TECHNICAL NOTE

Nanoparticle-based assay for the detection of virgin argan oil adulteration and its rapid quality evaluation

M. Zougagh · R. Salghi · S. Dhair · A. Rios

Abstract A new method, based on the formation of gold nanoparticles (AuNPs) and spectrophotometric analysis, is proposed to determine total phenolic acids in virgin argan oil samples. These compounds have reducibility due to the presence of the phenol group in their molecular structure, and a redox reaction occurs in the presence of $HAuCl_4$. The formation of AuNPs as a result of the redox reaction leading to colour changes can be visually observed, resulting in strong light signals that show absorption at 555 nm. As ferulic acid represents more than 95% of the total phenolic acid content of virgin argan oil, this compound was used as an adulteration marker to carry out the screening of samples for the evaluation of the authenticity of virgin argan oils. The analytical features of this screening method also allowed a *low precision quantization* of the quality of the product. Then, a reference HPLC-DAD/FD method was used to confirm the potential adulterated samples, as well as to provide a detailed quantitative analysis of the most representative phenolic compounds in the samples. The overall screening-confirmation strategy was validated by analysing pure virgin argan oil samples and argan oil samples adulterated with other commercial vegetable oils, demonstrating the reliability of the results. This approach is characterised by its simplicity, low cost, rapid information and responded to practical laboratories needs.

Keywords Gold nanoparticles · Liquid chromatography · Phenolic compounds · Virgin argan oil · Adulteration

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Introduction

Argan oil is produced from the fruits of the argan (*Argania spinosa*), a species of tree endemic to south-western Morocco and protected by UNESCO [1]. Argan oil is extracted, following multistep procedures, from the pit of the fruit by a traditional method or by press- or solvent extraction. The resulting oil is slightly darker than olive oil and has a reddish tinge. It is well known for its cosmetic, pharmaceutical and nutritional virtues. Of particular importance are its rich aroma and nutty flavour, which make this oil an exotic ingredient around the world. Recently, it was shown that the geographical origin of the argan fruit and the extraction method used to produce the oil have a considerable influence on its physicochemical composition and characteristics [2]. Argan oil is a relatively new international product and it is exported only by Morocco, although different companies in Europe and North America distribute the oil throughout the world. The price of argan oil in Europe is approximately 100 € per litre as it is considered a luxury food. However, it can be assumed that exports will increase in the near future due to the unique properties of the product [3]. Due to its relatively high price, there is always the possibility that illegal practices will occur, such as dilution with cheaper oils (olive oil, sunflower oil, coloured with paprika or other substances) [4]. Thus, due to the economic,

social and cultural importance of argan oil in Morocco, it is important to control the quality of this oil and prevent fraud by guaranteeing its origin and quality by controlling possible adulteration [4]. It has previously been reported that the organic composition of virgin argan oil, mainly the phenolic composition (principally ferulic and syringic acids at 3.147 and 0.037 µg g^{-1}, respectively) [4], leads to some relevant differences with respect to other edible vegetable oils.

There is considerable interest in phenolic acids due to their powerful antioxidant properties and, hence, their potential contribution to the defence against oxidative stress and protection against cancers and cardiovascular diseases. Substantial efforts have therefore been devoted to estimating the antioxidant power of these compounds and related food and beverage samples. The "total phenolics" determination is very difficult because of their chemical complexity, the difficulty in extracting them from the oil matrix, and the presence of complex interferences in oil samples. Therefore, it is important to underline that only an "index" can be accepted as an approach to ascertain the "total phenolic" content. Traditionally, the term "total phenolics" refers the total phenolic content obtained by spectrophotometric protocols, particularly that described by Folin-Ciocalteu [5, 6]. In this sense, Rojas et al. [7] evaluated the global phenolic content of argan oil and press cake samples using the Folin-Ciocalteu colorimetric method. This assay involves a standard procedure after the extract is obtained from the starting material. The analytical protocols for the colorimetric assay are modifications of the one presented by Gutfinger [8] for oil samples. Despite the widespread use of this assay, mainly owing to its simplicity, some inherent drawbacks have been repeatedly discussed—i.e. low specificity towards phenolic compounds and the use of reagents that are destructive for the sample. Other antioxidant activity assays with different advantages and disadvantages have been reported in the literature [9–12]. These include the Trolox equivalent antioxidant capacity radical scavenging assay, the oxygen radical absorbance capacity test, the copper reducing antioxidant capacity, or various electrochemical assays. Owing to the different drawbacks of these procedures, there is no standard quantitative method for the antioxidant activity or a "total antioxidant" nutritional index for food labelling (accounting for the level of reactivity of antioxidant compounds). In a search for a replacement for these assays by a more convenient procedure suitable for the assessment of total phenolic acids content, we propose a colorimetric method based on the formation of gold nanoparticles (AuNPs) as a result of the redox reaction carried out by phenolic compounds. The catalytic growth of gold nanoparticles has recently received considerable attention in connection with the colorimetric detection of oxidase or dehydrogenase biocatalytic processes [13, 14]. Such biosensing protocols rely on the reduction of HAuCl$_4$ by the enzymatically liberated hydrogen peroxide or NADH-reducing agents to enlarge Au NP seeds followed by optical detection of the corresponding plasmon absorbance. Similarly, Scampicchio et al. [15] demonstrated the use of a series of phenolic acids (propyl gallate, caffeic acid, protocatechuic acid, ferulic acid, vanillic acid) as active reducing agents for the catalytic growth of AuNPs and illustrated that the optical properties of the resulting polyphenol-mediated nanoparticles correlate well with the antioxidant (reducing) power of the corresponding phenolic acids.

