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Determination of Trigonelline in Seeds and Vegetable Oils by Capillary Electrophoresis as a Novel Marker for the Detection of Adulterations in Olive Oils

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A capillary electrophoresis method with UV detection was developed for the first time for the determination of the pyridine betaine trigonelline (*N*-methylnicotinic acid) in seeds and vegetable oils. Analytical characteristics of the method showed its good performance in terms of linearity ($r > 0.999$), precision (relative standard deviations < 5%), and limits of detection (up to 0.9 μM or 1 ng/g for oils). The developed method was applied to the analysis of soy and sunflower seeds, three varieties of olives, and sunflower, soy, and extra virgin olive oils. Trigonelline was determined in soy and sunflower seeds and their respective oils, whereas it was not detected in olives or olive oils. Different mixtures of extra virgin olive oil with seed oils were analyzed, detecting up to 10% of soy oil in olive oil. As a consequence, trigonelline is proposed in this work as a novel marker for the detection of adulterations of olive oils with other vegetable oils such as soy and sunflower oils.

KEYWORDS: Capillary electrophoresis; stacking; trigonelline; soy; sunflower; vegetable oils; olive oils

INTRODUCTION

Trigonelline (*N*-methylnicotinic acid, M_w = 137.14, CAS Registry No. 535-83-1) is an alkaloid belonging to the group of pyridine betaines possessing a quaternary amino group. Several health-promoting properties of trigonelline such as hypoglycemic, hypocholesterolemic, antitumor, antimigraine, or antiseptic effects have been reported (*1*). In addition, betaines are natural osmoregulators of many plants and are present in a high number of foods. Moreover, betaines have also been the subject of several investigations in view of their potentially positive biological effects in humans. In fact, they can act as osmolytes of kidney cells (*2*) and may have a role as a possible therapy in lowering the plasma concentration of homocysteine in homocystinuria (e.g., glycine betaine, proline betaine) (*3*, *4*).

Several methods have been reported for the determination of trigonelline, most of them employing a derivatization step. Thus, UV spectrophotometry (*5*), mass spectrometry (MS) (*6*), high-performance thin layer chromatography (HPTLC) with UV detection (*7*), high-performance liquid chromatography (HPLC) with UV detection (*8*−*18*) including methods with a previous derivatization with 2-naphthacyl trifluoromethane sulfonate (*12*, *15*) or phenylisothiocyanate (PITC) (*10*, *11*, *17*), HPLC coupled to MS (*19*, *20*), and capillary electrophoresis (CE) with UV detection and derivatization with *p*-bromophenacyl ester (*21*) have been employed. The described methods were applied both to plants (*7*, *9*, *19*, *21*) and to foods (*5*, *6*, *8*, *10*−*18*, *20*).

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In foods (as fruits, grains, vegetables, beverages, meat, seafood, or dairy products), trigonelline was found at low concentrations (e.g., chocolate) or it was not detected (e.g., olive oil sample), whereas it was found at high concentrations in coffee beans, lentils, and chickpeas (*12*, *15*). This was corroborated in other papers, where the content of trigonelline in edible lentil and garden pea seedlings was determined (*10*, *11*). What is more, in coffee beans, which present the highest content of trigonelline, it has been determined widely (*6*, *8*, *13*, *14*, *16*, *18*, *20*). Finally, trigonelline was also found in a considerable quantity in soy seeds and seedlings (*5*, *17*).

Although the presence of trigonelline in seeds was confirmed, there is no literature aimed to study the traceability of this compound from seeds to their oils. However, it is worth to noting that among oil minor components, peptides and proteins from seeds or fruits may be transferred to the edible oil together with fats (*22*).

The extra virgin olive oils are expensive oils that may become the object of adulterations for economical purposes. The most common method for adulteration is doping olive oils with cheaper oils such as seed oils, sunflower or soy oils being the most widely employed for this purpose. However, according to European regulations, the definition of virgin olive oils excludes "mixture with oils of other kinds" (*23*), and, on the other hand, at a national level (in Spain), it is specified that the mixture of olive oils with vegetable seed oils is forbidden (*24*). Thus, many powerful techniques for the detection of adulteration of virgin olive oil by edible oils have been described. They can be divided into "physical" and "chemical" methods. The first group is based on the total chemical makeup of the oil using spectrometric techniques such as fluorescence, nuclear magnetic resonance (NMR), Fourier transform Raman (FT-Raman), FT-infrared (FT-IR),

