = not detected.

Table 2
Analytical performance comparison of the proposed methodology with others studies found in the literature.

Analytes	Sample	Analytical Technique	Analysis time	PCA/ Classification	LODs (μmol L^{-1})	Ref.
Tartaric, malic, succinic, acetic and lactic acids	Wine	CE-UV	~6 min	No	0.1–0.9	[13]
Tartaric, malic, succinic, acetic and lactic acids	Wine	CE-UV	~8 min	No	29.7–407.8	[14]
Acetic, lactic, suberic, glutaric, succinic, malic, malonic, tartaric, oxalic, citric and *trans*-aconitic acids.	Wine, beer, and juice	CE-UV	~12 min	No	15.1–57.4	[20]
Tartaric, malic, succinic, acetic, lactic and citric acids	Wine	CE-UV	~3 min	No	0.4–4.8	[15]
Tartaric, malic, succinic, acetic, citric and lactic acids	Wine	CE-UV	~12 min	Yes	n.r.	[16]
Tartaric, malic, succinic, acetic, citric and lactic acids	Wine	CE-UV	~5 min	No	4.8–15.5	[17]
Oxalic, tartaric, formic, citric, malic, lactic, succinic and acetic acids	Wine and beer	CE-UV	~4 min	No	5.2–83.3	[19]
Lactic, succinic, malic, tartaric, shikimic, and citric acids	Wine	CE-MS	~4 min	No	n.r.	[10]
Acetic, lactic, oxalic, tartaric, malic, malonic, pyruvic, succinic, and citric acids, sulfate and phosphate	Wine	CE-CD	~200 s	Yes	1.6–5.7	[18]
Citric, tartaric, α-chetoglutaric, malic, pyruvic, succinic, fumaric, lactic and acetic acids	Wine and must	HPLC-UV-RI	~30 min	No	5.2–89.5	[21]
Acetic, lactic, tartaric, malic and cictric acids	Wine	HPLC-FTIR	~20 min	No	932.7–2237.1	[22]
Citric, tartaric, malic, lactic, acetic, caffeic, ellagic and gallic acids	Wine and must	HPLC-UV	~30 min	No	n.r.	[23]
Tartaric, malic, succinic, acetic, citric, oxalic and lactic acids	Wine and beer	HPLC-UV	10–20 min	No	n.r.	[24]
Tartaric, malic, citric, lactic, acetic, and succinic acids	Wine	HPLC-UV	~12 min	Yes	0.8–50.0	[25]
Tartaric, malic, citric, lactic, acetic, fumaric, gluconic and succinic acids	Wine and cava	HPLC-UV	~4 min	Yes	0.2–15.5	[26]
Tartaric, lactic, malic, malonic, gluconic, succinic, aspartic, ascorbic, sorbic and citric acids, sulfate, sulphite and phosphate	Wine	μITP-CD	10–15 min	No	n.r.	[31]
Tartaric, lactic, malic, succinic, acetic and citric acids	Wine	ME-CD	10–15 min	No	0.6–2.8	[32]
Tartaric, malic, succinic, acetic, citric, oxalic, formic, pyruvic and lactic acids, sulfate, chloride and phosphate	Wine	ME-C^4D	~100 s	No	2.2–33.3	[33]
Oxalic, tartaric, malic, succinic, citric, acetic, lactic and pyroglutamic acids, chloride, nitrate, sulfate and phosphate	Wine	ME-C^4D	~100 s	Yes	3.0–12.6	This study

n.r. = not reported.
CD = conductivity detector.
RI = refractive index detector.
FTIR = Fourier transform infrared spectroscopy.
μITP = isotachophoresis on chip.

3.4. Chemometric approach

Similarities among the anionic profile of wines considering the grapes varieties, the winemaking technique and the country of production (Brazil and Chile) were investigated by using a multivariate approach. PCA is a powerful tool to recognize patterns among samples when many variables are involved [45] and for this reason it was selected to analyze the data recorded by ME-C^4D measurements. A biplot scores graph for PC1 (34.85%) and PC2 (25.52%) containing all wine samples is shown in Fig. 5. The loadings plot (gray lines) was projected onto the same graph (right and top axis in gray color) to make easier the analysis of how variables influence the positioning of the samples. Although PC3 and PC4 presented 17.53 and 9.88% of the explained variance, respectively, the best samples grouping was observed by plotting PC1 versus PC2 (Fig. 5).

