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Analytical Methods

Rapid analytical method for the determination of organic and inorganic species in tomato samples through HPLC–ICP-AES coupling

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ABSTRACT

A HPLC–inductively coupled plasma atomic emission spectrometer (ICP-AES) hyphenation technique was used to determine the concentration of some organic (i.e., carbohydrates, carboxylic acids) as well as inorganic (metals and anions) compounds in tomato samples. A high efficiency nebulizer (HEN) coupled to a low inner volume cyclonic spray chamber (Cinnabar) was used to interface both techniques. The HPLC–ICP-AES chromatograms for organic compounds were obtained by plotting the 193.03 nm carbon emission intensity versus time. In the present work, it was also possible to obtain information about the concentration of several metals in foodstuffs. Finally, by registering the intensity at the sulphur and phosphorous emission wavelengths, the content of anions such as sulphate and phosphate was determined. In general terms, the results obtained with HPLC–ICP-AES did not differ significantly from those found with a refractive index detector. Due to the huge amount of information provided by this hyphenation, it was possible to apply it to the discrimination among different samples of native tomato cultivars.

1. Introduction

Carbohydrates, carboxylic acids, metals and inorganic anions are present in many food samples. The determination of these compounds is of capital importance, because they preclude some relevant properties of the foods, among them the sensorial quality (Roselló, Galiana-Balaguer, Herrero-Martínez, Maquieira, & Nuez, 2002) and they provide information about the nature and origin of the foods (Beullens et al., 2006; Ruiz, Valero, García-Martínez, Serrano, & Moral, 2006). Hence, the development of rapid analytical methods providing this kind of information is of capital importance for food quality control purposes.

High performance liquid chromatography (HPLC) has been extensively used for the determination of organic compounds such as carbohydrates and carboxylic acids in foodstuffs (Beullens et al., 2006). In most of the methods described in the literature these groups of compounds are sequentially determined. Separation of a mixture of carbohydrates and carboxylic acids has also been carried out by HPLC with ion exchange and ion exclusion columns consisting of sulphonated polystyrene-divinylbenzene resins with hydrogen (Molnar-Perl, 1999) or calcium (Blanco, Gutierrez, Mangas, & Noval, 1988) as counter ions. When the former was the counter ion, mobile phases usually consisted of diluted solutions of sulphuric (Glew et al., 2005), phosphoric (Chinnici, Spinabelli, Riponi, & Amati, 2005) or carboxylic acids (McFeeters, 1993) among others. In contrast, if Ca^{2+} was present as counter ion, a $Ca(Na)_2$ EDTA solution was the selected mobile phase (Blanco et al., 1988). In all these cases traditional chromatographic detectors have been used.

Additional methods have also been employed for determining sugars and carboxylic acids in foods. An 'electronic tongue' consisting of a series of 15 potentiometric sensors was recently applied (Beullens et al., 2006). Although the results for tomato samples were in agreement with those found with HPLC, this method exhibited low sensitivities for the sugars determination. The same authors also employed attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) and concluded that both methodologies were a promising alternative to chromatographic methods for sugar and carboxylic acids profiling (Beullens et al., 2006), but more research has to be done on this matter. Moreover, capillary zone electrophoresis (CZE) has been used for the determination of main carbohydrates and carboxylic acids involved in tomato flavour (Roselló et al., 2002). Comparatively, the method based on CZE provided lower limits of detection and higher resolution than HPLC equipped with either Refractive Index or UV–visible

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detectors. In contrast, no improvement in terms of analysis time was noticed.

In addition to the classical detectors, in a recent approach, a HPLC system was coupled to inductively coupled plasma atomic emission spectrometry (ICP-AES) for determining carbohydrates in fruit juices (Jinno, Nakanishi, & Nagoshi, 1984; Peters, Davis, & Jones, 2001). The solution leaving the column was directed towards the sample introduction system of the spectrometer. Carbohydrates were detected by measuring the carbon emission intensity at 193.03 nm. With this detector the background signal was high, because of the high amount of CO_2 delivered to the plasma coming from several sources (Peters, Hou, & Jones, 2003). Moreover, due to the fact that carbohydrates were detected by means of the measurement of the carbon emission intensity, the use of organic compounds in the mobile phase had to be avoided.

