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ORIGINAL ARTICLE

Free fluoride determination in honey by ion-specific electrode potentiometry: Method assessment, validation and application to real unifloral samples

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Abstract Surprisingly, a reliable method for measuring the concentration of free fluoride ions in honey is still missing from the literature, notwithstanding the generally recognized importance of the analyte and the matrix. To fill this gap, this study proposes and validates a straightforward ion-specific electrode potentiometric method for this task. The method offers very low detection and quantification limits (6.7 μg kg^{-1} and 25 μg kg^{-1}, respectively), good linearity ($R^2 > 0.994$), good sensitivity (typically 55 ± 3 mV for an order of magnitude of concentration) in an unusually low concentration interval (between 0.020 and 1 mg L^{-1}), and acceptable precision and bias. The method was applied to 30 unifloral (thistle, eucalyptus and strawberry tree) honey samples from Sardinia, Italy. The amount of free fluoride ions found in these honeys appears to be lower than

Abbreviations: FISE, fluoride ion-selective electrode; TISAB, total ionic strength adjusting buffer; EDTA, ethylenediaminetetraacetic acid disodium salt; CDTA, 2-[2-[bis(2-hydroxy-2-oxoethyl)amino]cyclohexyl]-(2-hydroxy-2-oxoethyl)aminoethanoic acid; EC, external calibration method; MSA, multiple standard additions method; CRM, certified reference material; LOD, limit of detection; LOQ, limit of quantification

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the range usually found in the literature; indeed, early results suggest a possible dependence of the analyte concentration on the honey's botanical origin.

1. Introduction

Fluoride ion is ubiquitous in water, minerals, foods and tissues of animals and plants. It is included in the list of elements considered essentials for animal life, but, at concentrations slightly higher than those of physiological action, it is considered harmful to animals, humans, and plants. In particular, it is demonstrated as one of the most phytotoxic air pollutants (Fornasiero, 2001). The major natural sources of fluoride ion are airborne HF and other fluorine-containing species like SiF_4, present in volcanic activity, ocean spray, and dust from the weathering of fluoride-containing rocks or soils. Anthropogenic sources of gaseous and particulate fluorides are represented by the airborne emissions by industrial plants or by combustion processes, mainly from coal combustion.

Honey, the chief hive product, essentially consists of a supersaturated aqueous solution of sugars, mostly fructose and glucose, but its composition includes a series of minor organic and inorganic species. The variety and abundance of these compounds are often related to the botanical and geographical origins of honey, but, as in the case of inorganic components, sometimes they may also reflect the existence of an environmental issue in the production area (Bogdanov et al., 2007; Fermo et al., 2013; Lambert et al., 2012; Leita et al., 1996; Meyer et al., 1988; Przybyowski and Wilczynska, 2001; Rashed et al., 2009; Rashed and Soltan, 2004; Rodríguez Garcia et al., 2006; Tong et al., 1975; Yücel and Sultanoğlu, 2012).

In the past, scarce attention has been devoted to the determination of the fluoride ion in apiary productions. Early, Tong et al. (1975) evaluated the concentration of 47 elements (including fluorine) in 19 honey samples produced near highways, industrial and mining areas using spark source mass spectrometry methods. Later, Meyer et al., 1988 measured the amount of fluoride in a number of honey samples produced in three sites localized in the Puyallup Valley, Washington, USA, along a three-year period. Unfortunately, no detail concerning the nature of the potentiometric method was provided in the study. More recently, Rashed and Soltan (2004) evaluated the concentration of this analyte in three unifloral Egyptian honeys (sesame, orange and clover). The fluoride ion-selective electrode (FISE) measurement has been performed on a solution obtained by extracting the "dry sample" with 1 M $HClO_4$ and adjusting the pH to 5.2 with 1 M CH_3COONa. "Good precision" and recoveries between 93% and 103% were claimed by the authors. The fluoride level measured in these studies spanned over a quite wide range, between 300 and 12500 $\mu g\ kg^{-1}$ (Meyer et al., 1988; Tong et al., 1975; Rashed and Soltan, 2004) probably depending both on natural causes (e.g. different floral and geographical origin of the analyzed samples) and anthropogenic factors, like the presence of industrial activities in the proximity of the apiary (Tong et al., 1975). On the other hand, the adoption of analytical methods not completely validated for this matrix cannot exclude the possibility that literature data were bias affected.