The detection of oil adulteration is a complex problem. Indeed, blending of two oils of similar composition can be hard to detect—as evidenced by the difficulties of detecting the adulteration of olive oil with hazelnut oil [16]. Authentication methods should allow a rapid screening, allow a simple sample preparation, and require readily available reagents. Authentication methods applied to oils are generally classified as physical (non-separative) [17, 18] or chemical (separative) [16, 19, 20]. The former technique requires a combination of measurements carried out on the sample whereas the latter technique focuses simply on the presence or absence of a specific constituent. Detection of the presence of a single constituent can be a misleading method since blending can easily alter the results. Detection of the absence of a constituent is more interesting if this constituent is commonly found in all the putative adulterants; the cost and technical difficulties associated with the removal of such a component from the adulterant make the adulteration unlikely. Other techniques based on the determination of metal content, in combination with different chemometrics approaches, have been also applied to analyse argan oil adulterated by the addition of cheaper vegetable oils [21].

With all these considerations in mind, we undertook the determination of phenolic acids in virgin argan oil. We observed that three phenolic acids are commonly found in argan oil: ferulic, vanillic and syringic acids. The ferulic acid concentration is at µg g^{-1} level, as determined by applying the method of Carbot et al. [22]. Hence, ferulic acid, a phenolic acid present as traces in most marketed oils, is found in high concentrations in virgin argan oil samples. We therefore selected ferulic acid as the adulteration marker. To validate the method, we used HPLC-DAD/FD to determine the ferulic acid level of mixtures of virgin argan oil and olive and sunflower oils, and consequently putative adulterated oils, at concentration ratios from 95/5 to 70/30 (argan oil/adulterating oil).

Experimental

Chemicals, material and samples

Phenolic standards (gallic acid, tyrosol, hydroxyphenyl acetic acid, vanillic acid, syringic acid, *p*-coumaric acid,

ferulic acid, *m*-coumaric acid and *o*-coumaric acid), cetyltrimethylammonium chloride (CTAC, 25% in water), hydrogen tetrachloroaurate (HAuCl$_4$ 3H$_2$O, 99.9%), sodium citrate (C$_6$H$_5$Na$_3$O$_7$ 2H$_2$O), sodium phosphate monobasic monohydrate and sodium phosphate dibasic, were received from Aldrich and Sigma and used without further purification. Standard stock solutions of the phenolic acids were prepared in methanol. All sample solutions were prepared by diluting the corresponding stock solutions in Milli-Q water. Solid-phase extraction diol cartridges (Vac 3 cc, 500 mg) from Waters (Milford, Ireland) were used to clean up and preconcentrate the oil samples.

Nineteen virgin argan oil samples (A1 to A19) from Morocco were studied. These commercial samples were purchased from local markets in Morocco. The samples were extracted by a cold-pressing process. The other edible vegetable oils (sunflower and olive oils) were acquired in Spanish markets and were used to compare the phenolic composition of Moroccan argan oil. The samples were stored in the dark at room temperature until the day of analysis. For the adulteration study, mixed oil samples were prepared by mixing argan oil with the sunflower (S1 and S2) and olive oils (O1 and O2) to give different samples in the appropriate proportions (5%:95%, 10%:90%, 15%:85%, and 20%:80%, 25%:75% and 30:70% *v/v*).

Instruments and apparatus

Samples were extracted using an SPE-Vacuum manifold from Supelco (Madrid, Spain). Absorbance measurements were performed using a DV-70 spectrophotometer (Beckman). HPLC separation and detection was performed using a modular liquid chromatographic system (Jasco) that consisted of a vacuum degasser (Jasco 2080-53), HPLC pump (Jasco PU-2080) with ternary gradient unit (Jasco, LG-2080-02), autosampler (Jasco, AS-2055), diode-array detector (Jasco, CD-2095) and fluorescence detector (Jasco, FP-2020). The entire system was coupled to LC-Net II/ADC (Jasco) for control, data acquisition and calculation using Chemnav software, which was run under Microsoft Windows XP on an IBM compatible personal computer. The analytical column was a reversed-phase SB-C18 column (Sorbax; 150 mm× 4.6 mm I.D., 3.5 μm) from Agilent technologies. A thermostatic bath and an ultrasonic bath were also used for sample preparation.

Sample pre-treatment

The oil samples were cleaned and concentrated in triplicate using SPE discovery DSC-diol cartridges according to a modification of the procedure described by Carbot et al. [22]. The cartridge was activated with 6 mL of *n*-hexane, 6 mL of methanol:water (80:20) and 3 mL of acetonitrile.

The oil (8 g) was dissolved in 10 mL of *n*-hexane and percolated into the cartridge. To remove the nonpolar fraction, the oil was washed under vacuum with 10 mL of *n*-hexane. The phenolic compounds were subsequently eluted with 8 mL of methanol:water. The eluent was evaporated to dryness on a rotary evaporator and the temperature was always controlled (<40 °C) to avoid the deterioration of phenols.

AuNPs spectrophotometric screening procedure

After the sample pre-treatment, the phenol compounds were dissolved in 0.5 mL of methanol and diluted to 25 mL with Au NP-growth solution consisting of AuCl$_4^-$ (10^{-3} M), CTAC (4×10^{-3} M), and sodium citrate (2×10^{-4} M) in phosphate buffer (pH 8.0; 10^{-2} M). The mixture was vortexed thoroughly and heated for 10 min at 45 °C. The absorbance spectra were recorded in visible region between 400 and 800 nm.