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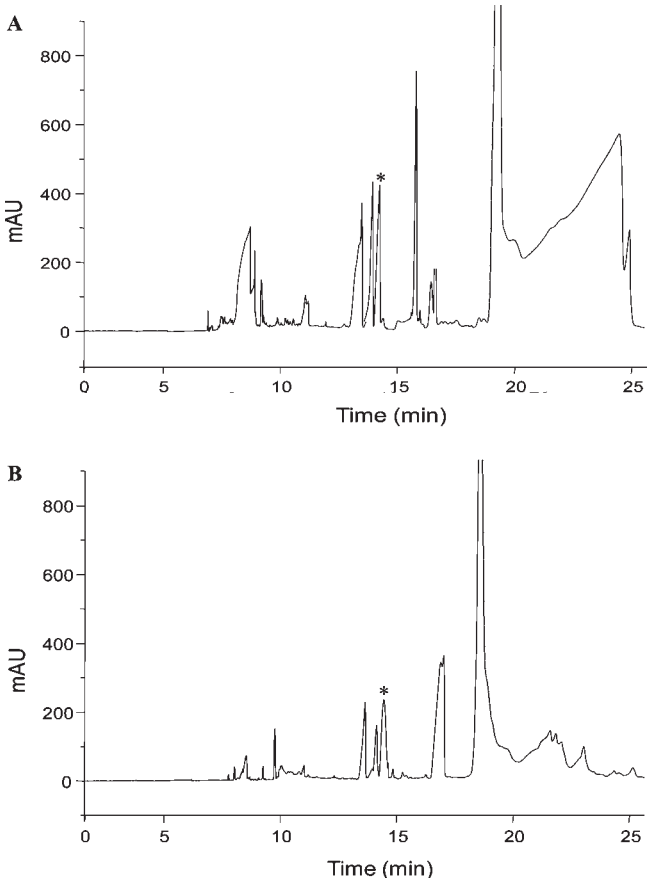

Figure 1. Electrophoretic profiles obtained for sunflower (**A**) and soy (**B**) seeds. Experimental conditions: BGE, 0.1 M formate at pH 2.0; uncoated fused-silica capillary, 75 cm (66.5 cm to the detection window) × 50 μm i. d.; temperature, 35 °C; applied voltage, 25 kV; injection by pressure at 50 mbar × 15 s. Samples were dissolved in acetonitrile/water (40:60, v/v). Asterisk marks trigonelline peak.

Raman, headspace-MS (HS-MS), or chemiluminiscence, among others (*25*). Most of these methods usually need sophisticated chemometric tools to carefully interpret the amount of accumulated data and to detect subtle differences that frequently exist between different oils. The second group of methods is based on the determination of particular chemical compounds in oils. They often use chromatographic techniques (such as HPLC, gas chromatography (GC), HPLC-MS, GC-MS, or CE), which provide useful information concerning authenticity and possible adulteration of oils (*26*). Until now, the saponifiable fraction (98.5−99.5% of the oil) has been the most studied part using fatty acids, triglycerides, sterols, tocopherols, or tocotrienols as markers of adulterations.

Capillary electrophoresis is a simple, rapid, and low-cost chromatographic technique. Moreover, it usually provides short separation times and high separation efficiency (*27*), and it has become one of the major choices for the separation of charged analytes and a solid alternative to other chromatographic techniques. To our knowledge, only one method by CE with a previous derivatization step has been described for the simultaneous determination of six betaines including trigonelline (*21*). However, only the presence of glycine betaine was detected when plant samples were analyzed. Therefore, the aims of this work were the following: (i) to develop for the first time a sensitive capillary zone electrophoresis−ultraviolet (CZE-UV) method without derivatization enabling the determination of trigonelline in seeds (such as soy and sunflower) and in their corresponding oils, (ii) to apply the developed method to investigate the presence of trigonelline in olives and olive oils, and (iii) to propose this compound as a novel marker for the detection of adulterations in olive oils.

MATERIALS AND METHODS

Chemicals and Samples. All reagents employed were of analytical grade. Methanol, chloroform, acetonitrile, *n*-propanol, isopropanol, and acetone were supplied from Scharlau Chemie (Barcelona, Spain). A 25% (v/v) ammonium hydroxide solution, sodium hydroxide, and hydrochloric acid were supplied from Merck (Darmstadt, Germany). Formic acid was from Riedel-de Häen (Seelze, Germany). Phosphoric acid was from Panreac Química S.A. (Barcelona, Spain). Trigonelline was from Sigma (St. Louis, MO). Distilled water was deionized by using a Milli-Q system (Millipore, Bedford, MA).

Arbequina, Picual, and Hojiblanca olives were taken from olive trees in Alcaudete (Jaén, Spain). Soy seeds, sunflower seeds, and virgin soy oil were purchased in a local herbalist (Madrid, Spain). Arbequina, Picual, and Hojiblanca extra virgin olive oils, refined sunflower oil, and refined soy oil were acquired in a local supermarket (Madrid, Spain).

Equipment. The analyses were carried out in a $HP^{3D}CE$ instrument (Agilent Technologies, Palo Alto, CA) equipped with an on-column diode array detector. Instrument control and data acquisition were performed with the $HP^{3D}CE$ ChemStation software. Separations were performed on an uncoated fused-silica capillary of 50 μm i.d. (375 μm o.d.), with a total length of 75 cm (66.5 cm to the detector) purchased from Polymicro (Phoenix, AZ). A pH-meter (Metrohm, Herisau, Switzerland) was used to adjust the pH of the running buffers.