Besides samples had been coded, in Fig. 5, they are shown with the color in accordance with the grape variety. Red cabernet, red merlot, white Chardonnay (W) and rosé Syrah wine samples are presented in purple, red, yellow and pink colours, respectively. In general, wine samples were well grouped according to their grape varieties. Maleate was responsible to discriminate white and rosé wines of red wines (Cabernet or Merlot). Only white and rosé wines contain this specie, with exception of wine RC3_BR, where 14.0 mmol L^{-1} of maleate was detected. In addition, the maleate concentration in white wines were slight higher than the concentration found in rosé wines, allowing to observe a subtle difference in both grouping. Another target OA that differentiated red wines of white and rosé wines was the lactic acid (quantified as lactate), since its concentration was considerably higher in red wines, while in white and rosé wines it was absent (W3_CH and RS1_CH) or present in concentration lower than LODs.

Organic acid content in wines depends on many factors. First, it is due to grape maturity and variety. However, the winemaking technique is fundamental for determination of organic acid concentrations, since acetic, succinic, and lactic acids are mainly obtained during the fermentation processes. Temperature, pressing, adjusting initial acids concentration before fermentation are some parameters that can be controlled by winemakers. Besides that, the origin of the wine is a parameter that also affects organic acids concentration [25,46].

The difference of maleate and lactate contents in red and white wines may be explained through the malolactic fermentation, a standard procedure for red wines and not necessarily employed to all white and rosé wines. In this process, the sour-tasting malic acid, naturally found in grape must, is converted to a softer-tasting lactic acid when a lactic acid bacteria is used in a secondary fermentation [9,47].

Lactate and tartrate were the most important species to differentiate Merlot of Cabernet Sauvignon varieties, which is mainly reflected by PC2. For both grapes varieties, these species are present but for Merlot the lactate contents (above 110 mmol L^{-1}) were higher than for Cabernet Sauvignon. Only one Chilean Merlot sample presented a lower level of lactate, i.e., RM6_CH with 10.5 mmol L^{-1}. On the other hand, the higher lactate concentration (77.5 mmol L^{-1}) in RC1_BR make this Cabernet wine appears in positive scores of PC1, closer to Merlot wines. Cabernet Sauvignon wines are close to each other, which means that their anionic profiles are similar. RC4_CH appears slightly above since it presents the highest acetate concentration (41.2 mmol L^{-1}).

It is important to mention that the knowledge of the wines anionic profiles, including inorganic anions and OAs, allows to access several information about the quality of final product, such as the acidity, taste and aroma features [9,47,48]. Moreover, from the qualitative and quantitative OAs information in wines, it is possible to make inferences on previous fermentative processes. Lastly, by means a multivariate study, using the anionic profiles of the wines, it is possible to discriminate samples according to grapes varieties and their winemaking techniques.