HPLC confirmation procedure

After sample pre-treatment, the residue was reconstituted in 1 mL of the initial HPLC mobile phase. The phenolic acids were injected, separated and quantified using a modification of the method of Carbot et al. [22]. The mobile phase consisted of a binary solvent system using water acidified with 2% acetic acid (solvent A) and 100% methanol (solvent B) at flow rate 0.8 mL min^{-1}. The gradient programme started with 90% eluent A and 10% eluent B and this was ramped linearly to 50% in 30 min. Initial conditions were reached in 5 min, so the total run time was 35 min. Wavelengths of 240, 280 and 320 nm were used for the diode-array detector. For the fluorimetric detection, it was necessary to monitor the first chromatographic peaks at λ_{ex}=278 nm and λ_{em}=360 nm during 15 min, and then at λ_{ex}=320 nm and λ_{em}=450 nm for the rest of chromatogram. The bandwidth was ±2 nm for both detectors and the injection volume was 20 μL. Chromatographic peaks were identified by comparing the retention times of samples with those of standard compounds. Efficient, reproducible, and sensitive separation and detection of the nine phenolic acids of interest (gallic acid, tyrosol, hydroxyphenyl acetic acid, vanillic acid, syringic acid, *p*-coumaric acid, ferulic acid, *m*-coumaric acid and *o*-coumaric acid) were obtained in less than 35 min.

Results and discussion

Figure 1 shows the overall strategy followed in this work. Argan oil samples were prepared according to the procedure described in the "Experimental" section ('sample pre-

Fig. 1 Scheme of analytical strategy for detecting virgin argan oil adulterations
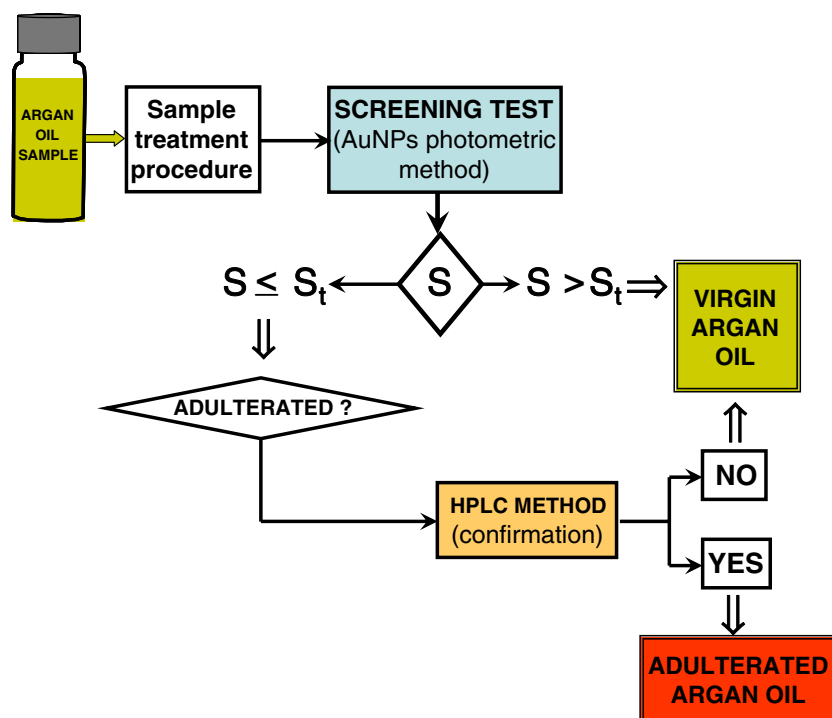
treatment'). Then, the screening test, based on AuNPs spectrophotometric method, was used for the analysis of the samples. This analysis allowed the classification of samples according to a threshold signal (S_t) indicating the characteristic high level of ferulic acid in virgin argan oil samples. Thus, samples presenting absorbance signals (S) higher than S_t were directly classified as 'virgin argan oil' samples, whereas samples presenting $S \leq S_t$ were potential candidates to be considered as 'adulterated argan oil' samples. In this case, HPLC confirmation method was used to evaluate the relative percentage of ferulic acid with respect the rest of phenolic compounds. Therefore, a minimum level of relative percentage of ferulic acid (% Min) must be established to definitively classify the samples by the confirmative HPLC method. Samples with lower percentages than % Min were clearly classified as 'adulterated', whereas samples with ferulic acid percentages≥% Min were considered as 'virgin' samples. The reliability of this strategy is consequently based on the correct establishment of the threshold values (S_t and % Min) for both classifications (screening and confirmation methods).

Screening test analytical features

Ferulic acid, vanillic acid, syringic acid, and tyrosol have different absorption spectra. The characteristic absorptions are located at 220 and 320 nm for ferulic acid, 220, 260 and 290 nm for vanillic acid, and 220 and 280 nm for syringic acid and tyrosol. The absorption features changed dramat-

ically after reaction with $HAuCl_4$, as shown in Fig. 2. The reactions of ferulic acid, vanillic acid, syringic acid and tyrosol with $HAuCl_4$ led to changes in the colour of the solutions for virgin argan oil samples. The four phenolic acids all possess a reducible phenolic group. More precisely, the latter turns into a phenate ion after heating and becomes reducible (Fig. 3) and therefore could be oxidised by $HAuCl_4$. As a result, $HAuCl_4$ is reduced and is present as gold NPs [14]. The absorbance signals of AuNPs are linearly dependent upon the concentration of the phenolic acids.

