

# Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols and phenolic compounds should confer valuable cancer chemopreventive effects

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The aim of this study was to evaluate the fatty acids, tocopherols, squalene, sterols and phenolic antioxidants in three types of argan oil (Moroccan food, Moroccan aesthetic and a French commercial variety) along with a basic comparison with extra virgin olive and sunflower oil. The fatty acid profiles in the argan oils were very similar, with oleic acid (43%) and linoleic acid (36%) and their respective monoacylglycerols predominating. The major vitamer identified was  $\gamma$ -tocopherol with a mean of  $483 \pm 11$  mg/kg, in contrast to  $\alpha$ -tocopherol, which is the major vitamer in olive ( $190 \pm 1$  mg/kg) and sunflower oil ( $532 \pm 6$  mg/kg). The squalene content of the argan oils was very similar with a mean of  $313 \pm 4$  mg/100 g, which is lower than that of the olive oil (499 mg/100 g) but significantly higher than in the sunflower oil (6 mg/100 g). In contrast to olive and sunflower oils in which  $\beta$ -sitosterol is predominant, the major sterols detected in the argan oils were schottenol (mean  $147 \pm 10$  mg/kg) and spinasterol (mean  $122 \pm 10$  mg/kg). The only phenolic compounds other than the tocopherol vitamers which could be readily detected and quantitated were vanillic, syringic and ferulic (probably conjugated to glucose) acids along with tyrosol.

## Introduction

Although data on the incidence of cancer in African countries is sparse and relates mainly to regions, it is evident that the diet of this continent, which is especially high in plant-derived products, confers cancer prevention properties on its inhabitants. For example, the incidence of breast and colon cancer is much lower than in Western Europe and North America (World Cancer Research Fund/American Institute for Cancer Research, 1997).

Lipid peroxidation, by the production of harmful reactive oxygen species (ROS) in humans, is now regarded to be a major cause of mutagenesis, carcinogenesis, cardiovascular disease and ageing. Antioxidants are believed to prevent or delay the onset of these processes. A treatise on the role of antioxidants in cancer prevention is given in Bartsch *et al.* (1999, 2002).

A source of antioxidants is seasoning oils, which have a high calorific value and contain varying amounts of

In contrast to the extra virgin olive oil (793 mg/kg), the concentration of total phenolic compounds is extremely low ( $< 5.0$  mg/kg). Nevertheless, argan oil with its high content of the vitamer  $\gamma$ -tocopherol, squalene and oleic acid is likely to enhance the cancer prevention effects of the Moroccan diet. *European Journal of Cancer Prevention* 12:67–75 © 2003 Lippincott Williams & Wilkins.

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essential fatty acids, such as linoleic acid necessary for the correct development of human tissue (FAO, 1978). An excellent example is extra virgin olive oil, which contains in addition an abundance of phenolic antioxidants including simple phenols, secoiridoids and lignans (Owen *et al.*, 2000a,b,c,d).

Argan oil is a product harvested from the fruits of the argan tree (*Argania spinosa*). Production of argan oil is endemic to south-west Morocco, where it plays a major ecologic and socio-economic role (Charrouf and Guillaume, 1999; Rezanka and Rezankova, 1999). However, in contrast to other seasoning oils, its potential health-protecting properties have been little studied. The traditional method for the production of argan oil is by hand, and is usually conducted by women. The fruits of the tree are harvested and allowed to dry in the sun before the pericarp is removed. The stones (nuts) are broken with rocks and the kernals (3–4 in number) are air-dried in clay containers and slowly roasted. The

roasted kernels are crushed and kneaded into a paste or dough with hot water. The resulting oil/water mixture is separated, furnishing a brown oil with a hazelnut taste. This is termed 'food' argan, which is used for culinary purposes. An 'aesthetic' variety of argan oil is also produced for cosmetic purposes without roasting the kernels. This form of the oil is used for the treatment of dermatologic disease (e.g. acne), hypercholesterolaemia and atherosclerosis among other maladies.

Argan oil is known to be a rich source of linoleic acid and tocopherols (Boukhobza and Pichon-Prum, 1988) and to contain the phenolic antioxidants caffeic acid and oleuropein (Chimi *et al.*, 1988) along with the  $\delta^7$ -sterols schottenol and spinasterol (Boukhobza and Pichon-Prum, 1988). However, definitive data on the lipid and antioxidant content of argan oil is sparse. The objective of this study was to conduct a comprehensive evaluation of the fatty acid, tocopherol, squalene, sterol and phenolic antioxidant content of this nutritionally important seasoning oil endemic to Morocco.