However, the ICP-AES detector showed several advantages compared to classical detectors. Thus, it can be considered as a universal detector. For non volatile organic compounds such as carbohydrates the emission intensity was independent of the compound for a given carbon concentration (Peters et al., 2001). Therefore, a calibration curve derived for a given carbohydrate could be used for the determination of other organics. Furthermore, it has been demonstrated that a calibration curve can be obtained with the injection of only one standard containing different concentrations of a set of non volatile organic compounds. This gives rise to a considerable shortening of the time of analysis. This is a very important issue when dealing with ICP-AES analysis, because the analysis costs are reduced. This calibration method has been called single injection calibration approach (SICA) (Paredes, Maestre, Prats, & Todoli, 2006). Finally, by a judicious selection of the stationary and mobile phases, it was possible to separate carbohydrates, carboxylic acids, aliphatic alcohols and metals in about 20 min.

The aim of the present work was thus to test the suitability of the HPLC–ICP-AES coupling for the rapid determination of carbohydrates, carboxylic acids and metals together with anions such as sulphate or phosphate in tomato cultivars. To the best of our knowledge there has not been any attempt to use an analytical technique to obtain information concerning compounds as different in nature as those considered in the present work. Because of the great deal of information that can be quickly obtained from such a coupling, a second goal of the present study was to demonstrate how to apply this assembly to the discrimination among several samples of tomato native cultivars.

2. Materials and methods

2.1. Samples and sample treatment

Eight different native tomato cultivars (i.e., Rosa, Baladre, Negro, Pera, Benavente, Muchamiel, Valenciano and Moncho) were selected. These varieties were cultivated in the same plot following an organic farming procedure. The tomato fruits were randomly collected at their commercial ripening state. Two of them (Valenciano and Muchamiel) were collected at two different ripening stages. Three pieces of every cultivar were selected and they were ground with a domestic grinder in order to obtain a homogeneous paste. A known amount of about 30 g of this paste was vacuum filtered. Afterwards, the solid residue was extracted three times with 20 mL of Milli-Q (<18 MΩ) water by sonication for 5 min. The three extracts were subsequently vacuum filtered. Finally, the filtered liquid fractions were mixed in a volumetric flask and the total volume was made up to 100 mL with Milli-Q water. Before being injected in the column, this solution was filtered through a Nylon filter with a 0.45 μm pore size.

2.2. Reagents and solutions

All reagents were of analytical grade. D(+)-glucose, D(−)-fructose, and succinic acid were purchased from Flucka (Buchs, Switzerland), D-sorbitol from Sigma–Aldrich Chemie (Steinheim, Germany), citric acid monohydrate from Prolabo (Val de Fontenay, France), L(+)-tartaric acid from Carlo Erba (Milano, Italy) and glycerol from Scharlau Chemie (Barcelona, Spain). Sodium sulphate anhydrous (Panreac, Barcelona, Spain) and ammoniun dihydrogen phosphate (Merck, Darmstadt, Germany) were also used.

Organic compounds were determined by HPLC–ICP-AES following a calibration method based on a single injection (i.e., SICA) (Paredes et al., 2006). A solution containing seven organic compounds at different concentrations (i.e., 15 μg mL^{-1} of glycerol, 30 μg mL^{-1} of citric acid, 50 μg mL^{-1} of L(+)-tartaric acid, 100 μg mL^{-1} of succinic acid, 310 μg mL^{-1} of D-sorbitol, 2530 μg mL^{-1} of D-fructose and 7500 μg mL^{-1} of D-glucose) was taken as standard.

Five standards ranging from 15 up to 170 μg mL^{-1} of sulphate and from 13 up to 140 μg mL^{-1} of phosphate were prepared to determine these inorganic anions. Finally, metals were determined by means of a standard additions procedure. Five solutions were prepared in which different volumes of a 20 μg mL^{-1} multielemental stock solution (prepared from a 1000 μg mL^{-1} multielemental solution, Merk IV) were added to 9 mL of the tomato extracts. Finally, Milli-Q water was added to a final volume of 10 mL.

The mobile phase consisted of a 0.007 mol L^{-1} nitric acid aqueous solution prepared from a 70% commercially available solution (Suprapure, Merck) and Milli-Q water.

2.3. Apparatus and procedure

A volume of 20 μL of either samples or standards was first injected in the system via an injection valve (Mod. 7725(i), Rheodyne, USA) placed before a 4-mm-length, 3-mm-i.d. guard column (Carbo-H^+, Phenomenex). A 300 × 3 mm cation exchange HPLC column with 8 μm-diameter particles (Rezex RHM, Phenomenex, Torrance, CA, USA) was used to separate these compounds. The mobile phase was pumped through the system by means of a HPLC pump model PU-2085 (Jasco Inc., Tokyo, Japan). The system was also equipped with a Gecko-2000 HPLC column oven (CIL Cluzeau, Sainte-Foy-La-Grande, France) which heated the column to a temperature of 80 °C. As metals do not need to be separated chromatographically, a second injection valve was connected after the column via a 90-cm-length, 0.254-mm-i.d. stainless steel tubing. The solution leaving this second injection valve (loop 20 μL) was directly driven into the ICP-AES spectrometer (i.e., Optima 4300 DV Perkin–Elmer, Überlingen, Germany). Therefore, two consecutive sample injections were required to perform a full data acquisition: the first one to determine organic compounds and inorganic anions and the second one to register the metal peaks (Paredes et al., 2006). No interferences were noticed between the metal peaks produced by the solutions being injected before and after the column.