According to AuNPs spectrophotometric screening procedure, the calibration curves of ferulic acid, vanillic acid, syringic acid or tyrosol were obtained. The correlations of absorbance with all studied phenolic acids were obtained at 555 nm. Linear relationships were found between the absorbance and the concentrations of phenolic acids over the range of 0.2–1.2 µg mL^{-1}, with correlation coefficients of 0.9991, 0.9980, 0.9986 and 0.9984 for ferulic, vanillic and syringic acids and tyrosol, respectively. The corresponding regression equation and other characteristic analytical parameters for the determination of these phenolic acids are shown in Table 1. The limits of detection, defined as the concentration of analyte giving a signal equivalent to the blank signal plus three times its standard deviation [23], were 0.038, 0.057, 0.047 and 0.050 µg mL^{-1} for ferulic, vanillic and syringic acids, and tyrosol, respectively. The limit of quantification, defined as the concentration of analyte giving a signal equivalent to the blank signal plus ten times its
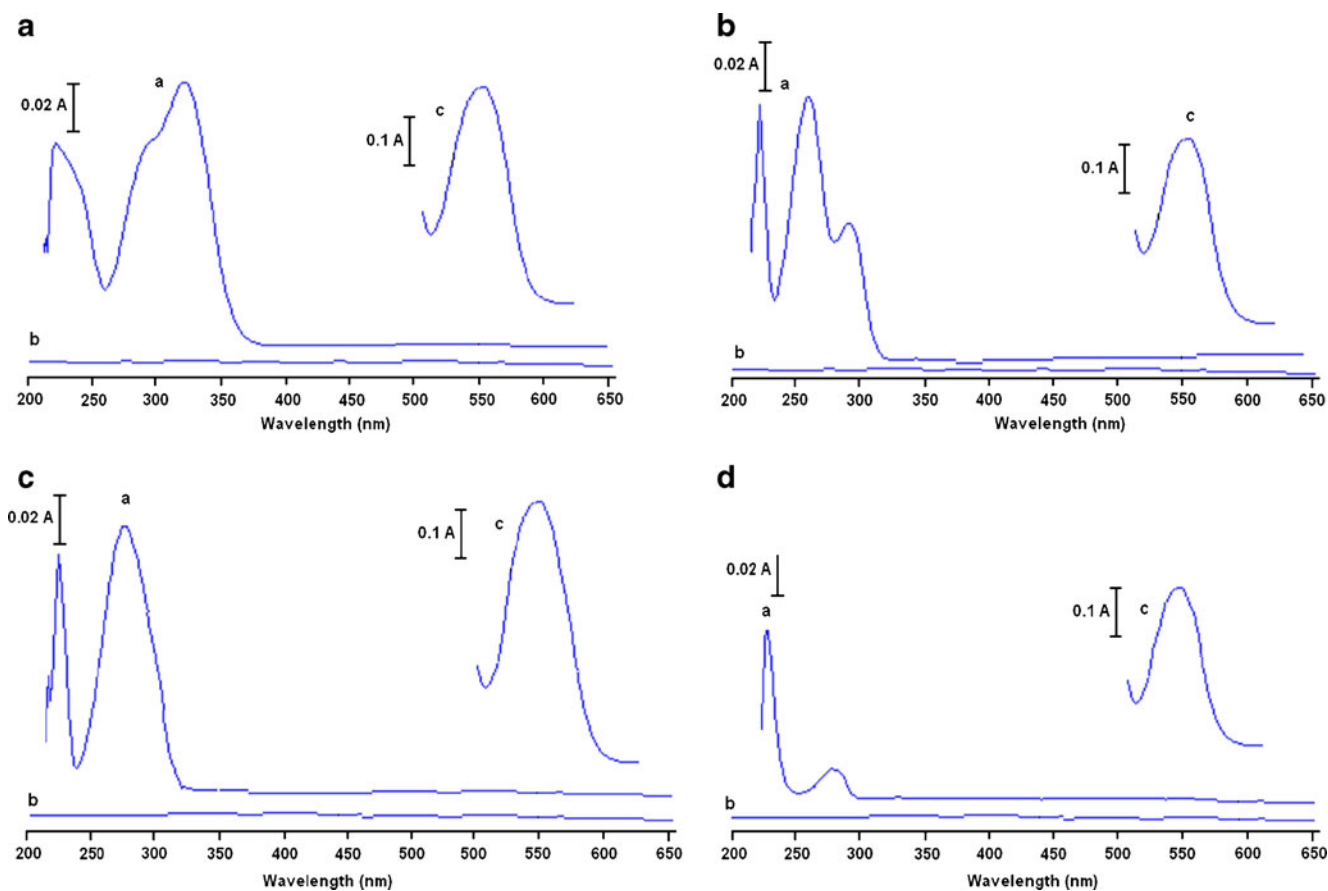
Fig. 2 Absorbance spectra of phenolic acids (*a*) and AuNPs formed in the absence (*b*) and presence (*c*) of 0.500 μg mL^{-1} of different phenolic acids: **a** ferulic acid, **b** vanillic acid, **c** syringic acid and **d** tyrosol