Following these introductory considerations and pursuing our interest in the qualitative and quantitative determination of minority compounds in beehive products (Ciulu et al., 2011, 2013; Sanna et al., 2000; Spano et al., 2006, 2008, 2009a, 2009b), we assessed and validated a potentiometric FISE method to directly measure the free fluoride concentration in honey. The procedure was also successfully applied to a number of selected unifloral honeys produced in different parts of the island of Sardinia, Italy.

2. Experimental

2.1. Equipment and labware

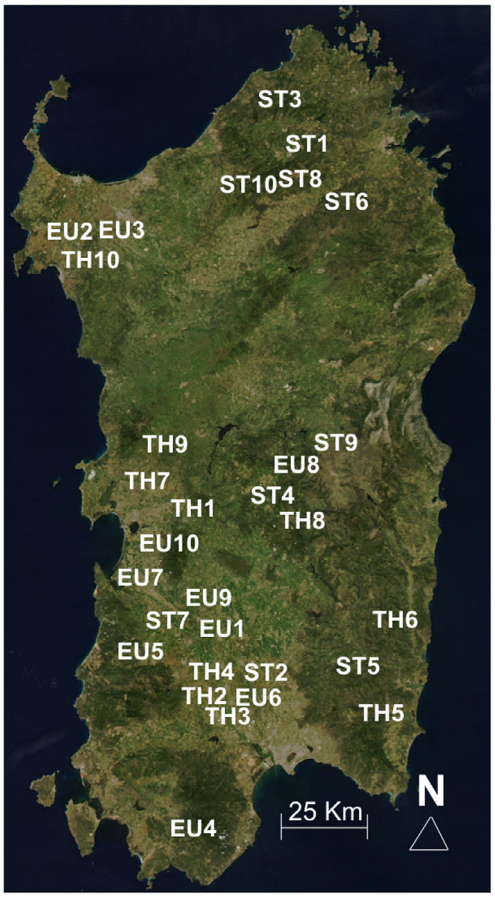
Potentiometric measurements were performed using a Fluoride Ion Selective Electrode (mod. DX219, Mettler Toledo, Switzerland) connected to an Ag/AgCl reference electrode (mod. 373/SSG/6 J, Amel s.r.l., Milan, Italy) and an ion analyzer (pH 1500 CyberScan, Eutech Instruments, the Netherlands). In addition, a combined glass-electrode (LIQ-GLASS 238000/08, Hamilton, Switzerland) was used with the ion analyzer to measure pH. Appropriate fixed volume Eppendorf Research Series 2100 pipettes were used. Everywhere possible, glassware was replaced with polyethylene labware.

2.2. Chemicals and reagents

All reagents were of analytical grade (Fluka, Milan, Italy), except NaF (99.99%, Sigma–Aldrich, Milan, Italy) and CH_3COOH (100% extra pure, Riedel-de Haën, Milan, Italy). Ultra-pure type I water (Merck, Milan, Italy) was used to prepare all the solutions. NaF was dried at 110 °C for two hours and cooled in desiccator before the preparation of 1000 mg L^{-1} F^- standard solution, which was then used to prepare diluted solutions. Total ionic strength adjusting buffer (TISAB) solution used in our study closely resembles that early proposed by Frant and Ross (1968), with the only difference being by the replacement of citric acid with EDTA. Hence, 58.0 g of NaCl, 37.0 g of NaOH, 57.0 mL of CH_3COOH were dissolved in 300 mL of 0.1 mol L^{-1} ethylenediaminetetraacetic acid disodium salt (EDTA). The pH was adjusted to 5.5 with 5.0 mol L^{-1} NaOH, then water was added to the solution up to a final volume of 1 L.