The sample introduction system consisted of either a high efficiency nebulizer (HEN, Meinhard Glass Products, Santa Ana, CA) or a PFA (Tetrafluoroethylene–perfluoroalkyl vinyl ether copolymer) pneumatic concentric micronebulizer (CPI, Santa Rosa, CA, USA) coupled to a spray chamber. Three different chambers were tested. These were a 20 cm^3 inner volume cyclonic spray chamber (Cinnabar, Glass Expansion, Australia), a 100 cm^3 inner volume Ryton double pass spray chamber (Perkin–Elmer) and a 40 cm^3 inner volume cyclonic spray chamber (glass expansion). Organic compounds, sulphates and phosphates were detected by means of the measurement of the carbon, sulphur and phosphorus emission lines, respectively. Table 1 summarizes the experimental condi-

Table 1
ICP-AES experimental conditions and emission wavelengths

Rf power (kW)	1.35
Argon outer gas flow rate (L min^{-1})	15
Argon intermediate gas flow rate (L min^{-1})	0.2
Argon nebulizer gas flow rate (L min^{-1})	0.6
Elements and emission wavelengths (nm)	C 193.090
	S 181.975
	P 213.617
	Mn 257.610
	Zn 206.200
	Fe 238.204
	Al 396.153
	Cu 324.752

tions as well as the wavelengths of the emission lines employed. The signals were taken under the axial plasma observation mode, because of the increased sensitivity. The sampling time was set at 1 s, so a point was acquired every 1.6 s. A total number of 15–20 points per peak were obtained. This allowed obtaining peak area RSDs lower than 5% for three signal replicates. A Waters 410 refractometer (Waters, Milford, MA) was used as a reference detector for the organic compounds determination.

3. Results and discussion

3.1. Effect of the ICP-AES sample introduction system

In the present work two pneumatic micronebulizers were tested. Note that, at a given flow rate, the micronebulizers showed higher sensitivities than conventional ones (Todolí, Hernandis, Canals, & Mermet, 1999). Furthermore, when coupling separation methods with ICP techniques, micronebulizers are preferred since they produce less significant peak dispersion, thus enhancing the chromatographic resolution (Woller et al., 1998). Six different nebulizer-spray chamber combinations were thus evaluated. The signal to noise ratio was obtained (RSD < 3%). The noise corresponded to the difference between the maximum and the minimum background measurements within a period of time of 30 s. For a given spray chamber the HEN provided higher values of this parameter than the PFA nebulizer operated under the same conditions. Nonetheless, as it has been previously reported for ICP-MS, the sensitivities for both nebulizers were comparable (Maestre, Todolí, & Mermet 2004). As expected (Todolí, Maestre, Mora, Canals, & Hernandis, 2000), for a given nebulizer, the conventional cyclonic spray chamber afforded higher emission intensities than both double pass and Cinnabar.

Several liquid flow rates ranging from 0.035 to 0.6 mL min^{-1} were also tested. An increase in the sample delivery rate led to sharp peaks. Thus on switching from the lowest to the highest liquid flow rate studied, the carbon peaks found by merely injecting 20 μL of a D-glucose standard solution in absence of column had a 2-fold improvement in their height whereas their width decreased by a factor of 2.5 (i.e., from 25 s to 10 s).

In order to evaluate the sample dispersion inside the introduction system, the chromatographic peaks obtained for different organic compounds (D-glucose, D-fructose and D-sorbitol) were registered for the three spray chambers evaluated. The band width was measured at the peak base (RSD < 1%). Thus, at a 0.6 mL min^{-1} liquid flow rate this parameter was slightly lower for the Cinnabar spray chamber than for the conventional cyclonic and double pass ones. The small differences in band widths were enough to affect the separation degree of the sugars.

Provided that the differences in sensitivity at the liquid flow rate tested were not significant, the device that provided the lowest dispersion degree among the considered systems was selected for performing the remaining experiments. Hence, the HEN was coupled to the Cinnabar spray chamber.