standard deviation [23], were 0.125, 0.189, 0.155 and 0.168 μg mL^{-1} for ferulic, vanillic and syringic acids, and tyrosol, respectively. The precision of the method, expressed as relative standard deviation, for the determination of total phenolic acid in virgin argan oil sample was 5.7% (*n*=10).
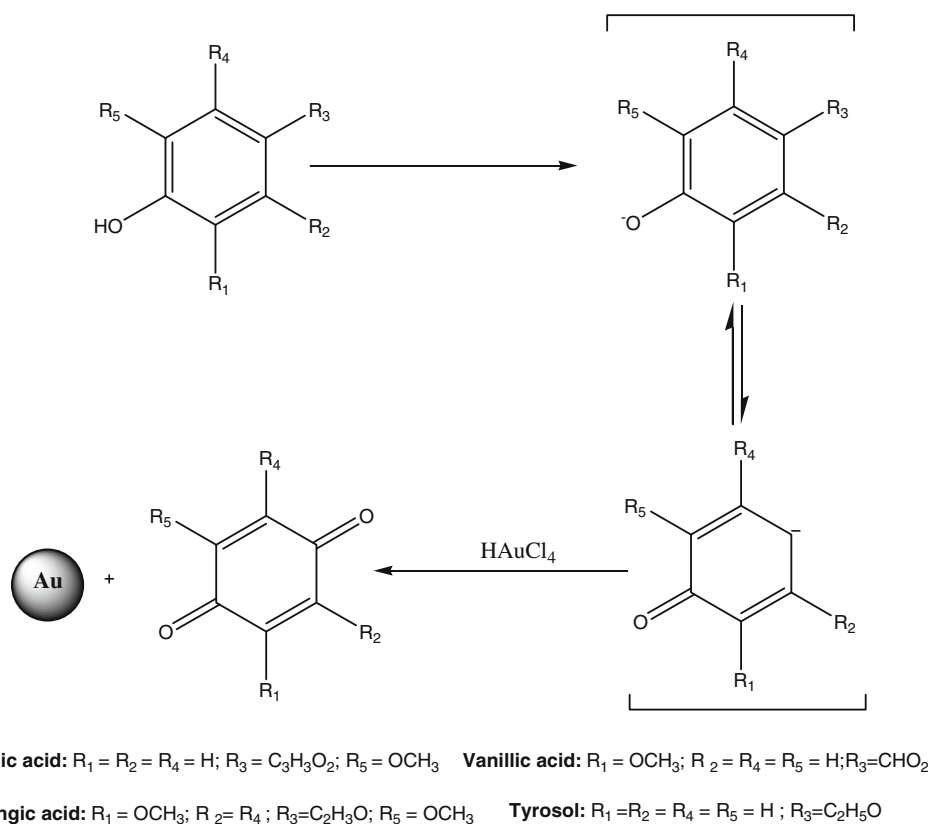
As the four phenolic acids present in virgin argan oil samples undergo the same redox reactions with HAuCl$_4$, it is impossible to separately detect one of them in a mixture. The crucial requirements for measuring the total phenolic acids content of virgin argan oil samples with AuNP measurements are that the ferulic acid, vanillic acid, syringic acid or tyrosol present the same absorbance and, as a result, the absorbance containing the overall response for the total amount of the four phenolic acids in the sample can be obtained. However, as can be seen from Table 1, there are some differences in the response factors (i.e. the slope of the calibration curves). However, it is possible to detect the total quantity of phenolic acids in virgin argan oil samples because the ferulic acid is present in these samples in a high quantity (until 97% of total phenolic acids) [3, 24, 25]. In order to quantify the total phenolic acid content, the "best worst-case scenario" approach can be used and this

involves assuming that the whole absorbance signal comes from the compound with the highest response (ferulic acid). Therefore, the ferulic acid calibration curve was chosen. The problem with this approach is that errors will occur resulting from the slightly different responses, whichever ferulic acid is chosen as the standard.

Analytical features of the HPLC confirmation method

Figure 4 shows a typical chromatogram of the separation of the phenolic compounds existing in virgin argan oil samples. The quantitative levels of the phenolic compounds found in the 15 samples examined in this study, using the HPLC method described in the "Experimental" section, are shown in Table 2. Only four phenolic compounds—ferulic acid, vanillic acid, syringic acid and tyrosol—were detected in these samples. The majority of the concentrations obtained are consistent with those found in the literature [3, 22, 23] and the differences can be explained in most cases by the different sample origin or the different extraction method employed. These oils are known as a rich source of ferulic acid (>95%), which is a more

Fig. 3 Molecular structures of ferulic, vanillic and syringic acids and tyrosol and a chart of their reactions with HAuCl$_4$

Ferulic acid: R$_1$ = R$_2$ = R$_4$ = H; R$_3$ = C$_3$H$_3$O$_2$; R$_5$ = OCH$_3$ **Vanillic acid:** R$_1$ = OCH$_3$; R$_2$ = R$_4$ = R$_5$ = H; R$_3$=CHO$_2$

Syringic acid: R$_1$ = OCH$_3$; R$_2$= R$_4$; R$_3$=C$_2$H$_3$O; R$_5$ = OCH$_3$ **Tyrosol:** R$_1$ =R$_2$ = R$_4$ = R$_5$ = H ; R$_3$=C$_2$H$_5$O

effective antioxidant than ascorbic acid and other phenolic acids such as gallic acid, tyrosol, hydroxyphenyl acetic acid, vanillic acid, syringic acid and coumaric acids. The virgin argan oil samples also contain other phenolic acids as minor components, including vanillic acid, syringic acid and tyrosol.

The differences obtained for total phenolic acids in the 15 virgin argan oil samples analysed by the screening method and by the HPLC method [22] are also listed in Table 2. As can be seen, an average error of 6.57% was obtained for the direct estimations of total phenolic acids in the samples. The statistical t_{exp} value was 0.1. This value is smaller than the corresponding tabulated t_{crit} value (2.06 for 28° of freedom at the 95% confidence level). Thus, it is feasible that the approximate calculation of total quantity of

phenolic acid can be performed using ferulic acid as the target compound.

Establishment of threshold values

As it can be seen in the results summarised in Table 2, the concentration of total phenolic acid (estimated as ferulic acid concentration) was in the range 2.5–3.9 μg g^{-1}. Other previous studies [3] revealed a maximum level of ferulic acid of 3.1 μg g^{-1} in virgin argan oil samples. Hence, we considered a value of 2.5 μg g^{-1} as the lowest concentration of ferulic acid in the total phenolic content, as acceptable for pure virgin argan oil samples. The threshold absorbance signal (S_t) was defined as the total phenolic acids concentration corresponding to 2.5 μg g^{-1}, expressed as

Table 1 Figures of merits for the most representative phenolic compounds by the AuNPs method

