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# Fast determination of cations in honey by capillary electrophoresis: A possible method for geographic origin discrimination

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ABSTRACT

This study reports the development and validation of a fast capillary electrophoresis method for cation determination in honey samples and the classification of honey by geographical origin using Principal Components Analysis (PCA). The background electrolyte (BGE) was optimized using the Peakmaster® software, which evaluates the tendency of the analytes to undergo electromigration dispersion and the BGE buffer capacity and conductivity. The final BGE composition was defined as 30 mmol $L^{-1}$ imidazole, 300 mmol $L^{-1}$ acetic acid and 140 mmol $L^{-1}$ Lactic acid, at pH 3,0, and the separation of $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $Mn^{2+}$ using $Ba^{2+}$ as the internal standard was achieved in less than 2 min. The method showed satisfactory results in terms of linearity ($R^2 > 0.999$), the detection limits ranged from 0.27–3.17 mg $L^{-1}$ and the quantification limits ranged from 0.91–10.55 mg $L^{-1}$. Precision measurements within 0.55 and 4.64%RSD were achieved and recovery values for the analytes in the honey samples ranged from 93.6%–108.6%. Forty honey samples were analyzed to test the proposed method. These samples were dissolved in deionized water and filtered before injection. The CE-UV reliability in the cation analysis in the real sample was compared statistically with ICP-MS methodology. No significant differences were found, with a 95% confidence interval between the methodologies. The PCA showed that the cumulative variance for the first two principal components explain more than 85% of the variability of the data. The analytical data suggest a significant influence of the geographical origin on the mineral composition.



## 1. Introduction

Honey is a natural food produced by honeybees from the nectar of plants. It is an aqueous supersaturated sugar solution, that contains a complex mixture of other minor substances (minerals, proteins, vitamins, organic acids, flavonoids, phenolic acids, enzymes and other phytochemicals) [1]. Since the presence of different minerals originating from the soil as well as the climate characteristics determine the melliferous flora, honey composition is directly affected by the geographical area where the honey is produced and the plants that the bees visited [1,2].

The mineral content of honey is considered to be very low, ranging from 0.04–0.2% (w/w). This content is dependent on the type of soil in which the original nectar bearing plant was located [1]. The mineral content of the soil is incorporated into the plant and can be excreted through specialized glands present in leaves [3]. Therefore, the mineral profile gives an indication of the geographical origin of honey [1].

Mineral composition has also been employed to discriminate honey produced in different geographical areas. For instance, Terrab et al. [4] determined 24 minerals in Spanish honeys, reporting a relationship between the mineral content and the geographical origin. Baroni et al. [2] also confirmed the authenticity of honey produced in the Province of Córdoba (Argentina) by analyzing the elemental composition of honey samples combined with multivariate statistical techniques.

The mineral content of honey can be determined by different methods. Küçük et al. [5] and Baroni et al. [2] used flame atomic absorption spectrometry (FAAS). Several researchers have determined minerals in honey through inductively coupled plasma-optical emission spectrometry (ICP-OES) [4,6,7], while other authors have employed inductively coupled plasma-mass spectrometry (ICP-MS) [8,9]. In addition, total reflection X-ray spectrometry (TXRF) [10–12], ion chromatography, and voltammetry [13] have also been used. Occasionally, capillary electrophoresis (CE) has also been applied for determining cations in several foodstuffs [14,15].

In order to investigate the geographical influence on mineral honey content, numerous honey samples must be tested. Consequently, only fast, inexpensive and repetitive methods are appropriate

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for this kind of research. Capillary electrophoresis (CE) is a powerful separation technique, with many advantages compared to other analytical techniques. These advantages include the ultra-small sample volume, low consumption of solvents and chemicals, reduced cost and short analysis time while maintaining high-resolution separation [16,17]. Furthermore, cation analysis by CE does not require sample mineralization and/or digestion steps. In this context, the goal of this study was to design a rapid method for the determination of cations in honey samples, using a CE methodology. To this aim, we determined the main cations in honey samples produced throughout the State of Santa Catarina (Brazil), and by means of multivariate statistical methods we correlated the cation profiles with the geographical origin of the samples.

## 2. Experimental

### 2.1. Materials

Analytical standard grade $K^+$, $Ba^{2+}$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$ and imidazole were purchased from Sigma Aldrich (Santa Ana, CA, USA). Acetic acid, lactic acid and sodium hydroxide pellets were analytical reagent grade and were acquired from Merck (Rio de Janeiro, RJ, Brazil). The water was purified by deionization (Milli-Q system, Millipore, Bedford, MA, USA).

A stock solution (100 mmol $L^{-1}$ in ultra pure water) of each analyte was prepared daily, stored at 4 °C, and diluted with ultra pure water to give the concentration required for the CE experiments. In the indirect cation analysis an optimal background electrolyte (BGE) was used, composed of 30 mmol $L^{-1}$ imidazole, 300 mmol $L^{-1}$ acetic acid and 140 mmol $L^{-1}$ lactic acid, at pH 3.0.