Before first use, a new capillary was conditioned by rinsing at 1 bar with 1 M NaOH for 30 min and then with water for 5 min, followed with 0.1 M HCl for 3 min, and finally with buffer for 60 min. After each run, the capillary was rinsed at 1 bar for 2 min with buffer. Injections were made at the anodic end using a N_2 pressure of 50 mbar for 15 or 50 s depending on the sample. The electrophoretic separation was achieved with a voltage of 25 kV (normal polarity). The temperature of the capillary was kept constant at 35 °C, and UV detection was performed at 195 nm with a bandwidth of 5 nm.

Separation Buffers and Standard Solutions. The buffer solutions were (i) 0.1 mM phosphoric acid adjusted at pH 2.5 with 25% (v/v) ammonium hydroxide solution and (ii) formic acid at different concentrations adjusted with 25% (v/v) ammonium hydroxide solution to reach different pH values. All of these solutions were filtered prior to use through 0.45 μm pore size disposable nylon filters from Titan (Eatontown, NJ).

The stock standard solutions of trigonelline were prepared from a 10 mM solution and diluted, in the appropriate solvent, to the desired concentration. These solutions were stored at 4 °C and warmed at room temperature before use.

Sample Extraction. The extraction method of Koc et al. (*28*) was slightly modified in this work. Sample treatment was different for seeds/olives and vegetable oils. *Sunflower seeds, soy seeds, and olives* were ground in a domestic miller (Kenwood Iberica, Barcelona, Spain) at room temperature and weighed (10 g) in polypropylene copolymer tubes of 80 mL. The extraction was performed with 40 mL of methanol/chloroform (2:1, v/v), and the samples were left at −20 °C overnight after their agitation in a vortex. Then, their centrifugation for 15 min at 11000*g* and 4 °C was carried out in an ultracentrifuge (multifuge 3LR Heraeus, DJB Labcare, Buckinghamshire, U.K.). The supernatant was collected in a new tube, and the residue was washed with 25 mL of methanol/chloroform/water (2:1:0.8, v/v/v) and centrifuged (15 min at 11000*g* and 4 °C). The supernatant obtained was combined with the first one, and to the product solution were added 4 mL of chloroform and 10 mL of water. A last centrifugation (15 min at 11000*g* and 4 °C) produced two phases; the upper aqueous phase was separated and evaporated to dryness in a concentrator (Labconco, Barcelona, Spain) at 80 °C. The final residue was reconstituted in 300 μL of acetonitrile/water (40:60, v/v). *Vegetable oils and extra virgin olive oils* were directly weighed (40 g) in polypropylene copolymer tubes of 500 mL. They were extracted with 160 mL of methanol/chloroform (2:1, v/v), vortexed vigorously, and left at −20 °C overnight. Then, centrifugation for 15 min at 4000*g* and 4 °C was carried out in a centrifuge (Heraeus Instrument, Hanau, Germany). The upper phase was collected in a new tube, and the bottom phase was washed with 100 mL of methanol/chloroform/water (2:1:0.8, v/v/v) and centrifuged (15 min at 4000*g* and