Phenolic acids	Linear range (μg mL^{-1})	$Y = (a \pm S_a)X + (b \pm S_b)$	R^2	$S_{y/x}$	LOD (μg mL^{-1})	LOQ (μg mL^{-1})	RSD (%)
Ferulic acid	0.2–1.2	$(0.7337\pm0.0110)X+(0.1418\pm0.0086)$	0.9991	0.0092	0.038	0.125	3.4
Vanillic acid	0.2–1.2	$(0.6334\pm0.0143)X+(0.0855\pm0.0111)$	0.9980	0.0120	0.057	0.189	4.3
Syringic acid	0.2–1.2	$(0.3245\pm0.0060)X+(0.0253\pm0.0047)$	0.9986	0.0050	0.047	0.155	5.2
Tyrosol	0.2–1.2	$(0.3907\pm0.0078)X+(0.1596\pm0.061)$	0.9984	0.0066	0.050	0.168	5.7

a slope, S_a standard deviation of the slope, *b* intercept, S_b standard deviation of the intercept, *R* regression coefficient, $S_{y/x}$ standard deviation of residuals, *LOD* limit of detection, *LOQ* limit of quantification, *RSD* relative standard deviation ($n=10$)

Fig. 4 Chromatogram obtained for a mixture of standard solutions of phenolic acids. The peaks labelled with numbers correspond to the following compounds: (1) galic acid, (2) tyrosol, (3) hydroxyphenyl acetic acid, (4) vanillic acid, (5) synergic acid, (6) p-coumaric acid, (7) ferulic acid, (8) m-coumaric acid and (9) o-coumaric acid. The concentration of every compound was 0.50 μg mL^{-1}
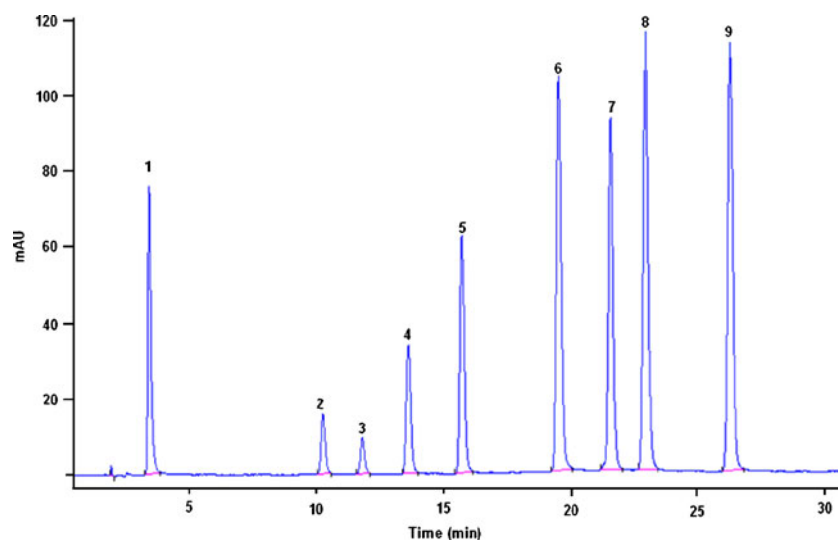
ferulic acid. In this study, we established a worst scenario for sample screening by selecting the total absorbance as a criterion to classify sample as negative (non-adulterated oil) if the absorbance signal at 555 nm was >0.25; or positive (adulterated oil) if the absorbance signal was <0.25. The final decision, however, can be made by subjecting the samples to the confirmatory HPLC method. In this case, a value of % Min=94% was established as the threshold for the confirmatory method. As an analytical quality control criterion for checking or correcting the threshold signals, control samples at concentrations of 2.2, 2.5 and 2.8 μg g^{-1} of ferulic acid were used, and proposed for routine work.

Validation of the strategy for the detection of virgin argan oil adulterations

Although the AuNPs photometric screening method was basically used as a qualitative method to classify samples as 'adulterated' or 'not adulterated', it can be also used to provide a *low precision quantization* (phenolic content expressed as ferulic acid). In fact, Table 2 includes the results obtained by the photometric method as a total determined concentration, for comparison to the total concentration obtained by the HPLC method (sum of the concentrations of the major phenolic compounds in argan oil samples). The differences between both values were expressed by the error (%) in Table 2. But, a more systematic statistical comparison was carried out by applying the t paired test to both series of results. Thus, by establishing the null hypothesis as the mean of differences equal to zero (experimental mean=0.0585; and standard deviation=0.2113), the experimental t value was 1.573, whereas $t_{crit}(n-1=14; \alpha=0.05;$ two tails) is 2.145. Therefore, results were statistically comparable at a 95% of confidence level; although the comparison by regression ($r=$

0.9365) corroborated the magnitude of the errors reported in Table 2. In any case, these statistical results demonstrated the usefulness of the screening method for both objectives: the classification of the samples (adulteration?), and for a rapid evaluation of the quality of virgin argan oil samples based on their phenolic contents expressed as ferulic acid.

The common oils used for virgin argan oil adulteration are olive and sunflower oils, which contain lower levels of ferulic acid. The screening method was tested by analysing 28 different samples. They were prepared by mixing argan oils (A16, A17, A18 and A19) with the sunflower (S1 and S2) and olive (O1 and O2) oils, to give different samples in the appropriate proportions (5%:95%, 10%:90%, 15%:85%, and 20%:80%, 25%:75% and 30:70% v/v) of adulteration. The results obtained for these samples are listed in Table 3. As can be seen, all of the samples were classified as positive or negative with 100% of reliability. Only those samples that have been adulterated gave a positive response with the spectrophotometric screening method. Average concentrations of positives and negative samples were calculated and the results are listed in Table 3. These samples were also processed following the confirmation method. The ferulic concentrations obtained are also listed in Table 3. As the data were quite consistent, it can be concluded that the proposed screening method is accurate and can, thus, be used for detection of virgin argan oil adulterations. With the information given by screening-confirmation strategy it is also possible to evaluate the quality of the non-adulterated argan oil samples. Thus, if 94% of ferulic acid threshold established the classification of samples for adulteration, the amount of ferulic acid (at % Min >94%) allowed to compare the quality of different samples of virgin argan oil. Thus, although for results in Table 3 there are some correlation between the percentage of ferulic acid and its concentration for samples A16, A17,