4. Conclusions

The use of ME-C^4D devices demonstrated to be a powerful technique

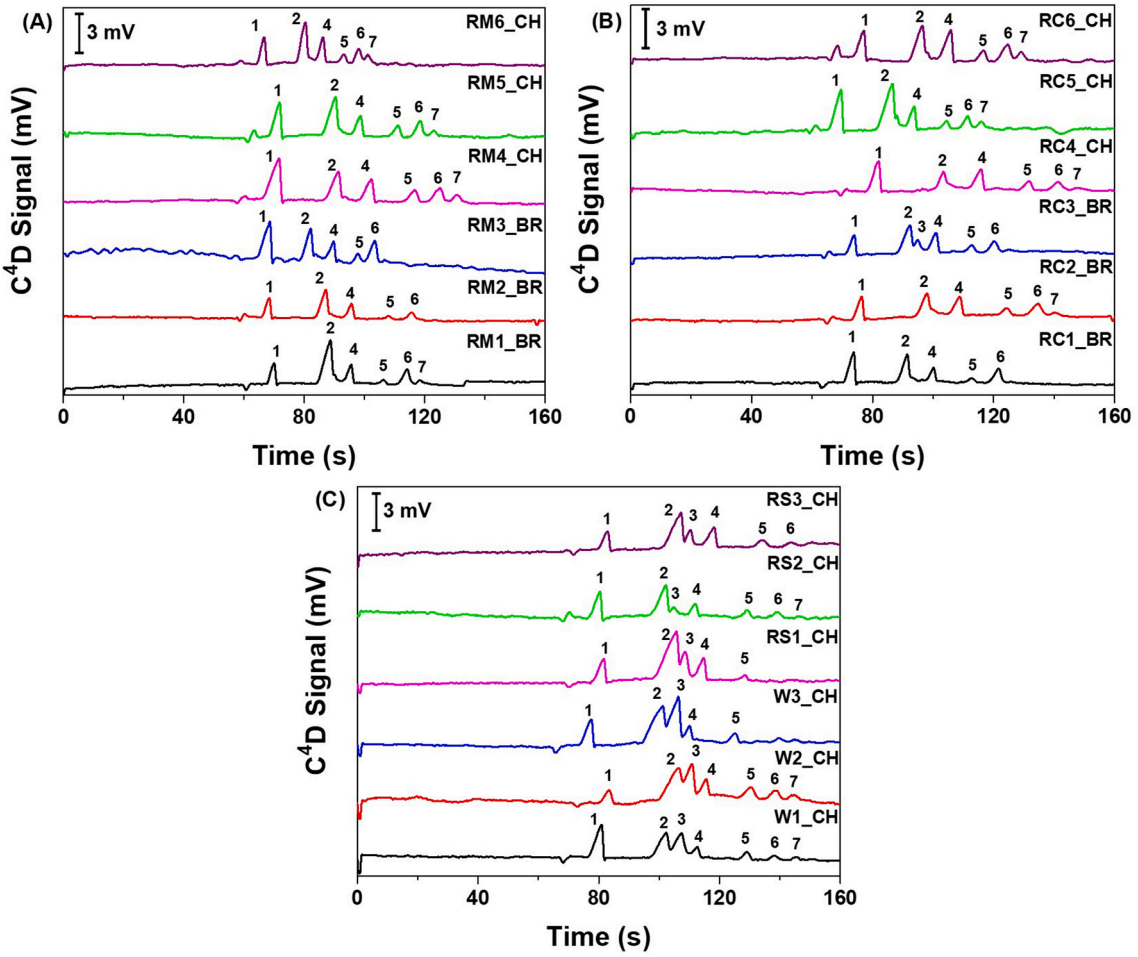

Fig. 4. Electropherograms showing the (A) red Merlot wines, (B) red Cabernet wines and (C) white and rosé wines analysis. (1) Sulfate, (2) tartrate, (3) maleate, (4) succinate, (5) acetate, (6) lactate and (7) phosphate. Other conditions: See Fig. 3. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Concentration levels (in mmol L-1) of the major anionic species in wines (n = 3).

Sample	Sulfate	Tartrate	Maleate	Succinate	Acetate	Lactate	Phosphate
RM1_BR	4.9 ± 0.6	123.3 ± 14.3	n.d.	14.8 ± 2.0	10.3 ± 0.4	141.6 ± 33.4	10.1 ± 1.0
RM2_BR	7.2 ± 1.0	24.9 ± 3.1	n.d.	12.0 ± 0.7	10.4 ± 2.1	112.7 ± 26.4	n.d.
RM3_BR	56.2 ± 4.6	49.6 ± 8.9	n.d	13.7 ± 1.2	11.2 ± 1.6	113.5 ± 8.8	n.d
RM4_CH	58.4 ± 2.2	14.6 ± 2.8	n.d.	58.8 ± 8.8	21.4 ± 2.8	118.5 ± 24.2	10.1 ± 0.4
RM5_CH	7.4 ± 1.4	33.5 ± 5.6	n.d.	11.9 ± 2.2	21.3 ± 1.3	117.0 ± 24.8	9.9 ± 0.8
RM6_CH	5.2 ± 0.2	13.8 ± 0.9	n.d.	8.7 ± 0.5	8.5 ± 0.3	10.5 ± 0.8	8.2 ± 0.4
RC1_BR	11.1 ± 1.0	36.8 ± 5.0	n.d.	22.1 ± 0.2	20.2 ± 4.4	77.5 ± 5.8	n.d.
RC2_BR	8.4 ± 1.2	20.8 ± 2.7	n.d.	21.7 ± 1.4	21.1 ± 3.9	24.3 ± 6.7	13.2 ± 1.4
RC3_BR	8.6 ± 0.9	28.9 ± 4.2	14.0 ± 1.8	29.5 ± 1.4	32.0 ± 0.8	48.5 ± 3.0	n.d.
RC4_CH	11.0 ± 0.9	29.3 ± 3.8	n.d.	28.0 ± 3.9	41.2 ± 7.5	42.6 ± 8.4	25.7 ± 4.8
RC5_CH	11.9 ± 1.4	33.1 ± 2.8	n.d.	15.2 ± 1.8	29.0 ± 4.1	36.6 ± 4.0	17.9 ± 3.0
RC6_CH	10.1 ± 1.2	28.3 ± 4.8	n.d.	17.0 ± 0.9	22.1 ± 3.0	38.5 ± 6.7	26.9 ± 4.0
W1_CH	10.0 ± 1.3	24.7 ± 1.1	25.1 ± 3.4	13.9 ± 2.4	29.6 ± 3.2	11.3 ± 1.9	15.1 ± 1.7
W2_CH	5.1 ± 0.4	25.3 ± 1.6	21.0 ± 3.0	6.9 ± 0.6	12.7 ± 0.7	11.8 ± 3.3	16.4 ± 2.4
W3_CH	4.4 ± 0.3	19.5 ± 1.3	30.1 ± 1.7	2.9 ± 0.3	11.9 ± 1.0	n.d.	5.2 ± 0.4
RS1_CH	8.9 ± 0.7	32.9 ± 3.5	17.2 ± 1.4	17.1 ± 0.5	10.2 ± 1.4	n.d.	n.d.
RS2_CH	14.4 ± 1.5	24.9 ± 4.3	6.0 ± 0.7	7.2 ± 1.1	15.6 ± 1.5	21.5 ± 4.2	10.5 ± 1.6
RS3_CH	3.6 ± 0.3	15.4 ± 0.6	5.4 ± 0.2	4.2 ± 0.3	5.5 ± 0.3	3.2 ± 0.2	3.5 ± 0.3