2.3. Samples

Thirty monofloral honey samples (thistle, *Carduus* L.; eucalyptus, *Eucalyptus camaldulensis;* strawberry tree, *Arbutus unedo* L.) were collected in different areas of Sardinia, Italy



Eucalyptus	City/town countryside
EU1	Samassi
EU2	Alghero
EU3	Alghero
EU4	Teulada
EU5	S.Gavino Monreale
EU6	Assemini
EU7	Arborea
EU8	Villanovatulo
EU9	Sardara
EU10	Palmas Arborea
Thistle	**City/town countryside**
TH1	Zerfaliu
TH2	San Sperate
TH3	Uta
TH4	Sardara
TH5	Castiadas
TH6	S. Vito
TH7	Bauladu
TH8	Mandas
TH9	Seneghe
TH10	Alghero
Strawberry tree	**City/town countryside**
ST1	Calangianus
ST2	Dolianova
ST3	Aglientu
ST4	Isili
ST5	Burcei
ST6	Monti
ST7	Gonnosfanadiga
ST8	Tempio
ST9	Sadali
ST10	Tempio

Figure 1 Geographical localization of the unifloral honey samples analyzed in the study. EU = Eucalypt honey, TH = Thistle honey, ST = Strawberry tree honey.

(Fig. 1), far at least 5 km in linear distance by every meaningful anthropic activity (e.g. cities and/or industries). They were directly provided by beekeepers. Qualitative melissopalynological analyses were performed following the method specified by the International Commission of Bee Botany (Louveaux et al., 1978) to verify the botanical origin of the honey samples. Finally, honey samples were stored in the dark at 3–4 °C until their analysis.

2.4. Sample preparation

Prior to analytical determination, each sample was first allowed to reach the room temperature and then homogenized for 15 min with an Ultra-turrax mixer mod.T18 (IKA, Staufen, Germany). An aliquot of honey, weighing 7.5000 g was dissolved in a 1:1 (v/v) TISAB: ultrapure water solution and diluted to a final volume of 10 mL with the same solvent. Each sample was analyzed at least in duplicate.

2.5. Electrodes cleaning

After each analytical session the fluoride ion selective electrode and the reference electrode were thoroughly cleaned with a cloth wetted with ultra-pure water.

3. Results and discussion

3.1. Method assessment

In spite of the importance of the fluoride ion in honey, until now only three studies (Meyer et al., 1988; Rashed and Soltan, 2004; Tong et al., 1975) have reported data on the concentrations of this analyte in this matrix. This lack of attention probably depends on the fact that fluoride ion is not routinely analyzed by the organizations responsible for food safety and health because of a number of analytical difficulties associated with its reliable quantification in foodstuffs (Rocha et al., 2013). Moreover, a careful analysis of the scarce literature concerning this aim allows us to conclude that a reliable and validated method devoted to the measure of the level of free fluoride ions in honey samples is at the moment absent.

Thus, in this work we present a new method for the determination of fluoride ions in honey based on direct potentiometry with FISE. In the past, this approach has been successfully used for the determination of fluoride ions in a number of foodstuffs (Kjellevold Malde et al., 2001; Oganessian et al., 2011; Ponikvar et al., 2007) since it provides some important advantages over other options, like the low cost of the apparatus, the simplicity of the analysis and its good sensitivity and

accuracy. One of the most significant steps involved in a reliable FISE method assessment is a proper choice of the TISAB solution, which should simultaneously fulfill different requirements, all aimed to maximize the amount of the free F^- ion (i.e., the only form of fluorine that can be detected by FISE). More specifically, the TISAB solution is simultaneously used as a pH buffer, an ionic strength buffer and a metal ion-complexing agent. The pH buffer choice should be a compromise between a value basic enough to completely deprotonate HF without increasing the interfering hydroxide ion concentration too much. Hence, a pH of the TISAB solution between 5.0 and 6.0 represented the best choice useful to prevent bias due to the competition effect by the OH^- on the acid-base equilibria of fluoride. In addition, substantial amounts of a neutral and strong electrolyte have to be added to optimize the background ionic strength needed to quickly reach the equilibrium potential and to have a constant activity. Finally, a specific chelating agent is often added to prevent the complexation of fluoride ion by interfering metal ions (e.g., Al^{3+} and Fe^{3+}). For these reasons, several different TISAB formulations have been proposed in the attempt to simultaneously address the various analytical complexities of each matrix.