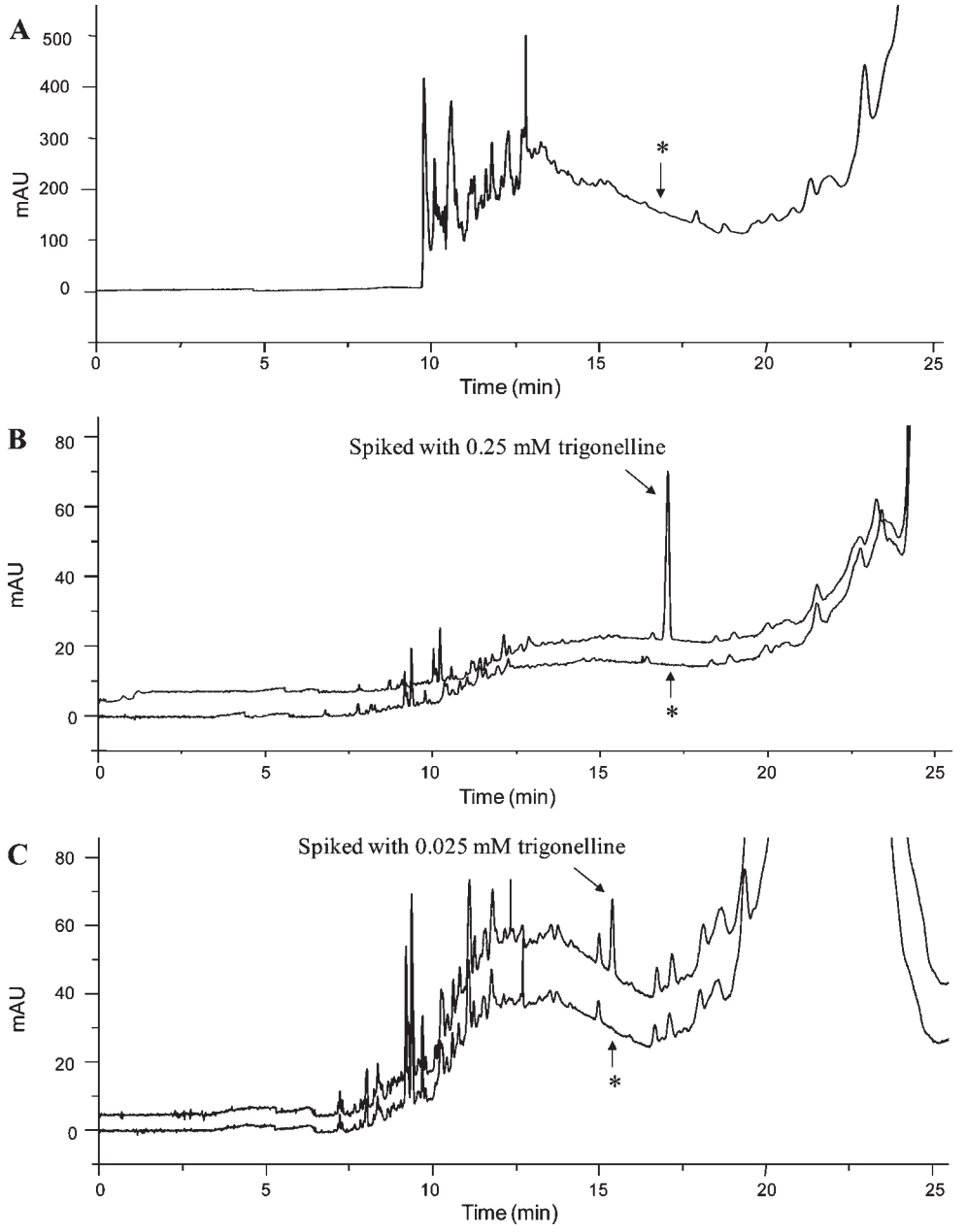

Figure 2. Electrophoretic profiles obtained for an olive sample (Picual variety): (**A**) undiluted sample injected by pressure at 50 mbar × 15 s; (**B**) 1/10 diluted sample in acetonitrile/water (40:60, v/v), nonspiked and spiked with 0.25 mM trigonelline standard, and injected by pressure at 50 mbar × 15 s; (**C**) 1/10 diluted sample in acetonitrile/water (40:60, v/v), nonspiked and spiked with 0.025 mM trigonelline standard, and injected by pressure at 50 mbar × 50 s. All other experimental conditions were as in **Figure 1**. Asterisk marks the position of the undetected trigonelline peak.

4 °C). The supernatant was mixed with the previous upper phase, and 100 mL of water and 40 mL of chloroform were added. The volume of these solvents was optimized because a higher proportion of water led to a cleaner aqueous phase. After other centrifugation (15 min at 4000*g* and 4 °C), the aqueous phase was taken and evaporated to dryness at 80 °C. The residue was reconstituted in 300 μL of acetonitrile/water (40:60, v/v).

The final solutions were stored frozen until analysis. They were thawed and centrifuged in Eppendorf vials in a minicentrifuge (Auxilab, Navarra, Spain) during 2 min at 1500*g* before injection in the CE system. In all cases, the samples were prepared in duplicate.

Plug Length Calculation. The injected lengths of the plugs were obtained from the Hagen−Poiseuille equation

$$l = \frac{t \times d^2 \times P}{32 \times L \times \eta}$$

where l is the length of the sample injection plug (mm), t is the time of duration of the pressure (s), d is the inner diameter of the capillary (mm), P is the injection pressure (Pa), L is the capillary length (mm), and η is the viscosity of the buffer (Pa s) (*29*).

RESULTS AND DISCUSSION

Development of an Analytical Method for the Determination of Trigonelline in Vegetable Samples. Due to the cationic nature of trigonelline, which possesses a permanent positive charge because of the presence of a quaternary ammonium group, acidic conditions were chosen to make negligible the electroosmotic flow and the interaction of this analyte with the capillary walls. Thus, two separation buffers at pH 2.5 (phosphate and formate at a concentracion of 0.1 M) were employed. Because formate buffer gave better peak symmetry, concentrations from 0.1 to 1 M and pH values from 2.0 to 3.0 were tested for this buffer. Formate at 0.1 M and pH 2.0 provided the shortest migration time and the best symmetry for trigonelline peak. Then, temperature values