Nitric acid (Merck, Darmstadt, Germany) was doubly distilled in a sub-boiling quartz distillation apparatus (Kürner Analysentechnik, Rosenheim, Germany). Hydrogen peroxide (Suprapur®, Merck) was used without purification. The concentrations of the standards used for the external calibration, prepared using stock solution Multi-element Calibration Standard 3 (PerkinElmer, Inc, Shelton, Ct, USA), ranged between 2.0 and 800 μg $L^{-1}$. Rh was chosen as an internal standard and added to a final concentration of 10 μg $L^{-1}$ in the standards, blank and sample solutions. Hydrogen peroxide (Suprapur®, Merck) was used without purification. The concentrations of the standards used for the external calibration, prepared using stock solution Multi-element Calibration Standard 3 (PerkinElmer, Inc, Shelton, Ct, USA), ranged between 2.0 and 800 μg $L^{-1}$. Rh was chosen as an internal standard and added to a final concentration of 10 μg $L^{-1}$ in the standards, blank and sample solutions.

### 2.2. Instrumentation

CE assays were conducted in a capillary electrophoresis system (model 7100, Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector (set at 215 nm; indirect detection, with a reference at 450 nm for peak inversion), a temperature-control device (maintained at 20 °C) and data acquisition and treatment software supplied by the manufacturer (HP ChemStation, rev. A.06.01). Uncoated fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with dimensions of 48.5 cm total length, 8.5 cm effective length, and 75 μm inner diameter were used. At the beginning of each day the capillary was conditioned by flushing with 1 mol $L^{-1}$ NaOH (10 min) followed by a 20 min flush with deionized water and the BGE solution (15 min). In between runs the capillary was reconditioned with the BGE solution (2 min flush). At the end of each working day the capillary was rinsed with 1 mol $L^{-1}$ NaOH (5 min) and water (10 min) and then dried in air (2 min). Standard solutions and samples were introduced at the extremity of the capillary nearest the detector and injected hydrodynamically (at 50 mbar for 3 s; 1 mbar = 100 Pa) with negative pressure. The applied separation voltage was 15 kV with positive polarity at the injection end.

In order to assess the accuracy of proposed procedure, the total concentration of K, Na, Ca, Mg and Mn was determined in honey samples using an ELAN 6000 Inductively Coupled Plasma Mass Spectrometer (Perkin-Elmer-Sciex, Thornhill, Ont., Canada), equipped with a pneumatic system. The instrument performance was assessed daily prior to its usage. Argon 99.996% (White Martins, SP, Brazil) was used. The instrumental parameters were as follows: RF power 1200 W; Sampler and skimmer cones Pt; scanning mode peak hopping; resolution 0.7 amu; readings per replicate 50; replicate 3; sweeps/reading 20; dwell time 50 ms; gas flow rates principal 15.0 L $min^{-1}$, intermediate 1.0 L $min^{-1}$ and nebulizer 1.07 L $min^{-1}$; internal standard 103Rh; isotopes 23Na, 24 Mg, 39 K, 43Ca,55Mn. Samples were digested using a MLS 1200 Mega microwave oven (Milestone, Sorisole, Italy).

### 2.3. Samples

The proposed method was applied to 40 honey samples, obtained from local producers, and their collection was organized through a state government research center called Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (EPAGRI). The honey samples were harvested in November and December 2010, from different locations across the state of Santa Catarina: Itaiópolis, Florianópolis, São Joaquim, Lauro Muller, Vidal Ramos, São Miguel do Oeste, Videira and Campos Novos (Table 1, Fig. 1) and stored at ambient temperature until the analysis.

Honey samples were accurately weighed (3.0 g), dissolved in deionized water in a 10 mL volumetric flask and the volume was properly completed. The honey sample solution was filtered through 0.45 μm membrane filters (Millipore, Bedford, MA, USA). An appropriate amount of the honey sample was placed in a CE vial and an aqueous solution of $Ba^{2+}$ was added as the internal standard (IS) to give a final concentration of 0.5 mmol $L^{-1}$of IS.

**Table 1**
Geographical origin of honey samples.

| Sample | City | Latitudes | Longitude | Altitude (m) |
|---|---|---|---|---|
| A ($n$=5) | Itaiópolis | 26° 20′24″ | 49° 53′ 4″ | 923 |
| B ($n$=5) | Florianópolis | 27° 35′ 49″ | 48° 32′ 56″ | 0 |
| C ($n$=5) | São Joaquim | 28° 26′ 13″ | 49° 95′ 6″ | 1.217 |
| D ($n$=5) | Lauro Muller | 28° 23′ 34″ | 49° 23′ 48″ | 220 |
| E ($n$=5) | Vidal Ramos | 27° 24′ 33″ | 49° 23′ 60″ | 661 |
| F ($n$=5) | São Miguel do Oeste | 26° 46′ 96″ | 53° 30′ 78″ | 606 |
| G ($n$=5) | Videira | 26° 58′ 40″ | 51° 11′ 35″ | 690 |
| H ($n$=5) | Campos Novos | 27° 22′ 59″ | 51° 13′ 16″ | 961 |


**Fig. 1.** Provenance of honey samples in Santa Catarina (Brazil). Cities: A—Itaiópolis; B—Florianópolis; C—São Joaquim; D—Lauro Muller; E—Vidal Ramos; F – São Miguel do Oeste; G—Videira; H—Campos Novos.

This solution was directly injected into the CE equipment with no other sample pretreatment.

Honey samples were digested prior to analysis ICP-MS. In brief, approximately 1 g of honey were weighed in PTFE flasks and 4 mL $HNO_3$ (65% v/v) and 2 mL $H_2O_2$ (30% v/v) were added. Samples were submitted to the following microwave program: 1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 400 W and 5 min at 650 W, followed by 5 min of ventilation. Deionized water was added up to the final volume of 20 mL. Before the analysis, the solutions were prepared in 1% (v/v) $HNO_3$ by proper dilution of the stock solution. Blank solutions were prepared in the same way.