Honey is an acidic foodstuff, and for this reason a TISAB characterized by a high buffering power is required. In addition, the literature data give account of uncontaminated honey samples characterized by low amounts of fluoride ion (less than 15 mg kg^{-1}, Bogdanov et al., 2008). Moreover, honey also contains a variable amount of different bivalent and trivalent metal ions that in principle can interfere with the accurate determination of fluoride (Sanna et al., 2000). Whereas the concentration of common trivalent ions appears to be quite low in honey (e.g., Fe^{3+} and Al^{3+} ions are usually less than 40 mg kg^{-1} and 24 mg kg^{-1}, respectively, Bogdanov et al., 2008), the amount of II group bivalent ions is not negligible (e.g., Ca^{2+} and Mg^{2+} ions can reach concentrations of several hundreds of mg kg^{-1}, Bogdanov et al., 2008).

In order to understand to what extent these metal ions can affect the measurements of free fluoride (the only species measurable by ISE) a speciation analysis was performed using the HYSS program (Alderighi et al., 1999). This program allows the calculation, as a function of pH and total concentrations of all the components, of the equilibrium concentrations of free and complexed species. The input data required by the program are: (1) the total concentrations of all ligands and metal ions; (2) the protonation constants of the ligands; (3) the formation constants of any possible complexes between metal(s) and ligand(s) in solution. The complex formation constants at 25 °C and 0.1 mol L^{-1} ionic strength, and the fluoride protonation constant were taken from IUPAC Database of Stability Constants (Pettit and Powell, 2001). The highest concentrations of interfering metal ions (transformed from mg kg^{-1} in mmol L^{-1} units according the procedure used for sample preparation) are reported in Table 1, second column.

In these conditions, the speciation plot in Fig. 2A is obtained.

The plot shows that the almost all fluoride is found, at the measurement pH 5.5, in complexed form, mainly as $[AlF]^{2+}$, $[AlF_2]^+$ and $[FeF]^{2+}$. The two trivalent hard metal ions, even if in lower concentrations than the bivalent calcium and magnesium, bind the hard fluoride making it unmeasurable by ISE. Actually, FISE measures only the free fluoride ion in solution. In absence of trivalent metal ions the calcium and magnesium interfere, masking about a 30% of the fluoride (Fig. 2B). The effects of adding ligands that form with the interfering metal ions more stable metal complexes stronger than F^- were also evaluated in the same conditions as in Fig. 2A. Fig. 2C and Fig. 2D show the effects of a 20 mmol L^{-1} concentration of EDTA and of another strong chelating agent like 2-[2-[bis(2-hydroxy-2-oxoethyl)amino]cyclohexyl]-(2-hydroxy-2-oxoethyl) aminoethanoic acid (CDTA), respectively. Although in these conditions the presence of an excess of any of these ligands is able to complex almost all the interfering metal ions, it is possible to observe that, at pH = 5.5, the pM values for Ca or Mg free ions (pM = −log[M], M = Ca^{2+}, Mg^{2+}) are higher when EDTA was used (pCa = 6; pMg = 4.3) rather than CDTA ligand (pCa = 4.8; pMg = 3.9). This implies that the conditional constants at pH = 5.5 for EDTA complexes with Ca^{2+} and Mg^{2+} must be higher than those by the complexes between CDTA and the same metal ions, and this allows to conclude that the use of EDTA at pH 5.5 is more effective than CDTA in maximizing the concentration of free fluoride ions. Given these results, and keeping into account also the easier commercial availability and the lower cost of EDTA than CDTA, we chose to use EDTA as a chelating agent in our TISAB formulation. The composition of the TISAB used in our study is completed by appropriate amounts of NaCl, NaOH and CH_3COOH to provide the needed pH and ionic strength buffer capabilities.