Table 2 Determination of phenolic compounds in virgin argan oil samples by the proposed methods (chromatographic and photometric)

Virgin argan oil samples:	A1	A2	A3	A4	A5	A6	A7
Vanillic acid (µg g^{-1})[b]	0.050±0.004	0.054±0.003	0.061±0.002	0.075±0.005	0.043±0.001	0.050±0.003	0.038±0.003
Syringic acid (µg g^{-1})[b]	0.074±0.005	0.060±0.004	0.078±0.007	0.090±0.006	0.043±0.004	0.055±0.004	0.048±0.002
Ferulic acid (µg g^{-1})[b]	3.484±0.055	2.466±0.047	2.494±0.052	3.219±0.030	3.406±0.036	2.850±0.041	2.747±0.031
Tyrosol (µg g^{-1})[b]	n.d.	0.019±0.001	n.d.	n.d.	0.020±0.002	n.d.	n.d.
Total calculated (µg g^{-1})[b]	3.608±0.061	2.598±0.057	2.632±0.051	3.384±0.041	3.512±0.031	2.955±0.030	2.833±0.049
Ferulic acid (%)[b]	96.57±0.21	94.90±0.17	94.74±0.13	95.13±0.31	97.00±0.25	96.46±0.23	96.98±0.27
Total determined (µg g^{-1})[c]	3.516±0.070	2.431±0.061	2.822±0.033	3.709±0.037	3.822±0.034	2.816±0.030	2.716±0.024
Differences[d] (%)	−2.56	−6.42	+7.21	+9.63	+8.84	−4.70	−4.13

Virgin argan oil samples:	A8	A9	A10	A11	A12	A13	A14	A15
Vanillic acid (µg g^{-1})[b]	0.060±0.004	0.072±0.006	0.062±0.003	0.068±0.001	0.056±0.004	0.069±0.001	0.088±0.004	0.061±0.003
Syringic acid (µg g^{-1})[b]	0.078±0.005	0.057±0.003	0.071±0.003	0.061±0.003	0.065±0.006	0.062±0.005	0.071±0.004	0.065±0.007
Ferulic acid (µg g^{-1})[b]	3.025±0.050	2.803±0.038	3.125±0.051	3.500±0.034	3.844±0.033	3.153±0.047	3.059±0.051	2.494±0.042
Tyrosol (µg g^{-1})[b]	n.d.	n.d.	0.019±0.001	n.d.	n.d.	0.020±0.003	n.d.	n.d.
Total calculated (µg g^{-1})[b]	3.163±0.037	2.933±0.032	3.277±0.034	3.629±0.045	3.964±0.038	3.304±0.037	3.218±0.043	2.620±0.034
Ferulic acid (%)[b]	95.65±0.33	95.58±0.35	95.37±0.13	96.46±0.22	96.96±0.20	95.43±0.19	95.08±0.24	95.19±0.28
Total determined (µg g^{-1})[c]	3.428±0.054	2.694±0.039	3.103±0.044	3.866±0.037	4.297±0.061	3.484±0.054	3.425±0.067	2.788±0.054
Differences[d] (%)	+8.39	−8.15	−5.30	+6.53	+8.39	+5.46	+6.44	+6.41

[a] Result plus SD ($n=3$)

[b] Percentage of ferulic acid calculated in the assayed samples using the HPLC-DAD/FD method

[c] Total of phenolic acids determined by AuNPs method using ferulic acid as standard. A1 to A15: virgin argan oil samples

[d] Differences between 'total calculated' and 'total determined'

Table 3 Application of the screening and confirmation methods to validate the analytical strategy developed to detect frauds (adulterated: yes/no), and to estimate the quality of virgin argan oil samples

Sample[a]	AuNPs screening method		HPLC confirmative method		Final decision (YES/NO)
	Screening result	Semi-quantitative concentration (μg g^{-1})[b]	Confirmation result	Concentration found of ferulic acid (μg g^{-1})	
A16	VIRGIN	2.70±0.10	VIRGIN	2.60±0.10	NO
A16-O1(5)	ADULTERATED	1.70±0.10	ADULTERATED	1.59±0.08	YES
A16-O1(10)	ADULTERATED	0.87±0.09	ADULTERATED	0.83±0.05	YES
A16-O1(15)	ADULTERATED	0.42±0.05	ADULTERATED	0.38±0.07	YES
A16-O1(20)	ADULTERATED	0.44±0.03	ADULTERATED	0.40±0.02	YES
A16-O1(25)	ADULTERATED	0.34±0.02	ADULTERATED	0.29±0.03	YES
A16-O1(30)	ADULTERATED	0.24±0.02	ADULTERATED	0.21±0.08	YES
A17	VIRGIN	2.80±0.10	VIRGIN	2.80±0.10	NO
A17-O2(5)	ADULTERATED	0.90±0.10	ADULTERATED	0.90±0.10	YES
A17-O2(10)	ADULTERATED	1.00±0.10	ADULTERATED	0.93±0.03	YES
A17-O2(15)	ADULTERATED	0.45±0.02	ADULTERATED	0.41±0.02	YES
A17-O2(20)	ADULTERATED	0.31±0.02	ADULTERATED	0.28 ±0.01	YES
A17-O2(25)	ADULTERATED	0.16±0.01	ADULTERATED	0.14±0.03	YES
A17-O2(30)	ADULTERATED	0.14±0.01	ADULTERATED	0.11±0.01	YES
A18	VIRGIN	3.40±0.2	VIRGIN	3.19±0.09	NO
A18-S1(5)	ADULTERATED	1.30±0.10	ADULTERATED	1.19±0.07	YES
A18-S1(10)	ADULTERATED	1.00±0.10	ADULTERATED	0.87±0.08	YES
A18-S1(15)	ADULTERATED	0.39±0.02	ADULTERATED	0.34±0.03	YES
A18-S1(20)	ADULTERATED	0.25±0.08	ADULTERATED	0.21±0.03	YES
A18-S1(25)	ADULTERATED	0.16±0.05	ADULTERATED	0.13±0.04	YES
A18-S1(30)	ADULTERATED	0.14±0.01	ADULTERATED	0.09±0.03	YES
A19	VIRGIN	3.40±0.20	VIRGIN	3.40±0.10	NO
A19-S2(5)	ADULTERATED	1.30±0.20	ADULTERATED	1.20±0.10	YES
A19-S2(10)	ADULTERATED	1.00±0.20	ADULTERATED	0.91±0.09	YES
A19-S2(15)	ADULTERATED	0.4 0±0.10	ADULTERATED	0.36±0.05	YES
A19-S2(20)	ADULTERATED	0.27±0.04	ADULTERATED	0.23±0.02	YES
A19-S2(25)	ADULTERATED	0.17±0.02	ADULTERATED	0.16±0.02	YES
A19-S2(30)	ADULTERATED	0.14±0.02	ADULTERATED	0.12±0.02	YES