## Materials and methods

### Standard compounds

Tyrosol (*p*-hydroxyphenyl)ethanol, syringic acid, *p*-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, Trolox, vanillic acid, vanillin,  $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\delta$ -tocopherol and  $\text{Fe}_2\text{Cl}_3 \cdot 6\text{H}_2\text{O}$  were obtained from Sigma-Aldrich (Steinheim, Germany). Ferulic acid and ethylenediamine tetra-acetic acid were obtained from Fluka Chemie (Buchs, Switzerland). Acetic acid, methanol, hypoxanthine, xanthine oxidase and salicylic acid were obtained from Merck (Darmstadt, Germany).

### Oils studied

The traditional food and aesthetic varieties of argan oil were kindly donated by the Argan Oil Women's Co-operative (Targanine), Tamanar (Essaouira region), Morocco. The commercial French variety was kindly donated by Mille et une Huiles Company, Paris, France. The extra virgin olive oil from Italy (Apulien) was a gift from Zait (67265 Grünstadt, Germany) while the sunflower oil was purchased from a local supermarket in Heidelberg, Germany.

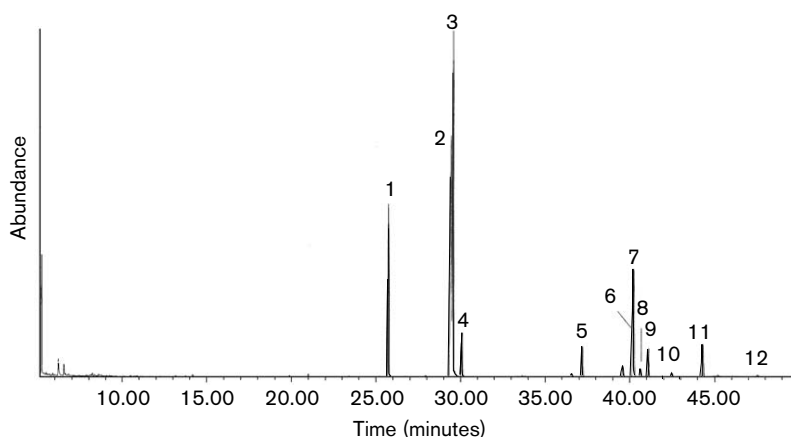
### Preparation of extracts

This was conducted as described by Owen *et al.* (2000c) with some modifications. The dry methanolic extracts were suspended in acetonitrile (2.0 ml) and extracted three times with hexane (1.0 ml) to reduce lipid components. The hexane extracts were discarded, and the acetonitrile solutions were dried under a stream of nitrogen and dissolved in methanol (1.0 ml) prior to analysis.

Table 1 Composition of lipids in the argan oils

Fatty acid	Argan oil composition (%)		
	Food	Aesthetic	Commercial
Palmitic acid	16.4	16.5	15.6
Stearic acid	4.2	3.7	8.5
Oleic acid	43.1	45.0	41.2
Linoleic acid	36.4	35.0	37.9
Monoacylglycerols			
Palmitoyl	15.9	14.0	18.0
Stearoyl	4.5	3.4	3.9
Oleoyl and linoleoyl	79.6	82.6	78.2

Fig. 1



The long-chain fatty acid and monoacylglycerol profile (TMS-ethers) in the aesthetic variety of argan oil as determined by GC/MS after saponification with KOH (10%) in distilled water. (1) Palmitic acid, (2) linoleic acid, (3) oleic acid, (4) stearic acid, (5) monoacylglycerol of palmitic acid, (6) monoacylglycerol of linoleic acid, (7) monoacylglycerol of oleic acid, (8) monoacylglycerol of stearic acid, (9) squalene, (10)  $\delta$ -tocopherol, (11)  $\gamma$ -tocopherol, (12)  $\alpha$ -tocopherol.

Table 2 Content of tocopherol vitamers in the oils

Oil type	Tocopherol vitamer			
	$\gamma$	$\alpha$	$\delta$	Total
Argan (food)	480 $\pm$ 7	35 $\pm$ 1	122 $\pm$ 10	636 $\pm$ 18
Argan (aesthetic)	465 $\pm$ 21	44 $\pm$ 3	120 $\pm$ 5	629 $\pm$ 28
Argan (commercial)	504 $\pm$ 4	46 $\pm$ 2	111 $\pm$ 4	660 $\pm$ 2
Olive	26 $\pm$ 1	190 $\pm$ 1	42 $\pm$ 2	257 $\pm$ 3
Sunflower	39	532 $\pm$ 6	11	582 $\pm$ 6

Data expressed in mg/kg  $\pm$  SEM from duplicate analyses determined by HPLC.