n.d. = not detected.

for fast and portable food analysis and quality control. The methodology provided the separation of 12 negatively charged species present in wines, including inorganic anions and OAs, within ca. 100 s. These anionic species are responsible for flavor, aroma, color and stability of wines. It was possible to detect and quantify sulfate, tartrate, maleate, succinate, acetate, lactate and phosphate in 18 samples. Anionic profile was used to discriminate samples by grapes varieties and by region of production, and their differences may be explained through fermentation processes.

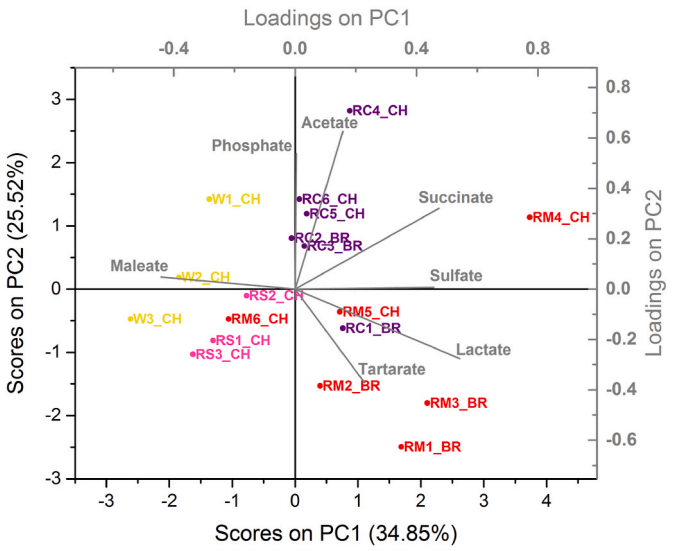

Fig. 5. Biplot scores graph with loadings projected to observe wine samples distribution according to their anionic profile (inorganic anions and OAs). Grapes varieties were: red Cabernet Sauvignon (RC, in purple), red Merlot (RM, in red), white Chardonnay (W) and rosé Syrah (RS). Brazilian (BR) and Chilean (CH) wines of different brands (1–6 for each grape variety) were considered. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.122381.

Credit author statement

Kemilly M. P. Pinheiro: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Validation; Visualization; Writing – original draft; Lucas M. Duarte: Conceptualization; Data curation; Formal analysis; Methodology; Software; Writing – review & editing. Gerson F. Duarte-Junior: Methodology; Formal analysis; Methodology; Writing – review & editing. Wendell K. T. Coltro: Resources; Funding acquisition; Project administration; Supervision; Writing – review & editing.

Novelty statement

Microchip electrophoresis devices have emerged as powerful platforms for rapid analysis using reduced amount of sample and when coupled to electrochemical systems including, for example, contactless conductivity detector, chip-based devices can be used as portable instruments for applications in different fields, like food science.

Wines are composed of a complex mixture of organic acids, carbohydrates, proteins, alcohol, minerals and polyphenols, of which organic acids are responsible for organoleptic properties, such as flavor, aroma, color and stability. For this reason, the development of new analytical methodologies based on emerging technologies may result in interesting tools for food quality control.

Glass electrophoresis microchip integrated with capacitively coupled contactless conductivity detection were employed for the rapid separation and determination of organic acids and inorganic anions in wine samples of different grape varieties. The optimized conditions ensured the simultaneous determination of chloride, nitrate, sulfate, oxalate, tartrate, maleate, succinate, citrate, acetate, lactate, pyroglutamate and phosphate within ca. 100 s with excellent analytical performance.

Based on the achieved data, a multivariate approach has successfully discriminated wine samples according to the grapes varieties, demonstrating to be useful for routine quality control of wines.

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