### 2.4. Analytical performance

In order to verify the method performance, the following quality parameters were evaluated: linearity, detection and quantification limits, precision (instrumental, intra-day and inter-day) and recovery. Linearity was evaluated using several different concentrations of a mixture of cation standard solutions. Each calibration sample was injected in triplicate. Linear calibration curves were constructed from the peak area ratios (analyte/IS) versus analyte concentration for each compound. The limit of detection (LOD) and the limit of quantification (LOQ) were determined for the honey samples, calculated based on signal-to-noise ratios of 3:1 and 10:1, respectively.

The instrumental precision was calculated considering the %RSD of the peak area ratio (analyte area/IS area) for twenty consecutive injections of a standard solution at the same concentration. Intra-day precision was evaluated by way of three injections of two solutions at the same level of concentration, providing six replicates. To evaluate inter-day precision, eighteen replicate determinations of the same solution were performed on three consecutive days (six replicates on each day). Intra and inter-day precision was also expressed in terms of the %RSD, of the peak area ratio.

The recovery was studied by fortifying a honey sample at three concentration levels for each cation, with 3 injections at each level. Recoveries were calculated on the basis of the difference between the total amount determined in the spiked sample and the amount determined in the non-spiked sample, divided by the amount added, and was expressed as % recovery.

### 2.5. Statistical analysis

The statistical package STATISTICA 7.0 for Windows (Statsoft) was used for basic statistical and multivariate analysis (PCA). All analyses were carried out in triplicate and the data were expressed as mean $\pm$ standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's HSD test was used to compare the data obtained for honey samples from different geographical origins. Differences between the means at the 95% ($p<0.05$) confidence level were considered statistically significant. PCA was used to derive the first five principal components from the data. These were used to visualize the relative distribution of the honey samples according their geographical origin.

## 3. Results and discussion

### 3.1. Method development

Several characteristics were considered in the selection of an appropriate BGE solution. Firstly, the BGE solution needs to provide an acid medium with pH below 5.0, at which all analytes are fully dissociated. This is advantageous since it makes the method robust [18]. $Ba^{2+}$ was chosen as the internal standard because it was not present in honey samples at the detection limit of this method. The cations $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$ and $Mn^{2+}$ were identified by comparison of the relative peak migration times with the IS migration time.

Imidazole at 30 mmol $L^{-1}$ was selected as the co-ion, because it has a mobility very close to that of cations, which is of interest in terms of reducing the electromigration dispersion (EMD). The EMD phenomenon for strong electrolytes can be described by models that are principally based on the difference between the effective mobility of the analyte and that of its co-ion in the BGE, resulting in a decrease in the peak symmetries [19,20]. Moreover, since the cations do not absorb UV light, the imidazole also acts as a chromophore, enabling the indirect detection of the cations. However, imidazole has no buffering capacity in the pH range required; and thus, acetic acid ($pK_a$ 4.75) was selected as the counter-ion for pH adjust. The ideal concentration of sodium hydroxide was chosen with the assistance of Peakmaster® software [20–22], which allows the influence of pH on the EMD values, effective mobility curves, buffer capacity, and BGE conductivity to be determined. Fig. 2, which was constructed using Peakmaster® software, shows the effective mobility curves, EMD, buffer capacity and conductivity for a BGE composed of a constant value of 30 mmol/$L^{-1}$ of imidazole and variable acid acetic values of 30–400 mmol $L^{-1}$, which generated different pH values.

As observed in Fig. 2, to promote a satisfactory separation of $K^+$, $Na^+$, $Ca^{2+}$ and $Ba^{+2}$ (IS), the calculated pH of 3.7, corresponding to 300 mmol $L^{-1}$ of acetic acid, seems to be appropriate,. This is because at this pH, the differences between the effective mobility of these cations are sufficiently large to allow their separation. Moreover, at pH 3.7 the BGE buffer capacity is quite high, and the EMD values are low enough to guarantee symmetric peaks. However, as shown in Fig. 2(b), the effective mobility curves of $Mg^{2+}$ and $Mn^{2+}$ are almost overlapping and therefore these cations are not separated under these conditions. In order to solve this problem we added lactic acid to the BGE, which acts as a complexing agent, modifying the mobility of the cations through metallic complex formation. Since the complexation reaction occurs very quickly, the free cation and the complex remain in equilibrium. The two species arrive simultaneously at the detector, generating only one peak on the electropherogram [23]. The lactic acid was tested at 100–300 mmol $L^{-1}$ and 140 mmol $L^{-1}$ appeared to best promote a satisfactory separation of $Mg^{+2}$ and $Mn^{2+}$. The final composition of BGE was defined as 30 mmol $L^{-1}$ of imidazole, 300 mmol $L^{-1}$ of acetic acid, and 140 mmol $L^{-1}$ of lactic acid, at pH 3.0.

After the co-ion, counter-ion and complex agent selection, other CE parameters, including tension and capillary length were optimized using the Peakmaster® software. An experimental electropherogram of a mixture of cation standard solutions under the optimized conditions is shown in Fig. 3. It can be observed that the separation of the analytes was achieved in less than 2 min.