### Acid hydrolysis

This was conducted exactly as described by Owen *et al.* (2000d).

### Unsaponifiable matter

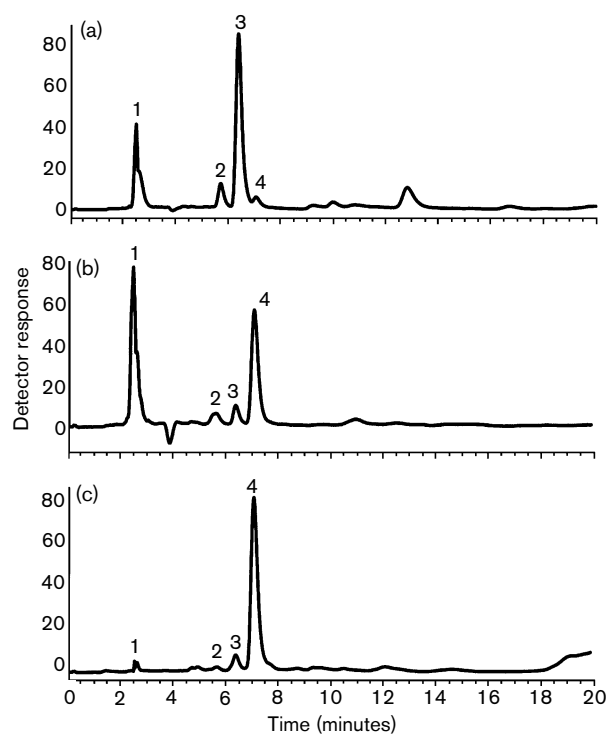
For the preparation of unsaponifiable matter containing predominantly squalene and sterols, each oil (400 mg) was well mixed with KOH (10%) in methanol (50 ml) with the use of sonication in Erlenmeyer flasks (100 ml), and horizontally shaken at 30°C for 24 h. After incubation, distilled water (10.0 ml), ethanol (1.0 ml) and hexane (20.0 ml) were added. The incubates were shaken vigorously in separatory funnels and after partition the hexane layer was collected. The remaining aqueous/alcohol phase was extracted twice more with hexane (20 ml). The hexane extracts were pooled, dried over anhydrous MgSO<sub>4</sub> and the solvent removed *in vacuo*. Dry extracts were suspended in dichloromethane (10.0 ml). Prior to GC-MS, 50  $\mu$ l (dried under nitrogen) of these suspensions were derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (100  $\mu$ l) at 37°C for 15 min.

For the preparation of unsaponifiable matter enriched in tocopherols, long-chain fatty acids and monoacylglycerols, the concentration of KOH (10%) remained the same but methanol was replaced by distilled water. After a similar incubation period, extraction was conducted with dichloromethane (50 ml) twice. The extracts were pooled, worked-up as before and suspended in methanol (2.0 ml) prior to GC-MS.

### High-performance liquid chromatography (HPLC)

Analytical HPLC of the methanolic extracts was conducted on a Hewlett Packard (HP), 1090 liquid chromatograph fitted with a reverse-phase, C-18 column (Latex-Eppelheim, Germany) as described by Owen *et al.* (2000c). Evaluation of the tocopherols (in duplicate), was conducted on the same machine and column using methanol (100%) as the mobile phase run isocratically for 20 min. After each run, the column was washed with dichloromethane for 10 min and the column was reconditioned with methanol also for 10 min prior to the next injection. Instrument control and data handling was by

Fig. 2



The tocopherol profile of the aesthetic variety of (a) argan compared to (b) olive (extra virgin) and (c) sunflower oil as determined by HPLC. (1) Void volume, (2)  $\delta$ -tocopherol, (3)  $\gamma$ -tocopherol, (4)  $\alpha$ -tocopherol.

means of an HP Chemstation operating in the Microsoft Windows™ software environment. Prior to analysis (20  $\mu$ l), the oils were dissolved in an equal volume of dichloromethane. The tocopherol content was monitored at 295 nm and quantitated with reference to standard curves of each vitamer in the range 0–500  $\mu$ g/ml.

### Squalene analysis

Squalene analysis by gas chromatography was conducted as described by Owen *et al.* (2000c).

