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Fast separation and determination of phenolic compounds by capillary electrophoresis–diode array detection Application to the characterisation of alperujo after ultrasound-assisted extraction

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Abstract

A dynamic approach has been proposed for the ultrasound-assisted extraction of twenty phenolic compounds from alperujo, a semisolid waste from the olive oil industry, that is a representative example of samples with a complex matrix. Multivariate methodology was used to carry out a detailed optimisation study of both the separation–determination and extraction steps in terms of resolution-analysis time and extraction efficiency, respectively. Consequently, the proposed method was able to extract the target analytes in 13 min; then, after dilution and centrifugation, the extract was injected into the capillary electrophoresis–diode array detection system for individual separation determination in 11 min. No cleanup of the extract was required. This method is less time-consuming, more selective and provides a larger information level than the Folin–Ciocalteau spectrophotometric method. Alperujo was demonstrated to be a powerful source of phenolic compounds, particularly as compared with olive oil—8680 versus 50–1200 μg/g.
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Keywords: Alperujo; Olive oil; Ultrasound-assisted extraction; Extraction methods; Polyphenols; Phenols

1. Introduction

In 1991/1992 continuous centrifugation using a two-phase decanter was introduced in the olive oil industry. Presently, this system is the most used, so 70% of Spanish olive oil is produced by this method. In this case, the semisolid residue obtained is called alperujo because it is an alpechin—liquid phase—and orujo—solid phase—mixture.

The high moisture level of alperujo—between 65 and 70%—hinders its use in the olive oil industry for a second extraction. The reason is the high cost of thermal drying operations, which is not compensated for the quality of the product obtained [1,2]. Moreover, this residue has a high polluting organic load owing to a high content of organic substances, including sugars, tannins, polyphenols, polyalcohols, pectins and lipids [3]. Therefore, the most general fate of alperujo is to be dumped in evaporation pools because of the prohibition of wasting into the public trenches.

One of the most conflictive fractions of alperujo corresponds to phenolic compounds. It is known that these compounds are major contributors to the toxicity and antibacterial and phytotoxic activity of black olive mill residues, which limit its microbial degradability [4]. However, these phenolic compounds possess strong antioxidant properties, which may turn the olive oil residues into a cheap source of natural antioxidants, in concentrations up to 100-times higher than in olive oil [3]. Phenolic compounds in plants are recognised as important compounds in conferring stability against auto-peroxidation of vegetable oils. The diverse group of phenolic compounds in plants include simple phenolics, phenolic acids, anthocyanins, hydrocinnamic acid derivatives and flavonoids. All the phenolic classes are endowed with the features for being free radical scavengers. However, the antioxidant activity of these

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compounds varies greatly, and some of them even exhibit pro-oxidant activity. Tocopherols are natural monophenolic compounds and, although they occur as minor constituents in vegetable oils, they are among the best known and most widely used natural antioxidants. α-Tocopherol predominates in virgin olive oil with a composition near to 95% of the total tocopherols. In addition, some of the other phenolic compounds may enhance oxidative stability of oils [5].

About the dietary intake for humans, the precise mechanism of in vivo is still not completely explained but it is inversely related to the risk of coronary heart disease—inhibition of the in vitro oxidation of human low-density lipoprotein (LDL)—and certain forms of cancer [6]. Besides, several compounds from *Olea europaea* L., such as hydroxytyrosol and oleuropein, have shown antimicrobial, hypoglycaemic, hypolipidemic and hypocholesterolemic properties [7,8].