3.2. Determination of organic and inorganic species

Fig. 1 shows a typical chromatogram obtained by HPLC–ICP-AES for a standard solution containing a mixture of metals, carbohydrates, carboxylic acids and anions. It is important to notice that the data included in this figure were obtained in about 18 min. Peaks #2–8 in Fig. 1 were obtained by plotting the carbon emission signal variation versus time. These peaks corresponded to the organic compounds present in the standard solution. It is worth mentioning that, for non volatile compounds, the ICP spectrometer acted as a universal detector in terms of peak area versus carbon

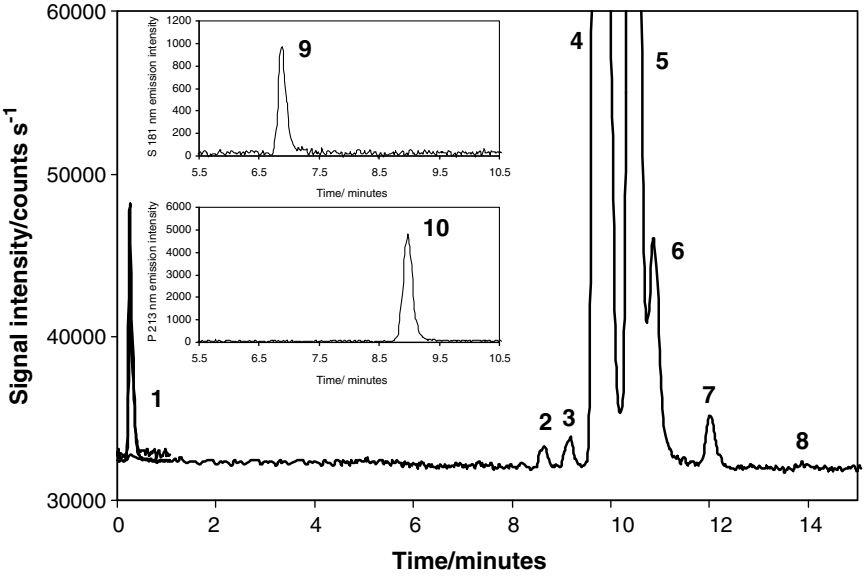

Fig. 1. Chromatogram obtained in ICP-AES for a standard solution containing carbohydrates, carboxylic acids, metals, sulphate and phosphate. Q_l = 0.6 mL min^{-1}. (1) Metals, 2 μg mL^{-1} each one; (2) citric acid, 30 μg mL^{-1}; (3) L-tartaric acid, 50 μg mL^{-1}; (4) D-glucose, 7500 μg mL^{-1}; (5) D-fructose, 2530 μg mL^{-1}; (6) D-sorbitol, 310 μg mL^{-1}; (7) succinic acid, 100 μg mL^{-1}; (8) glycerol, 15 μg mL^{-1}; (9) sulphate, 135 μg mL^{-1}; (10) phosphate, 108 μg mL^{-1}.

concentration. This made it possible to perform quantitative analysis by using a single calibration line obtained either from one (Paredes et al., 2006) or various injections (Peters et al., 2003). Indeed, if sensitivities (i.e., emission intensities of carbon peaks in Fig. 1 divided by the carbon concentration) were compared, the areas of the new peaks had 10% variability or less.

Sulphate and phosphate play a significant role on the distribution of other inorganic species in tomatoes (López, Tremblay, Voogt, Dubé, & Gosselin, 1996). By registering the emission intensity at their characteristic wavelengths (Table 1) the peaks corresponding to sulphur and phosphorous compounds were respectively obtained (peaks #9 and #10, Fig. 1). In the case of sulphates a single peak (peak #9, corresponding to bisulphate) was found in the chromatogram at 6.5 min. This result is expected owing to the characteristics of the stationary phase (i.e., weak cationic exchanger). Thus, anionic compounds were repelled by the stationary phase. In the case of phosphorous (peak #10, Fig. 1) a peak was found at a retention time close to 9 min. This peak corresponded to phosphate ion that, at the pH of the mobile phase (i.e., 2.15), was mainly present as a dihydrogen phosphate and phosphoric acid mixture. A single peak was observed at a retention time of 9 min, because these two species were in equilibrium and phosphoric acid was retained due to its inclusion into the pores of the stationary phase.

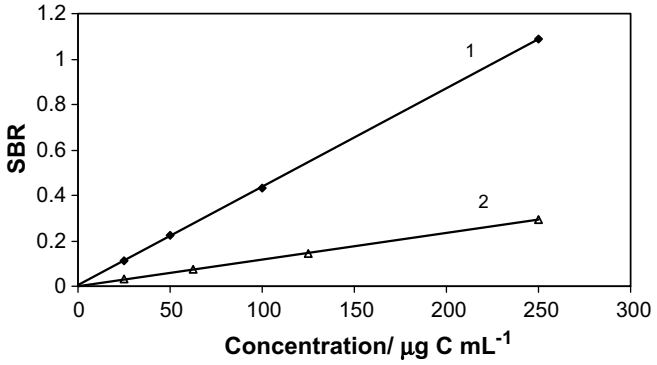

Fig. 2. Calibration lines obtained as the signal to background ratio (SBR) for the ICP-AES detector (1) and the RI detector (2). Compound: D-glucose. Mobile phase flow rate = 0.6 mL min^{-1}.