### 3.2. Analytical performance

Table 2 lists the results obtained for the quality parameters. The calibration curves showed good linearity, with correlation coefficients ($R^2$) being higher than 0.999 in all cases. LOD ranged from 0.27 mg $L^{-1}$ for $Mn^{2+}$ to 3.17 mg $L^{-1}$ for $K^+$ and the LOQ ranged from 0.91 mg $L^{-1}$ for $Mn^{2+}$ to 10.55 mg $L^{-1}$ for $K^+$. The relative standard deviation (RSD) of the instrumental precision was $\leq$4.64% for relative peak area. For intra-day and inter-day precision, the RSD values were $\leq$3.48% and $\leq$4.02% respectively. Precision did not exceed 5% in any case, demonstrating the acceptable repeatability and reproducibility of the proposed method. The accuracy of the cation analysis was determined

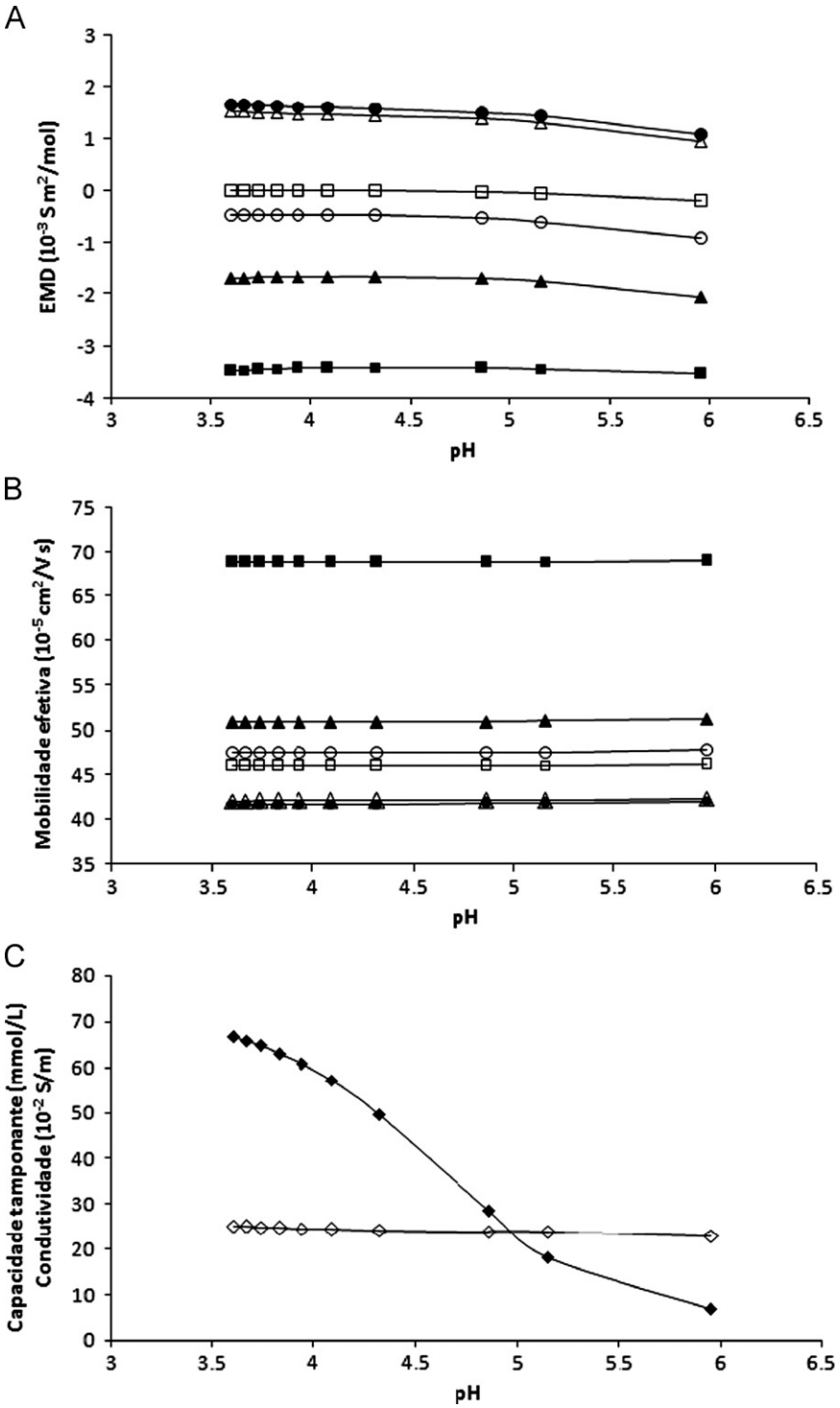


**Fig. 2.** Optimization of the BGE pH and composition using Peakmaster® software. Conditions: constant concentration of 30 mmol $L^{-1}$ imidazole and acetic acid varying from 30 to 400 mmol $L^{-1}$, which generates the pH values show in the figure (axis *x*). (a) EMD values versus pH curves for all analytes and IS, (b) versus pH curves for all analytes and IS, (c) BGE buffer capacity and conductivity versus pH curves. Legends: (■) potassium; (▲) barium (IS); (□) sodium; (○) calcium; (●) magnesium; (△) manganese; (◇) conducivity; (◆) buffer capacity.

using the recovery test. The average recoveries ranged from 93.6–108.6% for the three levels analyzed for each cation, with satisfactory RSD values.