The isolation and quantitation of the above compounds are, therefore, of high importance. Quantitative overall determination of phenolic compounds in oil is usually performed according to the Folin–Ciocalteau colorimetric method [9]. However, this method is not specific, as it gives no information of the nature of individual phenolic compounds. For this reason, a number of analytical methods have been proposed for the separation and determination of biologically active phenolic components in food. Separation and identification of individual phenolic compounds has been carried out by gas-chromatography (GC) and mass selective detector [10]. One problem associated with this option is that non-volatile phenolic compounds require derivatisation prior to the quantitation step. High-performance liquid chromatography (HPLC) is currently the most popular and reliable technique for the analysis of phenolic compounds. The technique has been mainly associated to UV absorption spectroscopy [3,11–14] or electrochemical detection (ED) [15,16] or coupled with colorimetric detection [17]. More recent development of LC–MS provides a useful tool for the determination of these compounds. However, LC–MS is very expensive equipment not common in routine laboratories [18].

Capillary electrophoresis (CE) has recently been used for this purpose [19–21]. CE is characterised by high separation efficiency, small sample and electrolyte consumption and rapid analysis, as the separation requires only several minutes. This last characteristic is the main advantage versus chromatographic methods, which makes CE of great utility in routine analysis and control and monitoring of processes in a number of industrial fields. Moreover, CE is relatively well suited to analysis of samples with complex matrices, as it allows in-capillary concentration through electrokinetic stacking [22].

Ultrasonic-assisted leaching is an effective way of extracting a number of analytes from different types of samples. The influence of extremely high effective temperatures, which result in increased solubility and diffusivity, and pressures, which favour penetration and transport, at the interface between an aqueous or organic solution subject to ultrasonic energy and a solid matrix, combined with the oxidative energy of radicals created during sonolysis of the solvent (hydroxyl and hydrogen peroxide for water), results in a high extractive power [23]. However, radicals created could degrade phenolic compounds owing to the antioxidant nature of the latter. In view of this problem, the multivariate methodology in the optimisation process is of paramount importance to select the optimal conditions that minimise potential degradation.

The overall aim of this research was to evaluate the potential use of alperujo as source of phenolic compounds. The different steps to be sequentially developed for achieving the aim were as follows: (a) optimisation of the most significant variables influencing the individual separation–detection of 20 phenolic compounds using CE–diode array detection (DAD); (b) design and optimisation of the most adequate method for the extraction of the target compounds from alperujo and preparation, if required, of the extract for proper introduction into the CE–DAD equipment.

2. Experimental

2.1. Samples

Alperujo samples from one olive oil mill in the Agriculture Cooperative “Nuestra Señora de la Consolación” (Doña Mencía, Córdoba, Spain) within the denomination of origin “Baena”, obtained in the 2003/2004 crop season were used for this research. Samples were taken directly from the production line and stored at −20 °C until analysis.

2.2. Reagents

The most representative phenolic compounds in olive oil, which are commercially available were purchased from different places. Thus, tyrosol, α-tocopherol, rutin, syringaldehyde, vanilline, catechin, *trans*-cinnamic acid, gentisic acid, ferulic acid, *o*-coumaric acid, *p*-coumaric acid, caffeic acid, vanillic acid, protocatechuic acid, *p*-hydroxyphenylacetic acid, quercetin and 3,4-dimetoxibenzoic acid from Sigma (St. Louis, USA), oleuropein from Extrasynthese (Genay, France) and gallic and tannic acids from Merck (Darmstadt, Germany).

The stock solutions were prepared by dissolving 0.2 g of each standard in 100 ml of 10% *N*,*N*-dimethylformamide in HPLC-grade methanol, both from Panreac (Barcelona, Spain). Eighteen microohms deionised water from a Millipore Milli-Q water purification system was used to prepare the water–methanol extractant mixture and buffer solutions for the optimisation study. The running buffer was 45 mM sodium tetraborate (pH 9.6), adjusted to pH 10 with sodium hydroxide and 5% HPLC-grade methanol as organic modifier.

2.3. Apparatus and instruments

Ultrasonic irradiation was applied by means of a Branson 450 digital sonifier (20 kHz, 450 W) equipped with a cylindrical titanium alloy probe (12.70 mm diameter), which was immersed into a water bath in which the extraction cell was placed. An extraction chamber consisting of a stainless steel cylinder (13 cm in length and 8 mm i.d.), closed with screws at either end, was used, allowing circulation of the leaching solvent through it. The screw caps were covered with a cellulose filter to ensure that the sample remained in the extraction chamber. Fig. 1 shows the experimental set-up used for the dynamic ultrasound assisted extraction of phenolic compounds from alperujo.