Limits of detection for these anions were calculated according to the 3 signal-to-noise ratio criterion. Under the conditions used in the present work, the LODs were 33 and 7 µg mL^{-1} for sulphate and phosphate, respectively.

Concerning metals determination, one peak per wavelength appeared at shorter retention times (peak #1, Fig. 1). Thus the time elapsed from the injection of the solution via the first valve to the time in which first chromatographic peak appeared was used to register the peaks for metals by injecting the solution through the second valve. In our study Mn, Cu, Zn, Fe and Al were detected at significant levels in tomato samples.

Obviously, the chromatogram found with the RI detector for the same standard contained less information, because data for metals and inorganic anions were not obtained. The same organic compounds were found with both detectors used which demonstrated the capability of the ICP-AES instrument for the determination of organic compounds. By comparing the chromatogram of Fig. 1 with that for the RI detector it was observed that, as previously indicated (Paredes et al., 2006; Peters & Jones, 2003), the background signal was higher for the former setup. In spite of this the use of an ICP-AES system did not induce any loss in sensitivity compared to a classical chromatographic detector. In order to test the sensitivity supplied by both methods calibration lines were obtained. Fig. 2 shows that the signal to background ratio was up to five times higher for the ICP-AES detector than that for the RI one. However, both systems afforded similar LODs (Paredes et al., 2006). These results owed to the extremely stable background that was found in the case of the RI detector.

3.3. Analysis of several tomato cultivars

The HPLC–ICP-AES coupling was applied to the analysis of tomato samples. Fig. 3 shows the chromatogram obtained for one of these samples. As mentioned before, five metals were present in tomato samples at detectable levels. The metals peaks appeared at the same time (peak #1, Fig. 3) because they were obtained by means of a second injection through the second valve (see Section 2). Note that the signal for each element was measured at a different wavelength (Table 1).

When the carbon emission signal was plotted versus time, a peak was found at the dead time (t_0 in Fig. 3) that corresponded to oxalic acid. However, as it will be discussed later for the phos-

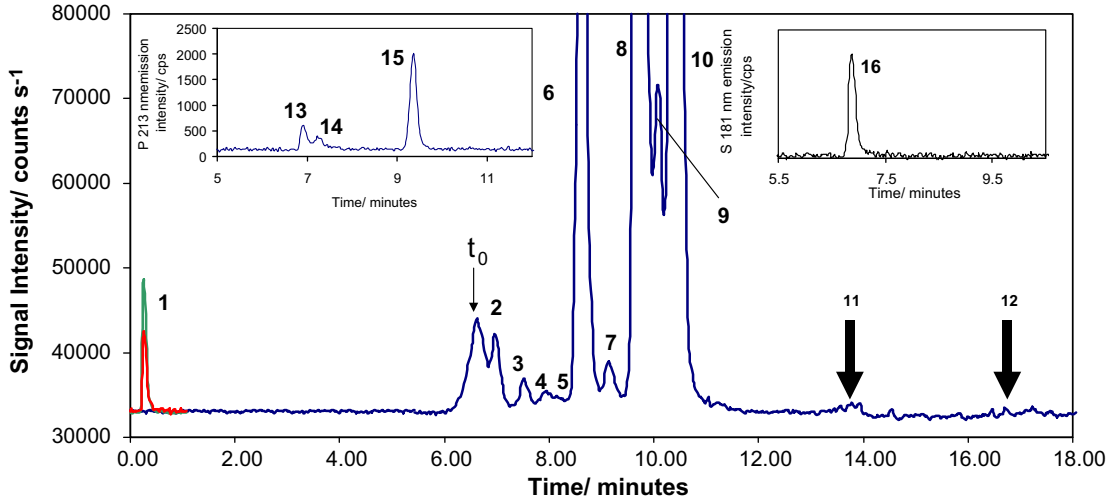

Fig. 3. Chromatogram obtained through ICP-AES for a tomato sample. Cultivar: Rosa. (1) metals; (2) dehydroascorbic acid; (3) unidentified; (4) unidentified; (5) unidentified; (6) citric acid; (7) unidentified; (8) D-glucose; (9) malic acid; (10) D-fructose; (11) glycerol; (12) unidentified: (13) unidentified; (14) unidentified; (15) phosphate; (16) sulphate.