Table 1. Characteristics of the CE Method Developed for the Determination of Trigonelline

time for hydrodynamic injection at 50 mbar	15 s	50 s
linear working concentration range[a]	0.025−1 mM (r > 0.999)	
LOD[b]	3.3 μM (0.46 mg/L)	0.9 μM (0.13 mg/L)
LOQ[b]	8.3 μM (1.15 mg/L)	3.0 μM (0.42 mg/L)
precision		
instrumental repeatability (n = 6)[c]		
Ac, RSD(%)		
soy	4.7	
sunflower	4.9	
refined sunflower oil		3.6
refined soy oil		3.9
virgin soy oil		4.1
t, RSD(%)		
soy	3.6	
sunflower	3.0	
refined sunflower oil		3.2
refined soy oil		4.3
virgin soy oil		2.2
intermediate precision (n = 9)[d]		
Ac, RSD(%)		4.2
t, RSD(%)		4.6

[a] Linear working concentration range studied included six concentration levels (0.025, 0.05, 0.1, 0.25, 0.5, and 1 mM). [b] The LOD (S/N = 3) and LOQ (S/N = 10) were determined by injecting trigonelline standard (5 μM). [c] Calculated from six consecutive injections in the same day for each sample. [d] Determined from three individual virgin soy oil samples injected five times in three consecutive days.

Table 2. Concentrations of Trigonelline Determined in Sunflower and Soy Seeds Analyzed in This Work (Experimental Conditions as in **Figure 1**)

assessment of matrix interferences[a]		correlation coefficient
slope by the external standard method	121.7 (±1.1)	0.9998
slope by the standard additions method in sunflower seed	76.3 (±8.2)	0.9923
slope by the standard additions method in soy seed	97.1 (±7.3)	0.9963
sample analyzed[b]		
sunflower seed	10.3 μg/g	
	15.8 μg/g	
soy seed	8.2 μg/g	
	8.1 μg/g	

[a] External standard method was evaluated with six standard solutions of trigonelline ranging from 0.025 to 1 mM. Standard addition method was achieved with four spiked levels of trigonelline (0.25, 0.5, 0.75, and 1 mM). Values in parentheses are confidence intervals at 95% for the slope. [b] Residue obtained from the extraction reconstituted in acetonitrile/water (40:60, v/v) and diluted 1/20 for sunflower and 1/5 for soy prior to quantitation to avoid distortions in the peak shape during the spiking due to its high concentration in the real sample. Samples were prepared in duplicate and injected three times.

from 15 to 35 °C and separation voltages from 15 to 25 kV were tested; 35 °C and 25 kV provided the baseline separation among trigonelline peak and the other sample peaks at the shortest migration time. In the case of the samples, trigonelline peak was identified on the basis of its characteristic UV spectrum and by spiking the sample with trigonelline standard after the extraction procedure. **Figure 1** shows the electropherograms obtained for samples of sunflower seeds (**Figure 1A**) and soy seeds (**Figure 1B**) at 15 s injections. Among the different absorption maxima of trigonelline, it was shown that at the highest wavelengths the selectivity increased but the sensitivity decreased significantly. Thus, peak area and peak height at 264 nm were about 8 times lower than at 195 nm. Therefore, the latter wavelength was selected to obtain the highest detection sensitivity.

When olive samples were analyzed under the above-mentioned conditions, a trigonelline peak was not detected in any of the three varieties of investigated olive samples. As shown in **Figure 2A**,**B**, trigonelline was not detected either in the undiluted or the diluted sample. To increase the detection sensitivity, different sample injection strategies based on in-capillary sample preconcentration approaches were employed for trigonelline standard. First, a *normal stacking mode* (*NSM*) was used. Hydrodynamic injections from 15 s (plug length about 1% of the total capillary length) to 200 s (plug length about 14% of the total capillary length) were tested. Different sample solvents (water, 10 mM formic acid, methanol, isopropanol, *n*-propanol, and acetonitrile) and different mixtures of them were investigated for trigonelline standard. The highest preconcentration factor (up to 30-fold for 200 s) was obtained for acetonitrile/water (80:20, v/v). On the other hand, a *field amplified sample stacking* (*FASS*) was tested using the same sample solvent (acetonitrile/water, 80:20, v/v), which was also selected to inject a plug (50 mbar for 4s) prior to electrokinetic injection at 10 kV from 15 to 200 s. Because preconcentration factors obtained by FASS were generally lower than those obtained by NSM for the same injection times (only 16-fold for 200 s) and the sample injection was less reproducible, NSM was chosen to be applied to the analysis of olive samples.

When samples of the three varieties of olives were analyzed with acetonitrile/water (80:20, v/v), a partial solubilization of samples was observed, which was attributed to the presence of other polar matrix components extracted with trigonelline. Thus, a decrease in the acetonitrile proportion was required, obtaining the best peak intensity for acetonitrile/water at 40:60 (v/v) as sample solvent. In addition, it was only possible to apply an injection of 50 s (plug length about 3.5% of the total capillary length) to achieve the baseline resolution between trigonelline peak and matrix peaks. Despite the improvement in sensitivity achieved with the NSM method, trigonelline was not detected in any of the three varieties of olive samples analyzed (see **Figure 2C**) so it could be concluded that trigonelline was not present in olive samples at least at concentrations above the limit of detection of the developed method.