### 3.3. Analysis of Honey samples

The results obtained in the univariate analysis of the cation concentrations in honey samples (mean, standard deviation, minimum and maximum values) are given in Table 3. The one-way ANOVA considering geographical origin as the main effect shows that statistically significant differences were found for all cations analyzed. This indicates that the location where honey is produced affects the mineral profile. Fig. 4 shows the typical electropherograms for sample B1 (Fig. 4a) and sample F3 (Fig. 4b). The observed difference in the peak areas illustrates the large variation in the cation content of these samples.

$K^+$ was the most abundant element in all samples analyzed, with values ranging from 507.77 to 1999.59 mg $kg^{-1}$. This is consistent with the findings of other authors who consider this mineral as the most quantitatively important in honey, accounted for around 50% of the total mineral content [2,6,8–10,12].

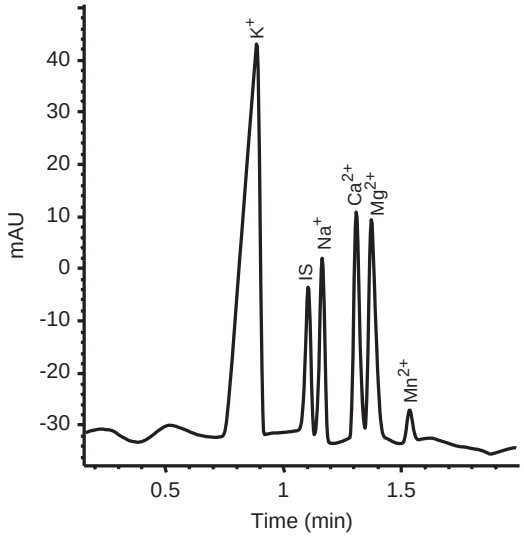


**Fig. 3.** Electropherogram of standard mixture of analyzed cations in honey samples. $Ba^{2+}$ was added as intern standard (IS). Separation conditions: 30 mmol $L^{-1}$ imidazole, 300 mmol $L^{-1}$ acetic acid and 140 mmol $L^{-1}$ lactic acid, at pH 3.0; injection at -50 mbar for 3 s; applied voltage of 15 kV; capillary 48.5 cm ($L_{tot}$) x 8.5 cm ($L_{det}$) x 75 μm (i.d.); 20 °C; indirect detection (imidazole) at 215 nm.

The second most abundant cation for all samples was $Ca^{2+}$, ranging from 32.18 to 165.01 mg $kg^{-1}$. These values are similar to those obtained by Pisani et al. [8], Kropf et al. [12] and Suarez-Luque et al. [14], for Italian, Slovenian and Spanish honeys, respectively.

The $Mg^{2+}$ and $Na^+$ concentrations were in the range of 8.44 to 68.03 mg $kg^{-1}$ and 5.91 to 64.17 mg $kg^{-1}$, respectively and the average contents of these elements in the honey samples varied according their geographical origin. In most of the honey samples analyzed (originating from regions A, B, C, F, G and H) the content of $Mg^{2+}$ was the third most abundant cation, while in honey samples from regions D and E, the third most abundant cation was $Na^+$. Fernández-Torres et al. [6] and Suarez-Luque et al. [14] found similar results, with contents of $Mg^{2+}$ and $Na^+$ varying among honey samples from different origins.

The concentration of $Mn^{+2}$ which was the lowest among the cations analyzed, ranged from 1.99 to 5.74 mg $kg^{-1}$. This was comparable to values recorded in other studies [6,8–10].

In order to show the CE-UV reliability in the cation analysis in a real sample, a comparison was performed using the ICP-MS methodology analysis. Thus, a paired-samples *t* test was carried out taking into account the five cations present in the sample: $K^+$, $Ba^{2+}$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$. The statistical results (for $n=3$) were *p*-value was higher than 0.05, no significant difference within the 95% confidence interval between CE-UV and ICP-MS methodologies was evidenced.

In order to establish differences between the geographical origin of honey samples, the multivariate technique of PCA was applied to the data.

**Table 2**
Analytical performance of the method.

| | Linearity | | | Precision (%RSD) | | | Recovery | |
|---|---|---|---|---|---|---|---|---|
| Cation | $R^2$ | LOD[a] | LOQ[a] | Instrumental | Intra-day | Inter-day | Rec. (%) | %RSD |
| $K^+$ | 0.9997 | 3.17 | 10.55 | 0.66 | 0.32 | 1.46 | 94.3–105.4 | 0.48–1.30 |
| $Ca^{2+}$ | 0.9998 | 1.17 | 3.91 | 0.57 | 0.55 | 0.91 | 94.3–108.4 | 0.25–0.87 |
| $Na^+$ | 0.9992 | 0.31 | 1.05 | 4.64 | 1.47 | 4.02 | 93.6–108.6 | 1.05–3.01 |
| $Mg^{2+}$ | 0.9996 | 0.42 | 1.42 | 0.82 | 0.73 | 0.95 | 95.0–107.5 | 0.43–1.65 |
| $Mn^{2+}$ | 0.9993 | 0.27 | 0.91 | 2.51 | 3.48 | 2.97 | 94.2–104.5 | 1.61–1.88 |

%RSD: relative standard deviation. Rec. %: recovery percentage.

[a] Values expressed as mg $L^{-1}$, determined in honey samples.