phorous chromatograms, it was concluded that other organic compounds were partially responsible for this peak. Some of the compounds yielding peaks were unidentified (see caption of Fig. 3). It was verified that peaks #3 and #4 were partially ionized compounds (e.g., carboxylic acids), since their retention times changed with the nitric concentration in the mobile phase. In contrast, the retention time of peak #5 did not change with mobile phase composition which suggested that this peak corresponded to a neutral compound (e.g., carbohydrates). The concentration of the compounds leading to peaks #4 and #5 appeared to be directly affected by the tomato growing because the ratio between their areas changed with the degree of ripening. Peak #7 was found to be a carboxylic acid. Its retention time was very close to that for L-tartaric acid. However, experiments carried out analyzing a tomato sample and a standard with more diluted mobile phases revealed that the compound providing the peak #7 appeared at a longer retention time than L-tartaric acid. Peak #7 appeared between the peak for citric acid (peak #6, Fig. 3) and that for malic acid (peak #9, Fig. 3). As it has been previously mentioned, with the chromatographic column used in the present work, for carboxylic acids, the higher the pk_a value is the longer the retention times are (Paredes et al., 2006). Therefore, peak #7 in Fig. 3 is likely to correspond to an acid with a pk_a value very close to that for citric acid (3.06). For some varieties (i.e., Rosa, Baladre, Pera, Negro) two small peaks appeared at retention times of 14 and 17.2 min (see arrows in Fig. 3). These compounds corresponded to either small carbohydrate molecules that, because of their size spent a longer time inside the column or long chain carboxylic acids that were strongly retained due to hydrophobic interactions with the stationary phase. Further experiments confirmed that peak #11 corresponded to glycerol, whereas peak #12 was a carboxylic acid.

Interestingly, for phosphorous three peaks were found. Peak #15 in Fig. 3 corresponded to dihydrogen phosphate. The two peaks found at short retention times (peaks #13 and #14 in Fig. 3) were expected to be organic compounds and did not interfere with dehydroascorbic acid when carbon signal is monitored (peak #3, Fig. 3), because they were present at very low concentrations.

Tables 2 and 3 summarize the values of the concentrations found for the compounds and elements determined in the different varieties of tomatoes. Two of these varieties were analyzed at two different ripening states representative of the fruit consumption. In these tables the results found for ICP-AES were validated by comparing them to those from other methodologies (i.e., HPLC with refractive index detection for organic compounds and ICP-AES by continuous mode introduction of the sample for metals). Thus, three of the six identified organic compounds were determined both by ICP-AES and RI. No significant differences were found between results for both detectors. Only these compounds are shown in Table 2, because the latter detector was not able to provide

Table 2
Concentration (in mg of carbon/kg tomato) determined through HPLC–ICP-AES for three of the identified compounds and the different tomato varieties analyzed[a]

Tomato variety	Citric acid	D-Glucose	D-Fructose
Pera	$(127 \pm 3) \times 10$	$(59 \pm 2) \times 102$	$(71 \pm 3) \times 10^2$
	$(118 \pm 2) \times 10$	$(62 \pm 2) \times 10^2$	$(72 \pm 2) \times 10^2$
Benavente	$(19 \pm 3) \times 10^2$	$(64 \pm 11) \times 10^2$	$(70 \pm 13) \times 10^2$
	$(172 \pm 2) \times 10$	$(64 \pm 2) \times 10^2$	$(69 \pm 2) \times 10^2$
Baladre	$(121 \pm 14) \times 10$	$(484 \pm 10) \times 10$	$(60 \pm 4) \times 10^2$
	$(119 \pm 2) \times 10$	$(51 \pm 2) \times 10^2$	$(66 \pm 2) \times 10^2$
Negro	$(139 \pm 9) \times 10$	$(50 \pm 3) \times 10^2$	$(63 \pm 2) \times 10^2$
	$(135 \pm 2) \times 10$	$(53 \pm 2) \times 10^2$	$(65 \pm 2) \times 10^2$
Rosa	$(180 \pm 9) \times 10$	$(45 \pm 3) \times 10^2$	$(569 \pm 12) \times 10$
	$(174 \pm 2) \times 10$	$(49 \pm 2) \times 10^2$	$(53 \pm 2) \times 10^2$
Moncho	$(22 \pm 2) \times 10^2$	$(69 \pm 3) \times 10^2$	$(79 \pm 3) \times 10^2$
	$(177 \pm 2) \times 10$	$(64 \pm 2) \times 10^2$	$(73 \pm 2) \times 10^2$
Valenciano ripening state 1	$(170 \pm 6) \times 10$	$(65 \pm 3) \times 10^2$	$(75 \pm 5) \times 10^2$
	$(144 \pm 2) \times 10$	$(60 \pm 3) \times 10^2$	$(68 \pm 2) \times 10^2$
Valenciano ripening state 2	$(145 \pm 5) \times 10$	$(64 \pm 5) \times 10^2$	$(78 \pm 4) \times 10^2$
	$(123 \pm 2) \times 10$	$(60 \pm 2) \times 10^2$	$(71 \pm 2) \times 10^2$
Muchamiel ripening state 1	$(115 \pm 9) \times 10$	$(51 \pm 5) \times 10^2$	$(68 \pm 8) \times 10^2$
	$(96 \pm 2) \times 10$	$(47 \pm 2) \times 10^2$	$(59 \pm 2) \times 10^2$
Muchamiel ripened ripening state 2	$(13 \pm 2) \times 10^2$	$(58 \pm 8) \times 10^2$	$(75 \pm 6) \times 10^2$
	$(104 \pm 2) \times 10$	$(51 \pm 2) \times 10^2$	$(64 \pm 2) \times 10^2$