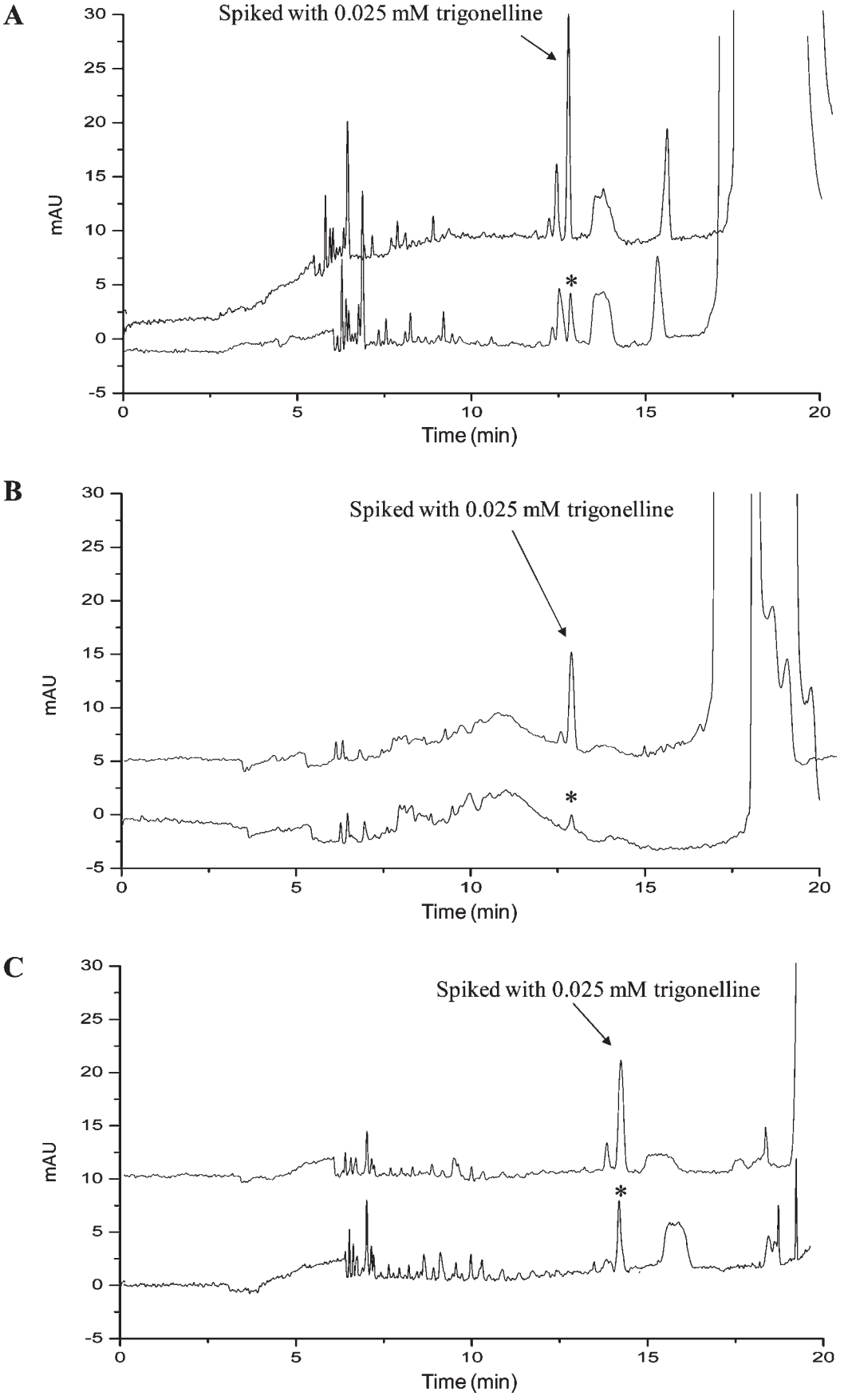

Figure 3. Electrophoretic profiles obtained for sunflower and soy oils, nonspiked and spiked samples with 0.025 mM trigonelline standard: (**A**) virgin soy oil; (**B**) refined soy oil; (**C**) refined sunflower oil. Experimental conditions: injection by pressure at 50 mbar × 50 s and all other experimental conditions as in **Figure 1**. Asterisk marks the position of the trigonelline peak.