### Gas chromatography-mass spectrometry (GC/MS)

This was conducted by the methods of Owen *et al.* (2000c) on a Agilent 5973 mass quadrupole spectrometer coupled to a Agilent 6890 gas chromatograph.

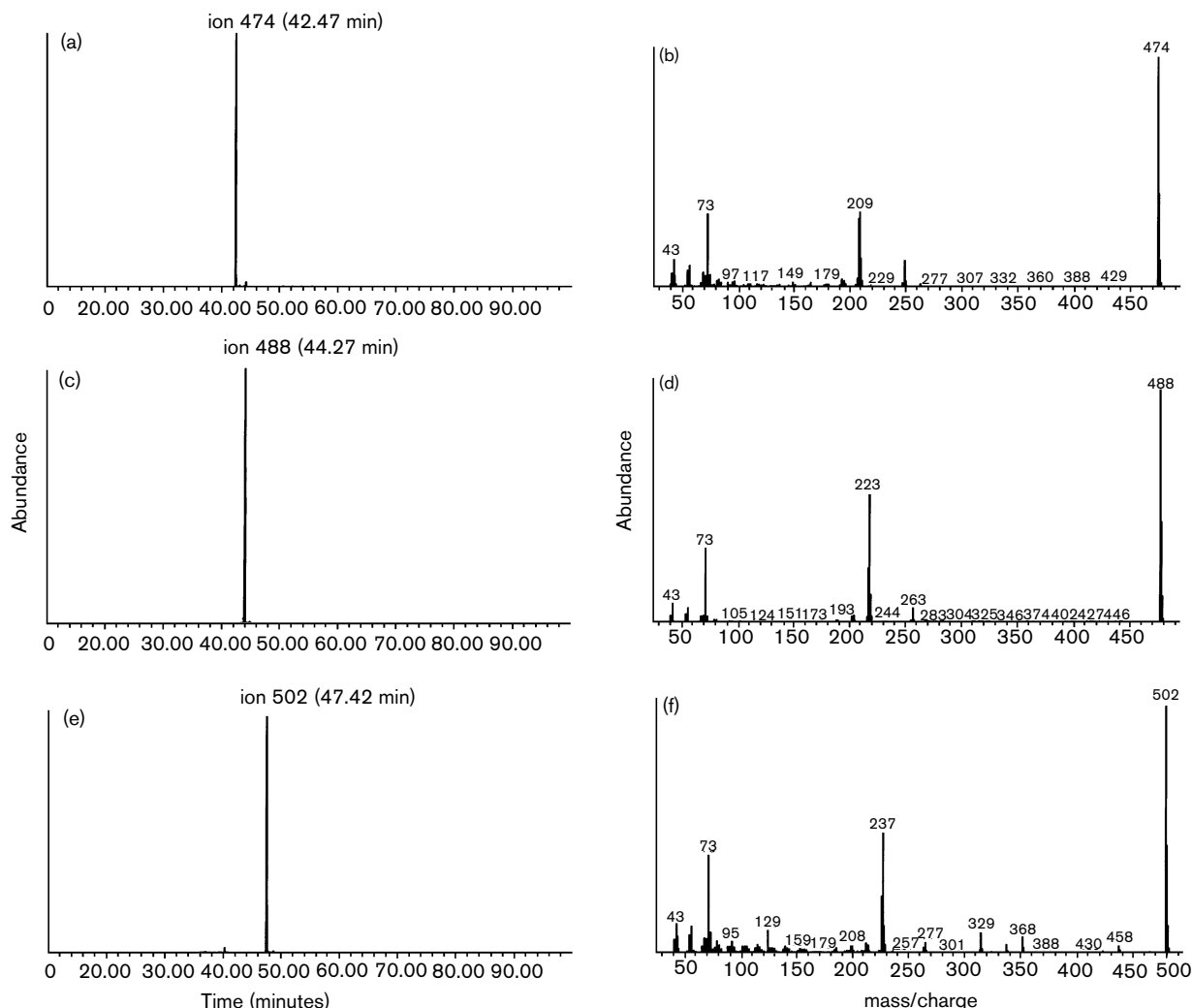
### Antioxidant assay

The antioxidant capacity of methanolic extracts of the oils and pure phenolic compounds was assessed as described by Owen *et al.* (1996, 2000c).