In order to determine the extent of a possible matrix effect, we performed a comparison between the external calibration method (EC) and the multiple standard additions method (MSA). The procedure adopted for the measurements using the EC method is as follows: a blank solution was prepared mixing 10 mL of ultra-pure water with 10 mL of the TISAB solution and the potential was measured; later, the fluoride concentration was gradually increased by six consecutive additions (0.08–1.40 mL) of a fluoride standard solution (5.0 mg L^{-1}), from 0.02 and 0.35 mg L^{-1}, and the resulting

Table 1 Concentrations (mmol L^{-1} units) of various ions used in speciation analysis.

Ion	Concentration Fig. 2A	Concentration Fig. 2B	Concentration Fig. 2C	Concentration Fig. 2D
F^-	0.59	0.59	0.59	0.59
Fe^{3+}	0.54	–	0.54	0.54
Al^{3+}	0.67	–	0.67	0.67
Ca^{2+}	5.6	5.6	5.6	5.6
Mg^{2+}	9.3	9.3	9.3	9.3
EDTA	–	–	20	–
CDTA	–	–	–	20

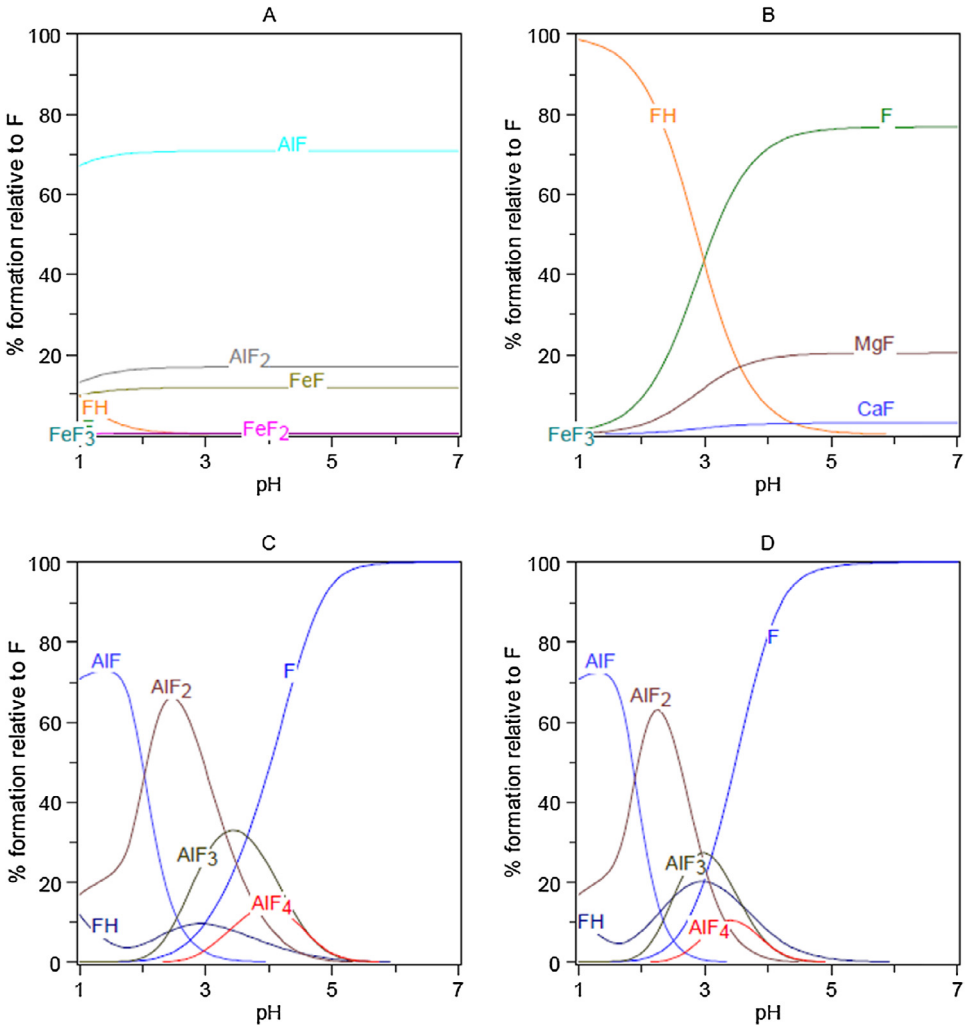


Figure 2 (A) Speciation plot of fluorides in presence of trivalent and bivalent metal ions; (B) speciation plot of fluorides in presence of bivalent metal ions; (C) speciation plot of fluorides in presence of trivalent, bivalent metal ions and EDTA 20 mmol L^{-1}; (D) speciation plot of fluorides in presence of trivalent, bivalent metal ions and CDTA 20 mmol L^{-1}.

potential was measured after each addition, under gentle and constant stirring until reaching the equilibrium value. A linear regression analysis of the resulting data provided the EC line used for the quantification. The fluoride concentration of the analytical samples, prepared as described in the Experimental section, was then evaluated in duplicate. The blank correction was performed according to Villa's procedure (Villa, 1988).

On the other hand, the procedure applied for the measurements using the MSA is as follows: the potential of each analytical sample, prepared as described in the Experimental section, was measured under gentle and constant stirring until reaching its equilibrium value. Afterward, six known amounts of analyte (containing 0.25, 0.50, 0.50, 1.00, 1.00 and 2.00 μg) from a standard fluoride solution were added, in the same conditions as described above, collecting the potential value after each addition. The Gran's-like linearization procedure provided the analytical concentration.