**Table 3**
Cation content (mg/kg) in honey samples analyzed.

| Geographical origin | | Cation (mg/kg) | | | | |
|---|---|---|---|---|---|---|
| | | $K^+$ | $Ca^{2+}$ | $Na^+$ | $Mg^{2+}$ | $Mn^{2+}$ |
| A | M ± SD | $625.0 \pm 73.6^{d}$ | $32.2 \pm 3.5^{b}$ | $5.9 \pm 2.8^{c}$ | $8.4 \pm 1.2^{d}$ | $2.0 \pm 0.9^{d}$ |
| | Range | 527.7–718.8 | 27.6–36.6 | 3.5–9.3 | 7.3–10.5 | 1.3–3.5 |
| B | M ± SD | $1999.6 \pm 129.8^{a}$ | $165.0 \pm 10.3^{a}$ | $64.2 \pm 5.6^{a}$ | $68.0 \pm 4.5^{a}$ | $4.4 \pm 0.5^{abc}$ |
| | Range | 1905.5–2221.1 | 157.2–182.8 | 58.9–72.3 | 65.0–75.9 | 3.73–4.81 |
| C | M ± SD | $1161.6 \pm 128.1^{b}$ | $154.5 \pm 60.5^{a}$ | $14.4 \pm 1.9^{b}$ | $45.9 \pm 22.4^{b}$ | $5.5 \pm 1.6^{ab}$ |
| | Range | 967.2–1289.7 | 93.2–229.8 | 12.7–17.5 | 25.4–74.0 | 3.9–8.2 |
| D | M ± SD | $894.3 \pm 110.1^{c}$ | $73.0 \pm 3.3^{b}$ | $55.8 \pm 5.4^{a}$ | $21.3 \pm 1.6^{cd}$ | $4.8 \pm 1.5^{ab}$ |
| | Range | 804.5–1040.6 | 67.9–75.9 | 49.8–62.0 | 18.9–22.7 | 4.0–7.6 |
| E | M ± SD | $586.8 \pm 17.4^{d}$ | $55.7 \pm 5.2^{b}$ | $15.2 \pm 4.8^{b}$ | $14.8 \pm 0.4^{cd}$ | $3.8 \pm 0.2^{bc}$ |
| | Range | 558.7–606.8 | 46.8–59.1 | 6.7–17.9 | 14.4–15.4 | 3.5–4.0 |
| F | M ± SD | $507.8 \pm 16.3^{d}$ | $49.2 \pm 2.6^{b}$ | $10.3 \pm 1.7^{bc}$ | $13.7 \pm 1.0^{d}$ | $3.1 \pm 0.2^{cd}$ |
| | Range | 481.1–525.2 | 45.5–52.1 | 8.1–12.6 | 12.1–14.5 | 2.8–3.4 |
| G | M ± SD | $1216.5 \pm 12.7^{b}$ | $75.1 \pm 1.9^{b}$ | $12.4 \pm 2.5^{bc}$ | $31.6 \pm 5.0^{bc}$ | $5.2 \pm 0.4^{ab}$ |
| | Range | 1205.1–1237.8 | 72.5–77.8 | 9.1–15.9 | 28.8–40.6 | 4.7–5.6 |
| H | M ± SD | $610.9 \pm 7.1^{d}$ | $59.1 \pm 1.5^{b}$ | $14.4 \pm 5.5^{b}$ | $22.9 \pm 0.4^{cd}$ | $5.7 \pm 0.3^{a}$ |
| | Range | 600.9–620.1 | 57.7–61.1 | 6.4–20.4 | 22.4–23.4 | 5.4–6.0 |

M ± SD: Mean ± standard deviation ($n=5$ for each region).

[a,b,c,d]Different letters in the same column indicate significant differences according to Tukey's test ($p < 0.05$).

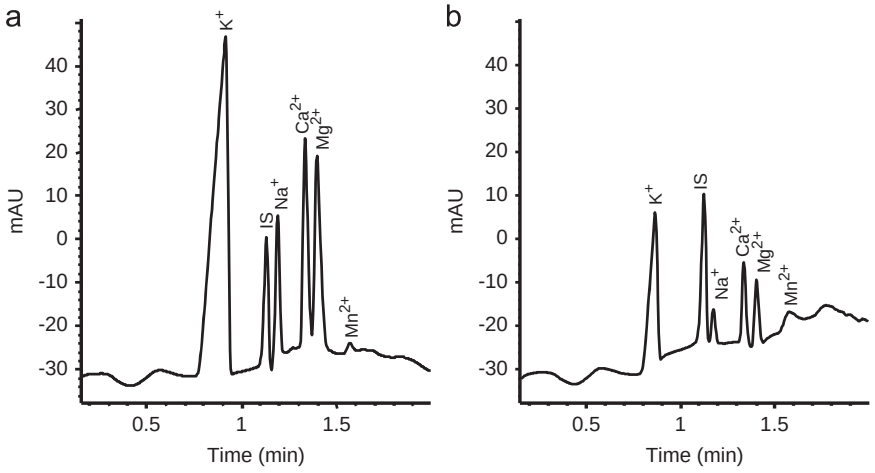


**Fig. 4.** Electropherogram of honey samples. (a) Sample B1. (b) Sample F3. $Ba^{2+}$ was added as intern standard (IS). Separation conditions: see Fig. 3.