A Gilson Minipuls-3 low-pressure peristaltic pump—programmed for changing the rotation direction a preset intervals—and PTFE tubing of 0.8 mm i.d. were used to build the flow manifold. The pump was operated through a personal computer and the associated software.

A Vac Elut SPS 24 (Varian, USA) vacuum station incorporated to an Eye14 A-3S evaporator from Rikakikai (Tokyo, Japan) and 500 mg C_{18} (not endcapped, 14% carbon content), C_{18} Hydra (special for polar analytes, not endcapped, 15% carbon content) and C_{18} ec (endcapped, 14% carbon content) sorption cartridges from Chromabond (Macherey–Nagel, Düren, Germany) were used in the cleanup and preconcentration steps.

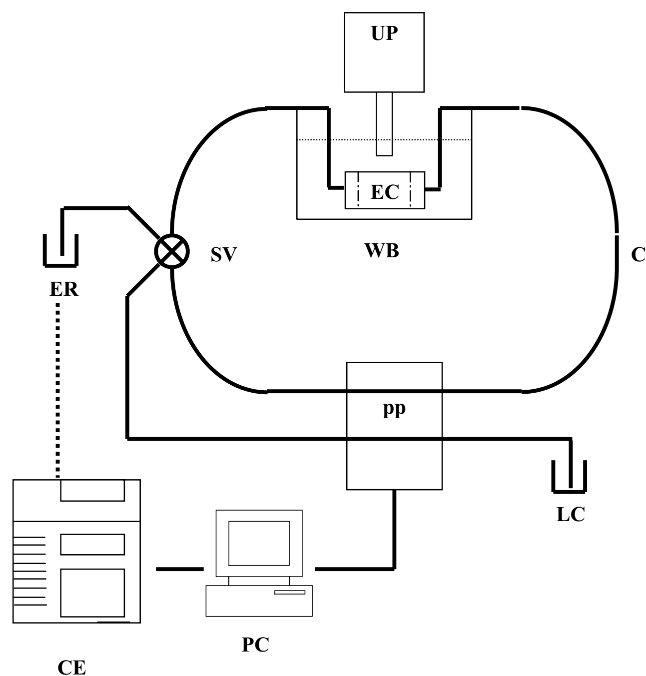

Fig. 1. Experimental set-up for the dynamic ultrasound-assisted extraction of phenolic compounds from alperujo. LC: leaching carrier; PP: peristaltic pump; UP: ultrasonic probe; EC: extraction chamber; WB: thermostatic water-bath; ER: extract reservoir; C: extraction coil; SV: selection valve; PC: personal computer; CE: capillary electrophoresis.

A 3D capillary electrophoresis Agilent G1600A Instrument (Waldbronn, Germany), equipped with a diode array detector (range 190–600 nm) and thermostated by a Peltier unit, was used to separate and quantify the analytes. Agilent capillary tubing of 48 cm (effective length 40 cm) × 50 μm i.d. × 375 μm o.d. was used.

2.4. Extraction procedure

Four grams of alperujo was placed in the extraction chamber, which was assembled and filled with the leaching carrier impelled by the peristaltic pump. After filling, the extraction chamber was immersed into the water bath at room temperature, maintained during the extraction time. The leaching carrier—methanol–water (1:3)—was then circulated through the solid sample for a 13 min preset time under ultrasonic irradiation (duty cycle 0.5 s, output amplitude 10% of the converter applied power 450 W with the probe placed at 3 cm from the top surface of the extraction cell). During extraction, the direction of the leaching carrier (at 2 ml/min flow-rate) was changed each 40 s, thus minimising both dilution of the extract and increased compactness of the sample in the extraction cell that could cause overpressure in the system.