The data in Table 2 substantiate the general absence of any significant differences (criteria: two tail *t*-test, $p = 0.99$) between analytical results obtained with the two methods. Once the absence of a matrix effect was ascertained, we were fully justified in adopting the simpler and faster EC method throughout the whole study.

3.2. Validation

3.2.1. Limit of detection and limit of quantification

The limit of detection (LOD) was determined using the procedure described by the Laboratory Certification Program of the Wisconsin Department of Natural Resources (Wisconsin Department of Natural Resources, 1996). Ten aliquots of 4 mL, with fluoride concentrations ranging from 0.02 and 0.1 mg L^{-1}, were obtained from a honey solution prepared by dissolving a 30.0000 g of sample in 50.0 mL of a 1:1 solution of ultra-pure water:TISAB mixture. For each aliquot, the fluoride concentration was measured and the standard deviation was evaluated. The LOD and the limit of quantification (LOQ) are defined as LOD = $s{\cdot}t_{v,\alpha}$, and LOQ = 10 s, where s is the sample standard deviation, t is Student's t value for v degrees of freedom and α is the confidence level, equal to 99%.

Table 2 Comparison between fluoride amounts measured in selected unifloral honey samples using: (a) external calibration (EC) and (b) multiple standard additions (MSA) quantification methods.

Floral origin	EC $C_F^- \pm SD^*$ ($\mu g\ kg^{-1}$)	MSA $C_F^- \pm SD^*$ ($\mu g\ kg^{-1}$)
Eucalyptus	363 ± 2	370 ± 20
Thistle	28 ± 2	30 ± 3
Strawberry tree	117 ± 1	110 ± 10

* SD = standard deviation; $n = 2$.

Hence, the experimental LOD value was 6.7 $\mu g\ kg^{-1}$, while LOQ was 25 $\mu g\ kg^{-1}$.

3.2.2. Linearity and sensitivity

To verify the linearity range, calibration lines were obtained using the procedure previously described, at fluoride ion concentrations spanning from 0.020 to 1 mg L^{-1}. Considering the very low concentrations involved in this method, good determination coefficients (R^2) were observed, ranging from 0.994 to 0.999, whereas the sensitivity – measured as the slope of the calibration line – was typically of −55 ± 3 mV for decade of analyte concentration. Furthermore, the residual analysis can exclude the possibility of any deviation from linearity of the calibration plots in the concentration range examined.

3.2.3. Precision

The precision of the proposed method was evaluated in terms of repeatability and intermediate precision.