[a] For a given variety the first row corresponds to the concentration obtained through ICP-AES, whereas the second row shows the concentrations obtained with the IR detector.

Table 3
Concentration (in mg of phosphate or element/kg tomato or in mmol sulphate/l) determined through HPLC–ICP-AES for the different tomato varieties analyzed

Tomato variety	Phosphate	Sulphate	Mn[a]	Zn[a]	Fe[a]	Al[a]	Cu[a]
Pera	3.3 ± 0.4	2.1 ± 0.7	0.35 ± 0.02	1.53 ± 0.07	1.22 ± 0.06	2.12 ± 0.11	0.39 ± 0.02
			0.420 ± 0.008	2.24 ± 0.08	0.93 ± 0.03	0.7 ± 0.3	0.55 ± 0.02
			0.5 ± 0.2	2.6 ± 0.3	0.8 ± 0.2	0.7 ± 0.2	0.55 ± 0.14
Benavente	2.0 ± 0.8	2.0 ± 0.6	0.32 ± 0.02	1.38 ± 0.07	0.85 ± 0.04	1.37 ± 0.07	0.27 ± 0.01
			0.41 ± 0.04	1.7 ± 0.2	0.65 ± 0.08	0.182 ± 0.003	0.62 ± 0.04
			0.6 ± 0.2	1.6 ± 0.3	1.2 ± 0.2	0.16 ± 0.08	0.85 ± 0.11
Baladre	3.5 ± 0.4	3.1 ± 0.6	0.24 ± 0.01	0.97 ± 0.05	0.81 ± 0.04	1.33 ± 0.07	0.32 ± 0.02
			0.34 ± 0.02	1.9 ± 0.3	0.70 ± 0.07	0.18 ± 0.08	0.70 ± 0.04
			0.32 ± 0.11	2.1 ± 0.5	0.7 ± 0.1	0.2 ± 0.2	0.6 ± 0.2
Negro	3.2 ± 0.6	2.2 ± 0.6	0.37 ± 0.02	3.1 ± 0.2	1.19 ± 0.06	3.1 ± 0.2	0.46 ± 0.02
			0.43 ± 0.03	3.3 ± 0.2	0.77 ± 0.13	0.9 ± 0.2	0.84 ± 0.04
			0.47 ± 0.10	3.6 ± 0.2	0.8 ± 0.2	1.1 ± 0.3	0.8 ± 0.2
Rosa	3.80 ± 0.08	3.6 ± 0.6	0.36 ± 0.02	1.21 ± 0.06	1.02 ± 0.05	2.02 ± 0.10	0.29 ± 0.01
			0.510 ± 0.004	1.89 ± 0.09	0.98 ± 0.11	1.7 ± 0.2	0.39 ± 0.03
			0.53 ± 0.08	1.9 ± 0.2	0.98 ± 0.10	1.8 ± 0.2	0.30 ± 0.09
Moncho	3.3 ± 0.6	3.3 ± 0.7	0.40 ± 0.02	1.44 ± 0.07	1.09 ± 0.05	0.64 ± 0.03	0.33 ± 0.02
			0.51 ± 0.03	n.a.	0.73 ± 0.13	2.2 ± 0.9	0.56 ± 0.06
			0.49 ± 0.10	2.2 ± 0.5	0.8 ± 0.2	n.a.	0.5 ± 0.2
Valenciano	4.6 ± 1.1	5.8 ± 1.1	0.40 ± 0.02	1.35 ± 0.07	1.38 ± 0.07	1.06 ± 0.05	1.06 ± 0.05
			0.51 ± 0.02	1.63 ± 0.09	0.86 ± 0.11	0.8 ± 0.2	1.39 ± 0.07
			0.5 ± 0.2	2.0 ± 0.3	0.8 ± 0.2	0.9 ± 0.2	1.2 ± 0.2
Muchamiel	5.0 ± 1.1	3.6 ± 0.5	0.35 ± 0.02	1.59 ± 0.08	1.29 ± 0.06	0.99 ± 0.05	0.44 ± 0.02
			0.40 ± 0.05	1.9 ± 0.3	0.62 ± 0.12	0.3 ± 0.2	0.63 ± 0.11
			0.42 ± 0.13	2.02 ± 0.10	0.7 ± 0.1	0.3 ± 0.2	0.74 ± 0.13