After extraction was complete, the extract (6 ml) was collected, and 1 ml of *N*,*N*-dimethylformamide was added; then, diluted to 10 ml as final volume using methanol. Prior to introduction into the CE system, the extract was centrifuged for 3 min at 3000 rpm in order to eliminate potential in suspension particles which could plug the capillary.

2.5. Total phenol content determination

After isolation of the phenolic compounds by the extraction method described in the previous section, the concentration of total polyphenols was estimated by the Folin–Ciocalteau method [24], with absorbance monitoring at 725 nm. The spectrophotometric measurement was repeated three times for each extract and the average datum was interpolated in a caffeic acid calibration curve and expressed as μg of caffeic acid per g of alperujo.

2.6. Operating CE conditions

The running buffer used was a solution of 45 mM H_3BO_3 (pH 9.6), adjusted with NaOH to pH 10 and with 5% of methanol as organic modifier. Extracts were electrokinetically injected by application of 25 kV for 4 s. The analysis voltage was 27 kV, being the average current ∼110 μA, temperature 30 °C, and the wavelength selected depended on the compound to be monitored because of the difference between the absorption maxima of the target analytes. In order to maintain the capillary under optimal working conditions, its surface was regenerated after each run by sequential washing with water (2 min), 0.1 M sodium hydroxide (2 min), 1 min waiting, followed by the running

buffer (10 min). In addition, the capillary was activated every day by sequential washing with water (1 min), 0.1 M sodium hydroxide (10 min), 5 min waiting, and water (1 min).

3. Results and discussion

The optimisation sequence followed in this study consisted of two steps: the first, focused at the best separation–determination of the compounds using CE–DAD, and, the second, at extraction of the target compounds under the optimal working conditions. The optimisation of the electrophoretic separation was carried out with both standards and extracts in order to take into account the presence of possible co-extracted interferences in the latter which could exert a significant influence on the results.

In preliminary experiments, the optimal wavelength for monitoring each analyte was selected. The aim was to obtain the maximum signal with the lowest contribution from potential interferences by comparison between standards and extracts spectra. The best modality between hydrodynamic and electrokinetic injection was also selected at this step. The conclusion was that electrokinetic injection provides better results as the hydrodynamic alternative is less selective and significant interferences contribution was found.

3.1. Optimisation of the electrophoretic separation

Electropohoretic separation is influenced by experimental variables such as voltage, temperature, injection time and mode, capillary characteristics and buffer composition (including pH, addition of organic solvents, and modifiers) [25]. Chemometrics was used since the preliminary stages for the establishment of the CE method and analysis of CE data to extract the maximum amount of significant information. The aim of chemometrics in this research was the optimisation of the electrophoretic conditions to achieve a successful resolution of the analytes in a time as short as possible. Therefore, a compromise was established between resolution and analysis time. With the use of experimental design techniques, the number of experiments to be carried out can be reduced drastically when chemometric strategies are used. First, factorial designs provide information about the most relevant variables as well as their possible interactions, so that the main effects and interactions can be statistically evaluated. Then, the variables found to be significant for the separation can be studied more exhaustively while irrelevant factors can be obviated.

For the optimisation of this separation, seven variables were considered; namely, analysis voltage, injection time, buffer pH, buffer concentration, percentage of organic modifier in the buffer, capillary temperature and injection voltage. The response variable was the peak half-width, which should be the minimum possible value to get the best resolution in the shortest time.

Table 1
Optimisation of the electrophoretic separation

Variable	Tested range		Optimum value
	First design[a]	Second design[b]	
Analysis voltage (kV)	20–25	25–27	27
Injection time (s)	1–3	4–6	4
Buffer pH	9.6–10	10	10
Buffer concentration (mM)	15–25	25–35	45[c]
Methanol (%)	5–15	5	5
Temperature (°C)	20–30	30	30
Injection voltage (kV)	15–25	25	25

[a] Corresponding to the Plackett–Burman design.
[b] Corresponding to the two level full factorial design.
[c] Obtained from the univariate study.