**Table 4**
Loadings of the variables for the each principal component.

| Variable | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| $K^+$ | 0.934 | −0.192 | −0.044 | 0.293 | 0.048 |
| $Ca^{2+}$ | 0.928 | 0.025 | −0.295 | −0.210 | 0.084 |
| $Na^+$ | 0.716 | −0.317 | 0.613 | −0.102 | −0.006 |
| $Mg^{2+}$ | 0.962 | −0.027 | −0.240 | −0.023 | −0.124 |
| $Mn^{2+}$ | 0.484 | 0.846 | 0.221 | 0.034 | 0.002 |

### 3.4. Multivariate analysis

Principal component analysis (PCA) is a pattern recognition technique commonly used for the discrimination between honey samples of different origins [4,12,24]. PCA was used to search for data trends and to provide a partial view of the data in space with reduced number of dimensions, while preserving most of their variability. This method provides new variables as linear combinations of the original descriptor, which are called principal components (PCs) [9].

Table 4 shows the loading of the variables for the PCA. It can be observed that for PC1, $K^+$, $Ca^{2+}$, $Na^+$, $Mg^{2+}$ are the most important variables that explain the separation in the honey samples according to geographical origin, while in PC2 the most important variable is $Mn^{2+}$. The variables are displayed in Fig. 5(a). Since a consistent model requires a number of PCs so that over 75% of the total variation can be explained [9], the model obtained can be considered reliable. This reliability is explained by the first two PCs accounting for more than 85% of the variation in the honey samples analyzed.

Fig. 5(b) represents the graphic distribution of the honey samples according to their factor scores, and shows that the honey samples can be differentiated according to their geographical origin. Inspection of Fig. 3(b) revealed that the honeys were divided into separate groups that were associated with the geographical origin of the samples.

Samples from region B had the highest concentration of all analyzed elements, and this can be explained by the fact that these originated from an island, as shown in Fig. 1. In honey from coastal regions or islands, the sea is also a geogenic source of mineral salts as a consequence of marine aerosols [25]. Furthermore, the soils of coastal regions have a high saline content, so the nectar also incorporates more minerals from the soil [3]. These samples, which appear as an isolated group in the plot, are strongly positively correlated with PC1, and negatively correlated with PC2.

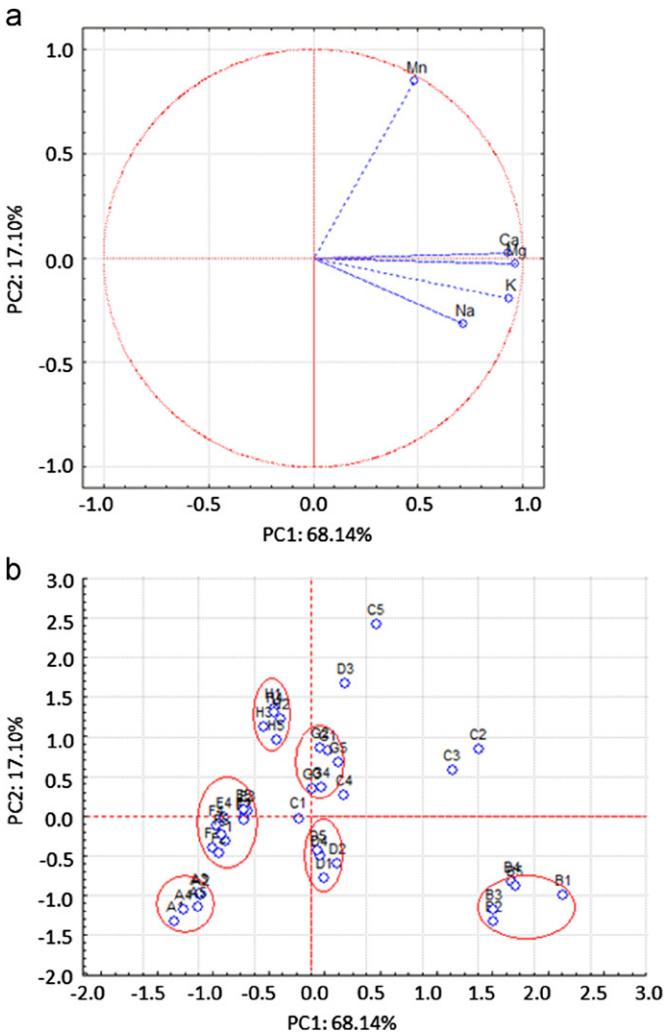


**Fig. 5.** (a) Loading plot showing first and second principal components for response values of the variables. (b) Score showing first and second principal components for the response values of honey samples. Geographical origin: A—Itaiópolis; B—Florianópolis; C—São Joaquim; D—Lauro Muller; E—Vidal Ramos; F—São Miguel do Oeste; G—Videira; H—Campos Novos ($n$=5 for each region).

The samples of regions A, E, F, and H had the lowest values for most of cations analyzed, and they lie within the left-hand side of the plot, having a negative correlation with PC1. These samples

can be differentiated according to $Mn^{2+}$ content: samples from region H are positively correlated with PC2 and had the highest $Mn^{2+}$ values, while samples from region A had the lowest $Mn^{2+}$ content and hence appear on the lower region of the plot.