[a] For a given tomato variety the numbers are given as: First row, external calibration in discrete mode; second row, calibration by standard additions in continuous mode; third row, standard additions in discrete mode.

quantitative information on the unidentified species. In the case of ICP-AES, it was possible to obtain the carbon concentration, since this method proved to be universal for non volatile compounds (Paredes et al., 2006; Peters et al., 2001). In the case of metals, the analysis was carried out by discrete mode using both external calibration and standard addition method. The results were validated by comparison against those obtained by ICP-AES using the standard addition method by continuous sample aspiration mode. By taking into account the confidence intervals it was found that there were significant differences between the results obtained with external calibration and standard additions (Table 3). However, the standard additions methods provided similar results irrespective of the injection mode used.

From the data obtained it was noticed that the concentration of inorganic as well as organic species varied as a function of the tomato variety. Thus, for example, it was possible to discriminate between the samples of Rosa and Pera cultivars by taking into account the concentration of D-glucose and D-fructose (Table 2). The concentration of these sugars was higher for the latter variety than for the former one. The content in citric acid permitted us to distinguish between varieties such as Pera and Moncho (Table 2).

Recently, it has been indicated that the content in trace elements (i.e., Fe, Cu, Zn, Mn) is more influenced by the cultivar than the concentration in major elements (P, Na, K, Ca, Mg) (Hernandez-Suarez, Rodríguez- Rodríguez, & Diaz-Romero, 2007). In agreement with these results, we found that the tomato cultivar precluded the concentration in these trace metals. Thus, if copper was taken into consideration (Table 3) the Valenciano variety had a higher copper concentration than the Rosa variety. Therefore, the results obtained through ICP-AES could be used for discrimination purposes. Metals determination is also important, because their concentration depends on variables such as cultivation method or period and region of sampling.

The aluminium concentration was the variable which best discriminated among the tomato samples as significant differences have been found among nearly all the cultivars. Moreover, the phosphate content showed significant differences between Benavente, Valenciano and Muchamiel from the rest of samples included in the analysis. On the other hand, the Mn composition gave only significant differences between Baladre and the whole cultivars except Muchamiel.

Another appreciation from the study of some organic compounds was that Muchamiel samples showed significant differences for dehydroascorbic acid, citric acid, D-glucose and D-fructose content at different maturity stages meanwhile Valenciano samples only were significantly different for dehydroascorbic acid and citric acid. Moreover, the dehydroascorbic acid content for a more advanced maturity stage showed significant higher values compared to the samples not so matured.

4. Conclusions

The use of an ICP-AES as detector for HPLC is a promising alternative to the already existing approaches. With this hyphenation, it is possible to determine the concentration of organic as well as inorganic compounds in samples of different nature thus giving rise to a higher amount of information than that obtained with classical chromatographic detectors. This fact would reduce the number of instruments and analysis methods required in a laboratory of food characterization.

As regards analytical figures of merit, it has been found that an ICP-AES provides sensitivities up to five times higher than a refractive index detector. However, due to the extremely stable background found with the RI detector, these two systems provide similar limits of detection.

In the present work, the developed coupling has been applied to the analysis of some native tomato samples. Due to the complexity of these specimens some peaks have not been identified. In the case of the HPLC–ICP-AES coupling this fact is not relevant, because for the purpose of discriminating different varieties it is possible to quantify, in terms of carbon concentration, the content of an unknown compound and to compare it among the different varieties of tomatoes.

Interestingly, a single chromatographic run provided quantitative information about compounds very different in nature such as sugars, carboxylic acids, inorganic anions and metals. This fact demonstrated the huge amount of information that could be derived from an ICP-AES system when used as chromatographic detector.

The present study has demonstrated the validity of the method for the determination of organic compounds and metals in food samples. Further studies would include the application of the described coupling to the fast discrimination between tomato varieties. In this case a larger number of samples would have to be analysed and multivariate statistical techniques should be applied to analyse the obtained data.

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