A Plackett–Burman design $2^7 \times 3/32$ type III resolution allowing four degrees of freedom and involving 12 randomised runs plus three centre points was built for a screening study of the behaviour of the seven variables potentially affecting the individual separation. The upper and lower values given to each factor were selected from the available data and experience gathered in the preliminary experiments. Both the tested and the optimum values obtained for each variable are shown in Table 1.

The conclusions of this screening study were that buffer pH, percentage of organic modifier in the buffer, capillary temperature and injection voltage were not statistically influential factors within the ranges under study. However, the results showed that the smallest peak half-width—and the best resolution as a result—were obtained with the maximum values of buffer pH, capillary temperature and injection voltage and with the minimum amount of modifier. Thus, the highest values tested for these variables—namely, pH 10, 30 °C and 25 kV, respectively—and the lowest value of the methanol content—5%—were selected for subsequent experiments.

Higher values of the analysis voltage, injection time and buffer concentration were tested using a two level full factorial design involving eight randomised runs plus three centre points. The first two variables were not statistically influential factors within the ranges under study, but better separations were obtained with the highest value tested of analysis voltage and the lower value of injection time—namely, 27 kV and 4 s, respectively. The buffer concentration was significant with a positive effect.

The influence of the buffer concentration was studied in a univariate way by fixing the other variables at their optimal values. Buffer concentrations between 35 and 45 mM were studied. The results obtained showed that the peak half-width was practically constant within this range, but the analysis time decreased from 20 min at 35 mM to 11 min at 45 mM. For higher concentrations, the resolution was affected and overlapped peaks were obtained, thus, 45 mM borate was selected.

Table 2
Optimisation of the ultrasound-assisted extraction of phenolic compounds

Variable	Tested range		Optimum value
	First design[a]	Second design[b]	
Radiation amplitude (%)	10–50	10	10
Duty cycle (%)	10–50	50	50
Irradiation time (min)	1–5	5–10	13[c]
Extractant flow-rate (ml/min)	1–2	2	2
Methanol (%)	100–50	50-25	25
Probe position (mm)	1–3	3	3
Extractant volume (ml)	2–4	4–6	6

[a] Corresponding to the Plackett–Burman design.
[b] Corresponding to the two level full factorial design.
[c] Obtained by the univariate study.

3.2. Optimisation of the ultrasound-assisted extraction of phenolic compounds

Once the electrophoretic conditions for separation of phenolic compounds were obtained, the next step was the study of the extraction in order to find the working conditions for optimal efficiency with minimal degradation of the phenolic compounds.

For the optimisation of this extraction step, seven variables were considered, namely: the probe position, ultrasound radiation amplitude, percentage of duty cycle of ultrasound exposure, irradiation time, extractant flow-rate, composition of the extractant and extractant volume. The latter was studied by changing the coil length. In this case, the response variable was the extraction efficiency expressed as the peak area for each compound under the capillary electrophoresis conditions previously optimised.

A Plackett–Burman design $2^7 \times 3/32$ type III resolution allowing four degrees of freedom and involving 12 randomised runs plus three centre points was built for a screening study of the behaviour of the seven factors affecting the extraction process. The upper and lower values given to each factor were selected from the available data and experience gathered in the preliminary experiments. The tested and the optimum values obtained for each variable are shown in Table 2.

The conclusions of this screening study were that the radiation amplitude, probe position, duty cycle and extractant flow-rate were not statistically influential factors within the ranges under study. However, the results showed better extraction efficiencies with the minimum value of radiation amplitude and maximum values of the other three variables. Thus, the lowest value tested for the first one—namely, 10% of radiation amplitude—and the highest values tested for the second ones—namely, 3 cm, 50% and 2 ml/min, respectively—were selected for subsequent experiments.