Honeys from regions D and G had intermediate contents of cations; thus, they appear in the center of the plot. The $Na^{+}$ content, may differentiate these regions samples. Samples from region D have contents almost five times higher than those from region G, and therefore, the contents are negatively correlated with PC2. The samples from region C do not form a homogeneous group, due to the high standard deviation values for the mean content of $Ca^{2+}$, $Mg^{2+}$ and $Mn^{2+}$ observed for these samples.

## 4. Conclusions

An indirect method based on CE has been developed for the determination of cations in honey samples from different geographical origins within the State of Santa Catarina (southern Brazil). This technique allowed the separation and quantification of $K^{+}$, $Na^{+}$, $Ca^{2+}$, $Mg^{2+}$ and $Mn^{2+}$ cations in honey samples. The method was optimized through the simulation software Peakmaster®, and validated, with satisfactory results being obtained in terms of linearity, precision and accuracy. The LOD values ranged from 0.27 mg $L^{-1}$ for $Mn^{2+}$ to 3.17 mg $L^{-1}$ for $K^{+}$. The method was applied successfully to the analysis of five cations in fourteen honey samples in less than two minutes. Only a simple treatment of dilution and filtration of the honey samples was required. The results obtained showed that the cation content differed significantly ($p < 0.05$) among the honey samples from different geographical origins. Honey samples from coastal regions contain the highest quantities of cations, probably due to the influence of salinity from the sea. PCA analysis appears to represent a useful tool for the discrimination of honey samples from different geographical origins based on the cation contents.

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## References

[1] E. Anklam, Food Chem. 63 (1998) 549–562.
[2] M.V. Baroni, C. Arrua, M.L. Nores, P. Fayé, M.P. Díaz, G.A. Chiabrando, D.A. Wunderlin, Food Chem. 114 (2009) 727–733.
[3] J.M. Alvarez-Suarez, S. Tulipani, D. Díaz, Y. Estevez, S. Romandini, F. Giampieri, E. Damiani, P. Astolfi, S. Bompadre, M. Battino, Food Chem. Toxicol. 48 (2011) 2490–2499.
[4] A. Terrab, D. Hernanz, F.J. Heredia, J. Agric. Food Chem. 52 (2004) 3441–3445.
[5] M. Küçük, S. Kolayh, S. Karaoğlu, E. Ulusoy, C. Baltaci, F. Candan, Food Chem. 100 (2007) 526–534.
[6] R. Fernández-Torres, J. Pérez-Bernal, M.A. Bello-López, M. Callejón-Mochón, J.C. Jiménez-Sánchez, A. Guiraúm-Pérez, Talanta 65 (2005) 686–691.
[7] A. Terrab, A.F. Recamales, M.L. González-Miret, F.J. Heredia, Food Chem. 92 (2005) 305–309.
[8] A. Pisani, G. Protano, F. Riccobono, Food Chem. 107 (2008) 1553–1560.
[9] M. Chudzinska, D. Baralkiewicz, D. Food Chem Toxicol 48 (2010) 284–290.
[10] T. Golob, U. Doberšek, P. Kump, M. Nečemer, Food Chem. 91 (2005) 593–600.
[11] M. Nečemer, I.J. Košir, P. Kump, U. Kropf, M. Jamnik, J. Bertoncelj, N. Ogrinc, T. Golob, J. Agric. Food Chem. 57 (2009) 4409–4414.
[12] U. Kropf, M. Korošec, J. Bertoncelj, N. Ogrinc, M. Nečemer, P. Kump, T. Golob, Food Chem. 121 (2010) 839–846.
[13] P.L. Buldini, S. Cavalli, A. Mevoli, J.L. Sharma, Food Chem. 73 (2001) 487–495.
[14] S. Suárez-Luque, I. Mato, J.F. Huidobro, J. Simal-Lozano, J. Chromatogr. A 1083 (2005) 193–198.
[15] S. Suárez-Luque, I. Mato, J.F. Huidobro, J. Simal-Lozano, Talanta 68 (2006) 1143–1147.
[16] X. Cheng, S. Zhang, H. Zhang, Q. Wang, P. He, Y. Fang, Food Chem. 106 (2008) 830–835.
[17] V.M. Rizelio, L.V. Gonzaga, G.S.C. Borges, G.A. Micke, R. Fett, A.C.O. Costa, Food Chem. (2012)http://dx.doi.org/10.1016/j.foodchem.2011.11.058.
[18] M. Piovesan, A.C.O. Costa, A.V. Jager, M.A.L. Oliveira, G.A. Micke, Anal. Chim. Acta 673 (2010) 200–205.
[19] V.M. Rizelio, L. Tenfen, R. Silveira, L.V. Gonzaga, A.C.O. Costa, R. Fett, Talanta (2012)http://dx.doi.org/10.1016/j.talanta.2012.01.034.
[20] B. Gaš, P. Coufal, M. Jaroš, J. Muzikář, I. Jelínek, J. Chromatogr. A 905 (2001) 269–279.
[21] M. Štědrý, M. Jaroš, B. Gaš, J. Chromatogr. A 960 (2002) 187–198.
[22] M. Štědrý, M. Jaroš, K. Včeláková, B. Gaš, Electrophoresis 24 (2003) 536–547.
[23] A.V. Jager, M.F.M. Tavares, Química Nova 24 (2001) 363–373.
[24] E. Corbella, D. Cozzolino, Food Sci. Technol. 39 (2006) 534–539.
[25] P. Pohl, Trends Anal. Chem. 28 (2009) 117–128.