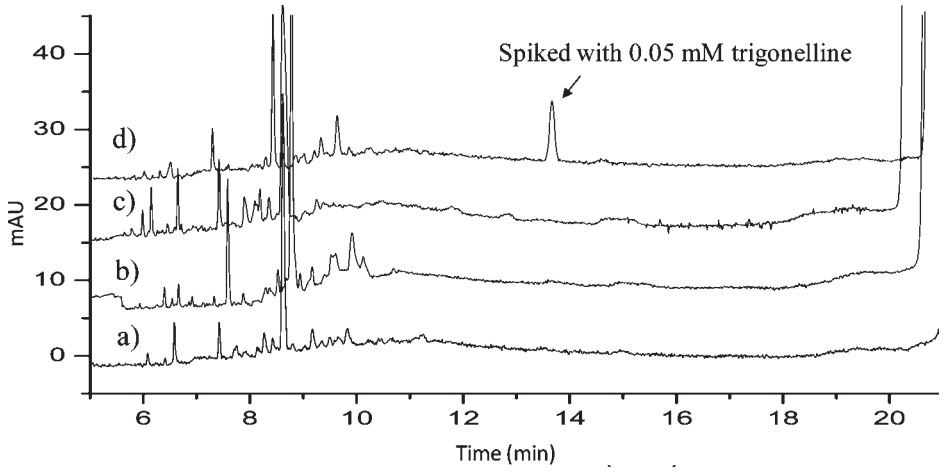

Figure 4. Electrophoretic profiles obtained for olive oils: (a) Picual, (b) Hojiblanca, (c) Arbequina, and (d) Hojiblanca olive oil spiked with 0.05 mM trigonelline standard. All other experimental conditions were as in **Figure 3**.

Using these final optimized conditions, some analytical characteristics of the method were evaluated prior to its application to the determination of trigonelline in sunflower and soy seeds as well as to the investigation of its presence in oil samples. **Table 1** groups the linear working concentration range studied, the limits of detection (LOD) and quantitation (LOQ) for trigonelline, and the precision of the developed CE method. A linear response was obtained in the linear working concentration range comprised from 0.025 to 1 mM. Appropriate precision (RSD < 5%) in migration times (t) and corrected peak areas (Ac, relationship among peak area and peak migration time) was obtained for real samples. Similar results on precision were reported (RSD values from 1 to 5%) when trigonelline was determined both in plants and in foods using the methods cited in the Introduction. The LOD for trigonelline was about 0.9 μM, that is, 0.13 mg/L. This instrumental LOD corresponded to 14 ng/g for seeds (sunflower and soy), 4 ng/g for olives, and 1 ng/g for oils of trigonelline in the samples analyzed. These values are among the lowest LODs reported for trigonelline using UV detection (ranging from 4.5 to 1000 ng/g). However, one method with MS detection allowed instrumental LODs 30 times lower (3×10^{-8} M) to be obtained.

Quantitation of Trigonelline in Sunflower and Soy Seeds. The optimized method was applied to quantify trigonelline in soy and sunflower seeds. As shown in **Figure 1**, trigonelline was identified in soy and sunflower seeds. Although the presence of trigonelline in soy seeds had previously been reported (ranging from 40 to 240 μg/g referred to dry mass) (*20*, *21*), the presence of this betaine in sunflower seeds has not been reported to date. Thus, this is the first time that trigonelline has been determined in sunflower seeds. **Table 2** shows the concentrations of trigonelline determined in sunflower seeds and soy seeds using the standard addition calibration method. As shown in this table, statistically significant differences were found between the slopes of calibration lines obtained by the external standard and the standard addition methods. Therefore, the presence of matrix interferences was demonstrated. Thus, the concentration of trigonelline in the analyzed samples was determined by means of the standard additions method, the concentration of trigonelline in these two seeds being about 10 μg/g. This concentration is slightly lower than the minimum concentration reported in the literature for mature soy seeds (*20*).

Investigation of the Presence of Trigonelline in Sunflower Oil, Soy Oil, and Olive Oil: Proposal of Trigonelline as a Novel Marker for the Detection of Adulterations of Olive Oils with Soy or Sunflower Oils. Because trigonelline is present in the sunflower seeds and soy seeds, this betaine is expected to be present also in the oils obtained from these seeds, confirming its traceability. As a consequence, the developed method was applied to study the presence of trigonelline in refined oils of sunflower and soy and in virgin soy oil. Trigonelline was detected in the sunflower and soy oils analyzed (see **Figure 3**). **Table 3** shows the concentrations of trigonelline determined in a refined sunflower oil and two soy oils (one refined and one virgin) using the standard additions method. Refined soy oil was the only case in which no matrix effects were observed. The highest concentration of trigonelline was found in virgin soy oil, which is elaborated under a physical process that does not decrease the content of the cationic trigonelline in oils. However, refined oils, which are subjected to a solvent extraction, presented a lower concentration of trigonelline, which can be explained because the content of free compounds, such as this betaine, could be reduced during the solvent extraction procedure. As expected from taking into account the method for obtaining the oil, the concentration of trigonelline in virgin soy oil was about 4 times higher than in refined soy oil. In addition, according to the higher trigonelline content in sunflower seeds with respect to soy seeds, trigonelline concentration in refined sunflower oil was higher than in refined soy oil. Nevertheless, it is important to remark that trigonelline concentrations determined in oils were about 1000 times lower than in their respective seeds.