Higher values for the irradiation time, extractant volume and composition of the extractant, in terms of higher water content, were tested using a two level full factorial design involving eight randomised runs plus three centre points. The extractant volume and its composition were not significant factors within the ranges under study. Better extraction efficiencies were found with the highest values tested of both variables—namely, 6 ml and water–methanol (75:25), respectively. The irradiation time was significant with a positive effect. Therefore, a kinetics study was made testing different sonication times in order to determine the time necessary for total removal of phenolic compounds in the alperujo samples, which was obtained after irradiation for 13 min. In view of these results, this sonication time was selected and used for further experiments. The extracts obtained with longer extraction times provided similar results with no detectable degradation. The kinetic curves practically showed the same evolution for all the target compounds.

3.3. Influence of a cleanup-preconcentration step prior to CE

A solid-phase extraction (SPE) step was assayed to clean and preconcentrate the phenolic compounds from the extracts before injection into the CE system. The results were compared with those from extracts directly injected into the capillary. Taking into account the variability of the target

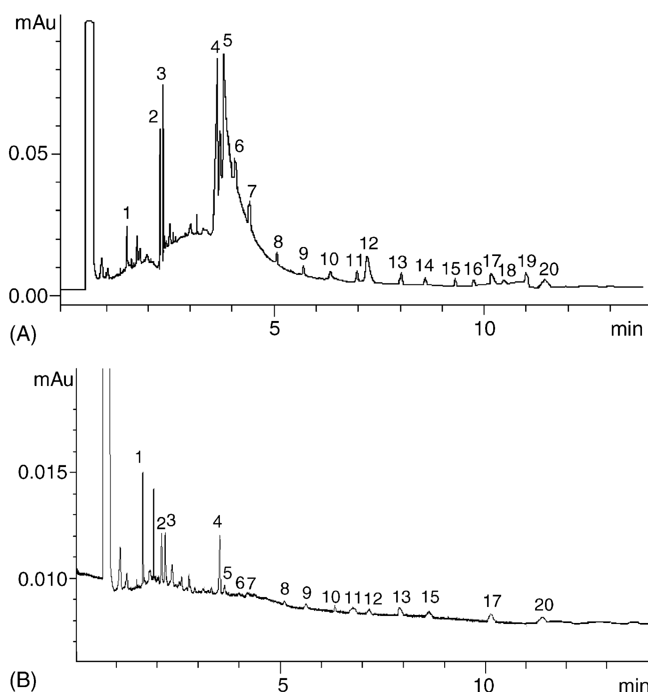

Fig. 2. (A) Electropherogram of extract from alperujo injected directly by CZE using the optimal working conditions (see Section 2.6). 100 µg/ml for all analytes at 210 nm. Peak identification numbers: 1, tyrosol; 2, α-tocopherol; 3, oleuropein; 4, rutin; 5, syringaldehyde; 6, vanilline; 7, catechin; 8, *trans*-cinnamic acid; 9, *p*-hydroxyphenylacetic acid; 10, gentisic acid; 11, ferulic acid; 12, tannic acid; 13, 3,4-dimetoxibenzoic acid; 14, *o*-coumaric acid; 15, *p*-coumaric acid; 16, quercetin; 17, caffeic acid; 18, vanillic acid; 19, gallic acid; 20, protocatechuic acid. (B) Electropherogram of extract from alperujo after SPE treatment with C_{18} hydra (see Section 3.3) using the optimal working conditions as in (A).

compounds in terms of molecular structure and functional groups, three types of SPE cartridges with different properties were used, following a similar procedure for all them. The off-line trace enrichment process was carried out passing different volumes of sample (1–6 ml) through the cartridge and the phenolic compounds retained were eluted with different volumes of methanol (1–10 ml). The best results were obtained passing all the extract (6 ml) and eluting with 3 ml of methanol. The tests carried out with C_{18} and C_{18} ec showed worse results practically for all analytes than those provided by C_{18} hydra. As the former are recommended for non-polar compounds, only those analytes which were able to link hydrophobically with the sorbent remained in the cartridge. Besides, no cleanup effect was observed owing to the high quantity of non-polar interferents. In the case of C_{18} hydra the polar interactions provided better results in terms of cleaning and preconcentrating effects. Nevertheless, direct determination provided better results due to either incomplete retention or elution of some analytes in the SPE step. These results are clearly shown in Fig. 2A and B.