[a] A16, A17, A18 and A19 are virgin argan oil samples, which were mixed (adulterated) with olive oil samples (O1 and O2), and sunflower oil samples (S1 and S2) in different proportions (percentage of adulterant oil in brackets)

[b] Concentrations of phenolic compounds expressed as ferulic acid (result plus SD (n=3)

A18, and A19, Table 2 clearly illustrates the different quality of samples A7 (97% of ferulic acid and 2.833 μg g^{-1}) and A12 (also 97% of ferulic acid, but 3.964 μg g^{-1} of phenolic concentration). In addition, as the screening method also presented *low precision quantization* features, it can also provide approximate information about the quality of virgin argan oil samples (samples from A1 to A15 in Table 2, and samples A16 to A19 from Table 3). For this reason, the values of concentration found by the screening method has also been included in Table 3, as an approximate, but rapid and cost-effectiveness evaluation of the quality of virgin argan samples based on the phenolic composition expressed as ferulic acid. In fact, there was a

good correlation between results reported in Table 3 by both methods (correlation coefficient=0.9991; slope= 1.018; and intercept=0.038). This correlation predicted very well the results which can be inferred in the confirmative method by using the information of the AuNPs photometric screening method. Table 4 illustrates this possibility. As it can be seen, predictions were very consistent for virgin argan oil samples and samples with the lowest level of adulteration. When adulterations were higher than 25–30%, differences increased as the concentrations of phenolic compounds in samples were very low. Nevertheless, the low concentration of phenolic compounds in samples clearly demonstrated adulteration and, conse-

Table 4 Correlation between the screening and the confirmative method used for predicting the total phenolic content by the confirmative HPLC method from the screening method

Sample	Screening method ($\mu g\ g^{-1}$)	Predicted value ($\mu g\ g^{-1}$)	Confirmative method ($\mu g\ g^{-1}$)	Prediction error (%)
A16	2.70	2.61	2.60	0.5
A16-O1(5)	1.70	1.63	1.59	2.6
A16-O1(10)	0.87	0.82	0.83	−1.6
A16-O1(15)	0.42	0.37	0.38	−1.4
A16-O1(20)	0.44	0.39	0.40	−1.4
A16-O1(25)	0.34	0.30	0.29	2.1
A16-O1(30)	0.24	0.20	0.21	−5.7
A17	2.80	2.71	2.80	−3.16
A17-O2(5)	0.90	0.85	0.90	−6.0
A17-O2(10)	1.00	0.94	0.93	1.5
A17-O2(15)	0.45	0.40	0.41	−1.4
A17-O2(20)	0.31	0.27	0.28	−4.7
A17-O2(25)	0.16	0.12	0.14	−14.6
A17-O2(30)	0.14	0.10	0.11	−9.2
A18	3.40	3.30	3.19	3.5
A18-S1(5)	1.30	1.24	1.19	4.1
A18-S1(10)	1.00	0.94	0.87	8.5
A18-S1(15)	0.39	0.35	0.34	1.6
A18-S1(20)	0.25	0,21	0.21	−1.0
A18-S1(25)	0.16	0.12	0.13	−8.1
A18-S1(30)	0.14	0.10	0.09	10.9
A19	3.40	3.30	3.40	−2.9
A19-S2(5)	1.30	1.24	1.20	3.2
A19-S2(10)	1.00	0.94	0.91	3.8
A19-S2(15)	0.40	0.36	0.36	−1.3
A19-S2(20)	0.27	0.23	0.23	−1.1
A19-S2(25)	0.17	0.13	0.16	−19.2
A19-S2(30)	0.14	0.10	0.12	−16.8

All values are in $\mu g\ g^{-1}$, and samples are those used in Table 3

quently, a very poor quality of argan oil sample, with independence of the level of error in the correlation with the confirmative method.

Conclusions

In this work, we develop an analytical strategy, combining both screening and confirmatory methods, to evaluate the adulteration of virgin argan oil samples. Ferulic acid has been confirmed as the marker phenolic compound to control the authenticity of this Moroccan food and cosmetic product. The overall strategy demonstrated to be simple, sensitive and reliable to be used in practice, and hence with a high potential to be transferred to routine laboratories. The capacity of the screening method, not only to classify samples as positives or negative according to the preset threshold, but also to provide *low precision quantization* information, provided rapid information on both, the authenticity of samples and also the intrinsic quality of

virgin argan oil samples. Then, the confirmative HPLC method allowed to corroborate the adulteration and, if required, to carry out a detailed quantification of the phenolic compounds present in the samples (included the ferulic acid used as the marker phenolic compound).

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