With respect to olive oils, as expected from taking into account the absence of trigonelline in olives, this betaine was not detected in olive oils from Picual, Hojiblanca, and Arbequina varieties (see **Figure 4**). As a consequence, because trigonelline is present in sunflower oils and soy oils but is not present in olive oils, this compound is proposed as a novel marker for the detection of adulterations of olive oils with seed oils. Thus, evaluations of mixtures of extra virgin olive oil with sunflower or soy oils at known w/w proportions (10, 20, 50%, w/w) were carried out. **Figure 5** shows the electrophoretic profiles obtained for oil mixtures of extra virgin Hojiblanca olive oil with seed oils. In the case of mixtures of virgin olive oil with virgin soy oil up to 10% of seed oil was detected. On the other hand, mixtures containing 20% (w/w) of seed oils are shown. Although in the literature, methods capable of detecting < 10% (w/w) of seed oils in olive oils have been reported (*26*), the percentage detected with the CE method developed here is according to the current official methods where percentages between 10 and 20% can be detected using free fatty acids or sterols as markers (*30*, *31*). The method developed in this work shows a high potential due to the use of a unique compound, trigonelline, as novel marker for the

Table 3. Concentrations of Trigonelline Determined in the Sunflower Oil and Soy Oils Studied in This Work (Experimental Conditions as in **Figure 3**)

assessment of matrix interferences[a]		correlation coefficient
slope by the external standard method	358.8 (±4.1)	0.9998
slope by the standard additions method in refined sunflower oil	333.6 (±12.8)	0.9986
slope by the standard additions method in refined soy oil	366.8 (±13.1)	0.9987
slope by the standard additions method in virgin soy oil	267.3 (±7.1)	0.9993
sample analyzed[b]		
refined sunflower oil	20.6 ng/g	
	21.5 ng/g	
refined soy oil	9.0 ng/g	
	11.2 ng/g	
virgin soy oil	34.4 ng/g	
	37.4 ng/g	

[a] External standard method was evaluated with six standard solutions of trigonelline ranging from 0.025 to 1 mM. Standard additions method was achieved with five spiked levels of trigonelline (0.025, 0.05, 0.1, 0.25, and 0.5 mM) added to oils. Values in parentheses are confidence intervals at 95% for the slope. [b] Residue obtained from the extraction and reconstituted in acetonitrile/water (40:60, v/v) and diluted 1/2 prior to quantitation. Samples were prepared in duplicate and injected three times.

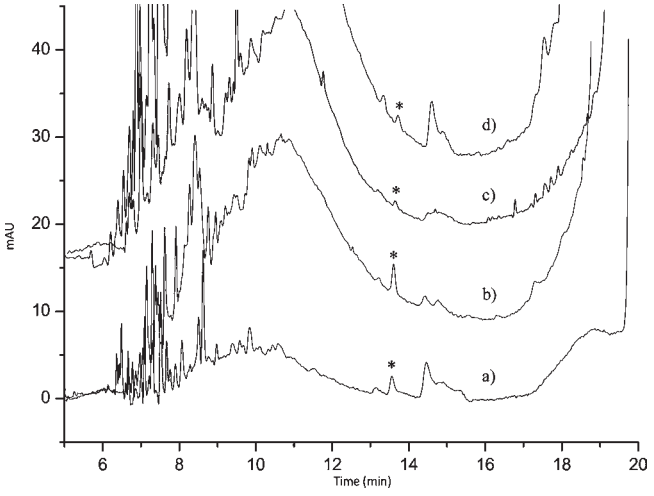

Figure 5. Electrophoretic profiles obtained for undiluted samples of oil mixtures of extra virgin olive oil Hojiblanca with (a) 20% (w/w) of refined sunflower oil, (b) 20% (w/w) of virgin soy oil, (c) 20% (w/w) of refined soy oil, and (d) 10% (w/w) of virgin soy oil. All other experimental conditions were as in **Figure 3**. Asterisk marks the position of the trigonelline peak.

adulterations of olive oils with sunflower and soy oils. This method requires an eletrophoretic separation to obtain the trigonelline peak as target signal without further treatment. There are other authentication methods based on spectrometric techniques that study oil fingerprints without any separation and sample pretreatment steps. However, the spectral differences between most vegetable oils are quite small, and the high amount of data obtained by these methods requires the use of chemometric tools (*26*). On the other hand, adequate analysis time, limit of detection, and acceptable precision of the CE method in comparison with other chromatographic methods to determine trigonelline both in plants and in foods were achieved. As a consequence, the developed method may be presented as an alternative technique to assess easily the quality of olive oils following a novel marker detected in sunflower and soy oils but not in olive oils.

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