3.4. Characterisation of the method

Calibration plots with standards were run for all analytes by using the peak area as a function of the concentration of each compound. The regression coefficients ranged between 0.9996 and 0.9999 for all analytes. The linear dynamic ranges are shown in Table 3.

The limit of detection (LOD) for each analyte was expressed as the mass of analyte which gives a signal that is 3σ above the mean blank signal (where σ is the standard deviation of the blank signal). The LODs obtained ranged between 2.07 and 6.95 μg/g. The limits of quantification, expressed as the mass of analyte, which gives a signal 10σ above the mean blank signal, ranged from 6.82 to 22.94 μg/g (Table 3). LODs and LOQs were estimated from alperujo extracts and standard solutions of these compounds.

3.5. Evaluation of the precision of the method

In order to evaluate the precision of the proposed method, within-laboratory reproducibility and repeatability were evaluated in a single experimental set-up with duplicates [26]. The experiments were carried out using 4 g of alperujo under the optimum working conditions. Two measurements of each compound per day were performed on 7 days. The results obtained are listed in Table 4. The repeatability, expressed as relative standard deviation, was from 1.49 to 8.97%; meanwhile, within-laboratory reproducibility ranged from 2.80 to 11.60%. These values qualify the proposed method as suitable for routine analysis of these compounds in almost any type of sample, as demonstrated with the example of a sample with a complex matrix as alperujo.

3.6. Determination of phenolic compounds in alperujo

Due to the complexity of the matrix of alperujo the quantitation of the target analytes was based on the standard addition method. For this purpose, different amounts of stock standard solution (2000 μg/ml) were added to 10 aliquots of

Table 3
Linear dynamic ranges, limits of detection (LODs), limits of quantification (LOQs) measurement wavelength and concentration of each analyte found in alperujo with the proposed method

Compound	Linear dynamic range[a,b]	LOD[b]	LOQ[b]	Wavelength[c]	Concentration[d]
Tyrosol	2000	6.50	21.52	210	1610 ± 59
α-Tocopherol	1500	3.25	10.79	290	434 ± 39
Oleuropein	1000	2.60	8.71	290	644 ± 35
Rutin	1200	4.33	14.29	270	479 ± 15
Syringaldehyde	1600	3.27	10.80	350	365 ± 25
Vanilline	2000	6.44	21.26	210	694 ± 37
Catechin	1800	2.65	8.74	210	1590 ± 120
trans-Cinnamic acid	1000	4.23	13.96	210	363 ± 28
p-Hydroxyphenylacetic acid	500	6.56	21.65	310	149 ± 5
Gentisic acid	800	4.38	14.45	210	198 ± 13
Ferulic acid	500	4.43	14.62	310	24.8 ± 0.8
Tannic acid	1800	3.40	11.22	210	1490 ± 125
3,4-Dimethoxibenzoic acid	800	3.96	13.07	310	212 ± 11
o-Coumaric acid	500	2.69	8.88	350	14.5 ± 0.8
p-Coumaric acid	600	2.07	6.82	350	56.6 ± 3.3
Quercetin	600	2.35	7.75	350	18.3 ± 0.8
Caffeic acid	600	3.35	11.06	210	248 ± 17
Vanillic acid	500	3.60	11.88	350	99.1 ± 1.47
Gallic acid	500	3.48	11.48	210	12.48 ± 0.4
Protocatequic acid	600	6.95	22.94	210	29.7 ± 1.7

[a] The linear dynamic ranges were from LOQ to the value indicated in the table.
[b] Expressed as μg/g.
[c] Expressed as nm.
[d] Expressed as μg/g ± S.D.