Repeatability (**r**) was evaluated from eight consecutive replications of the whole analytical procedure within the same analytical session, performed on different aliquots of the same sample of Eucalyptus honey (i.e., the sample in which the concentration of the analyte was found to be the closest to the average value of all samples analyzed). The precision value – expressed in terms of experimental variation coefficient of repeatability ($CV\%_{exp,r}$) – was 9.1%.

Furthermore, the intermediate precision (Magnusson and Örnemark, 2014) value (**IP**) was calculated as the experimental variation coefficient ($CV\%_{exp,IP}$) and was determined to be 12.7%. It was measured on analytical data obtained from six analyses of different aliquots of the same sample, performed over a number of analytical sessions within several weeks.

The acceptability of the repeatability data was verified through Horwitz's theory (Horwitz, 1982) and, more specifically, in terms of $HorRat_r$ ratio, calculated as follows:

$$HorRat_r = CV\%_{exp,r}/CV\%_{H,r}$$

where CV%H,r is the repeatability value predicted by Horwitz's theory as a function of the analyte concentration, expressed as a mass fraction (e.g., 1 mg $g^{-1} = 10^{-3}$). In this case the HorRatr value is 0.6, well below 1.5, which is the upper limit of acceptability of HorRat,r values.

3.2.3. Bias

Due to the fact that no certified reference material (CRM) is currently available and no reliable and independent analytical method has been previously described in literature for comparison with the proposed method, bias had to be estimated only by recovery tests, which were performed as follows. After homogenization, ca. 37.5 g (exactly weighted) of a honey sample were dissolved in 50.0 mL of a 1:1 solution of ultra-pure water:TISAB mixture. From this solution, four 10.0 mL aliquots were taken. The aliquots, 2, 3, and 4, were treated with an increasing volume of freshly prepared bulk solution containing 10 mg L^{-1} of fluoride ion, while no addition was made to aliquot 1. Each aliquot underwent the analytical procedure previously described and was analyzed in triplicate. The experimental concentration of the analyte measured in each aliquot was then plotted versus the q/m ratio, where q is the mass of added fluoride ion and m is the mass of honey in each aliquot. The average recovery (i.e., the percentage of the slope of the least squares regression line ($Y = aX + b$) obtained from this plot) was 110 ± 4%. The acceptability of this bias value was positively verified according to the guidelines described in the manual of the Association of Official Analytical Chemists (AOAC) for peer-verified methods (AOAC Peer-Verified Methods Program, 1998).

3.3. Free fluoride content of unifloral honey samples

The proposed method was tested on thirty Sardinian honey samples from three different botanical origins (thistle, eucalyptus, and strawberry tree) and collected in areas not interested by any meaningful anthropic activity. Table 3 shows the concentration of free fluoride ions for each sample and the typical range and the average concentration for each floral origin.

The comparison between our observations and literature data allows us to show that free fluoride concentration in Sardinian honey samples lies in the lower part of the concentration interval previously reported by other Authors (between 5000 and 12,500 $\mu g\ kg^{-1}$ in Egyptian honeys of three different botanical origins (Rashed and Soltan, 2004); between 300 and 1400 $\mu g\ kg^{-1}$ in honeys kept near an aluminum smelter in the State of Washington, (Meyer et al., 1988); between 1 and 8900 $\mu g\ kg^{-1}$ in nineteen samples from United States analyzed by Tong et al. (1975), collected in four different places around an aluminum plant in Puyallup Valley, USA). Regarding the samples analyzed in our tests, since they all came from areas of Sardinia not interested by any significant anthropogenic form of pollution, the wide variability of the measured amounts potentially reflects contributions from both their geographical and botanical origins (Rashed and Soltan, 2004).

A more detailed analysis of the data obtained in this study shows that the average concentration of free fluoride ions for each group of honey samples discriminates the botanical origin of the honey, whereas the range of the concentrations might be in principle associated to the different geographical origin of each sample analyzed. In particular, the average amount of free fluoride found in eucalyptus honeys was significantly higher than in thistle and strawberry tree honeys. On the other

Table 3 Fluoride amount, range and average concentration for thirty unifloral honey samples from Sardinia, Italy.