Table 4
Results obtained from the evaluation of the precision of the proposed method in terms of repeatability relative standard deviation (s_r) and within-laboratory reproducibility relative standard deviation (s_{WR}) obtained for each analyte

Compound	s_r (%)	s_{WR} (%)
Tyrosol	3.66	4.56
α-Tocopherol	8.97	10.32
Oleuropein	5.41	7.39
Rutin	3.17	7.40
Syringaldehyde	6.86	11.60
Vanilline	5.27	7.71
Catechin	7.63	11.06
trans-Cinnamic acid	7.60	11.45
p-Hydroxyphenylacetic acid	7.51	8.45
Gentisic acid	8.47	9.84
Ferulic acid	3.29	3.74
Tannic acid	7.63	9.29
3,4-Dimethoxibenzoic acid	5.42	7.22
o-Coumaric acid	5.30	8.77
p-Coumaric acid	5.79	8.43
Quercetin	4.55	8.29
Caffeic acid	6.97	7.94
Vanillic acid	1.49	2.80
Gallic acid	3.09	7.83
Protocatequic acid	5.78	8.64

extract in order to construct the corresponding calibration curve for each analyte. The results showed a high content of phenolic compounds—specifically, 8680 μg/g. This value is close to that obtained with the Folin–Ciocalteau spectrophotometric method, which was 9785 μg/g (absorbance $= 0.0924477C + 0.039647$, being C the total concentration of polyphenols expressed as μg of caffeic acid per g of alperujo, with a regression coefficient of 0.9978). Therefore, the compounds selected represent the profile of total phenolic compounds contained in alperujo. Some compounds, particularly, tyrosol, catechin and tannic acid, showed concentrations higher than 1000 μg/g. Concerning other compounds, their contents ranged between 149 and 694 μg/g, and only three of them were present in a concentration lower than 100 μg/g (see Table 3).

In comparison with the Folin–Ciocalteau method, the proposed method is less time-consuming—11 min versus 1 h—and more selective because it provides information of each analyte without spectral interferences.

4. Conclusions

A dynamic ultrasound-assisted approach has been proposed to extract the phenolic fraction from alperujo, which is a residue from the olive oil elaboration. Only 13 min are necessary for complete extraction without degradation by ultrasounds. The individual quantification is performed by CE–DAD, step previously optimised by a multivariate methodology and requiring only 11 min. Therefore, the overall analysis time was less than 30 min.

It is worth to emphasising that no extract treatment as cleanup was required, so direct determination after dilution and centrifugation of the extract drastically simplify the analytical process. This is a representative example of direct analysis by CE of extracts from complex samples.

Concerning the determination step, the results obtained were close to those provided by the Folin–Ciocalteau method, based on spectrophotometric monitoring of the overall phenolic content at 725 nm. The proposed method is more selective as enables the identification of each analyte, allowing its specific quantitation without interferences. This is not case of the Folin–Ciocalteau method, where other compounds can produce positive interferences. Moreover, the time required by the Folin–Ciocalteau method is 1 h versus the 11 min necessary for individual separation–determination of the method proposed here. As compared to HPLC methods, the proposed separation is also shorter as the duration of the former is about 1 h. Thus, the use of CE is an advantageous alternative to HPLC to quantify the antioxidant profile in complex samples.

The extraction method here reported demonstrates that alperujo is a powerful source of phenolic compounds. In comparison with olive oil, alperujo has higher values than olive oil of these compounds, which normally range between 50 and 1200 μg/g—the latter being infrequent [27]. This fact can be explained by the polar nature of the alperujo phase versus the non-polar of olive oil; thus, most phenolics remain in the residue during olive oil extraction. So, this research could be the basis for further extension of the extraction process to a pilot-plant scale for subsequent industrial exploitation of this very contaminant residue of the olive oil industry.

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