Floral origin Eucalyptus	C_F^- ± SD[a] (μg kg^{-1})	Floral origin Thistle	C_F^- ± SD[a] (μg kg^{-1})	Floral origin Strawberry tree	C_F^- ± SD[a] (μg kg^{-1})
EU1	159 ± 1	TH1	28 ± 2[c]	ST1	*25.8 ± 0.2*[c]
EU2	*47.4 ± 0.3*	TH2	64 ± 2[c]	ST2	*117 ± 1*[c]
EU3	78.7 ± 0.2[c]	TH3	*73 ± 2*[c]	ST3	36 ± 1
EU4	298 ± 9[c]	TH4	34.7 ± 0.1	ST4	46.7 ± 0.4
EU5	160 ± 10[c]	TH5	*< 25*[c]	ST5	109.2 ± 0.3
EU6	*363 ± 2*[c]	TH6	*< 25*	ST6	28 ± 1[c]
EU7	70 ± 2	TH7	*< 25*	ST7	69.4 ± 0.2
EU8	101 ± 2[c]	TH8	*< 25*[c]	ST8	34.9 ± 0.7[c]
EU9	113 ± 3[c]	TH9	*< 25*[c]	ST9	61.5 ± 0.9[c]
EU10	68 ± 2	TH10	*< 25*	ST10	43 ± 1
EU_{AVE}[b]	**146 ± 14**	**TH_{AVE}**[b]	**< 35 ± 2**	**ST_{AVE}**[b]	**57 ± 2**

$n = 3$

The highest and the lowest fluoride concentrations delimiting the range of each floral origin of honey samples appear in an *italic* typeface.

[a] SD = standard deviation.

[b] AVE = average value.

[c] $n = 2$.

hand thistle honey appears to show the lowest levels of free fluoride ions (six samples out of ten show analyte concentrations lower than the LOQ), whereas all the strawberry tree honey samples show levels that are quite low but still quantifiable.

The fact that element composition in honey can vary as a function of both the environmental conditions of the production site and its geographical location has been known for a long time (Anklam, 1999). However, more recently a number of studies have been published concerning the effective relationship between the elemental (and/or ionic) composition of honey and its botanical origin – often obtained through a chemometric analysis of the data (Camina et al., 2012; Chen et al., 2014; Chudzinska and Baralkiewicz, 2010, 2011; de Alda-Garcilope et al., 2012; Grembecka and Szefer, 2013; Lachman et al., 2007; Pisani et al., 2008; Necemer et al., 2009; Sahinler et al., 2009; Wang and Li, 2011; Yücel and Sultanoğlu, 2012 and 2013). To the best of our knowledge, no previous study has ever ascertained a possible contribution of fluoride ion to the definition of the botanical origin of the honey. However, it is evident that additional experimental work is still needed in order to confirm the possible relationship between the amount of this analyte in honey and its floral origin.

4. Conclusions

This study presents a direct and simple ISE potentiometric method for the determination of free fluoride ion in honey. Quantification was performed by external calibration, given the absence of any matrix interference – as shown by the comparison with analytical data obtained with a multiple additions method. The proposed method was successfully validated in terms of LOD, LOQ, linearity and sensitivity, precision and bias measurements, and making it fit for the purpose. The applicability of the method to real samples was verified by analyzing thirty unifloral (Thistle, Eucalyptus and Strawberry tree) honey samples from different regions of Sardinia, Italy. The amount of fluoride ion in Sardinian honeys appears to be low in comparison with the range defined by literature data. Among the honey samples analyzed in this work, the Eucalyptus honeys appear to be the richest in free fluoride ions. They presented an average concentration of 146 ± 3 μg kg^{-1}, which is higher than the average concentrations of both the Thistle honeys (⩽35 ± 3 μg kg^{-1}) and the Strawberry tree honeys (57.1 ± 0.7 μg kg^{-1}). The data presented in this study seem to suggest that the concentration of free fluoride ions could also play a role in identifying the botanical origin of honey, in addition to its geographical origin and the environmental state of the location. However, additional experimental work is needed to confirm these preliminary findings.

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