### Statistics

Differences in the antioxidant parameters were calculated by multiple linear regression. Inhibitory concentra-

Fig. 3



The tocopherol profile in the aesthetic variety of argan oil as determined by GC/MS of the unsaponifiable fraction. (a) Selected ion ( $m/z$  474) of  $\delta$ -tocopherol and (b) its mass spectrum. (c) Selected ion ( $m/z$  488) of  $\gamma$ -tocopherol and (d) its mass spectrum. (e) Selected ion ( $m/z$  502) of  $\alpha$ -tocopherol and (f) its mass spectrum.

tions ( $IC_{50}$  values) were determined using the Table Curve programme (Jandel Scientific, Chicago, IL, USA).

## Results

### Long-chain fatty acid and monoacylglycerol profiles

The profiles of the long-chain fatty acids in the three argan oils (Figure 1) studied were very similar and in accordance with previously published data (Table 1). The major unsaturated fatty acid detected was oleic (C18-1  $n$ -9) followed closely by linoleic acid (C18-2  $n$ -6). Linolenic acid (C18-3  $n$ -3) was not detected. On average, of the saturated long-chain fatty acids, palmitic acid (C16-0) predominated with minor amounts of stearic acid (C18-0), representing 16 and 5.5% of the total respectively.

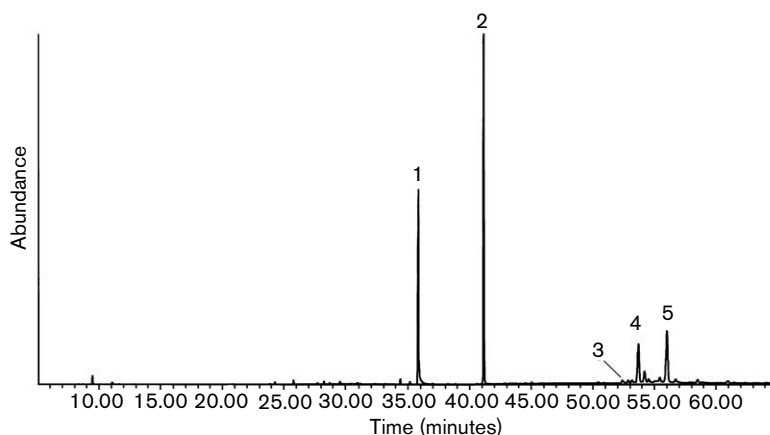
Compared with olive oil (13.5%) argan contains rather more linoleic (36%) but less oleic acid (43 versus 73%). Furthermore, the argan oils contained less linoleic (36% versus 53%) but similar levels of oleic acid (43 versus 39%) compared with sunflower oil.

The profiles of the monoacylglycerols in argan oils closely mirrored those of the long-chain fatty acids (Table 1, Figure 1), with oleoyl and linoleoyl predominating.

### Tocopherol profiles

Because saponification of the oils resulted in degradation of the tocopherol vitamers, especially using 10% KOH in methanol, a softer technique was sought so that the

Fig. 4



Squalene and sterol profile of the aesthetic variety of argan oil as determined by GC/MS of the unsaponifiable fraction after saponification with KOH (10%) in methanol. (1) 5 $\beta$ -Androstan-3,17-dione (internal standard), (2) squalene, (3)  $\delta^{8,22}$ -stigmastadiene-3 $\beta$ -ol, (4) spinasterol, (5) schottenol.

Table 3 Content of squalene in the oils

Oil type	Method	
	GC-MS	GC
Argan (food)	314 $\pm$ 1	319 $\pm$ 3
Argan (aesthetic)	321 $\pm$ 6	311 $\pm$ 2
Argan (commercial)	303 $\pm$ 4	275 $\pm$ 1
Olive (extravirgin)	499	nd
Sunflower	6	nd

Data expressed in mg/100 g  $\pm$  SEM for duplicate samples. Calculated against the internal standard 5 $\beta$ -androstan-3,17-dione (GC-MS, 50  $\mu$ g/ml; GC, 1.0 mg/ml). Correction factor for TIC response = 0.83; for FID response = equivalent. nd, not determined.

absolute values in the oils could be reliably determined. For this we turned to HPLC, and the total amounts of tocopherols detected were very similar in the three types of argan oils (Table 2). Total tocopherol content in the food, aesthetic and a commercial French variety of argan oil represented 636  $\pm$  18, 629  $\pm$  28 and 660  $\pm$  2 mg/kg respectively. This is very close to data previously reported. Until now, however, the major tocopherol reported in argan oil has been  $\alpha$ -tocopherol but in the current study, the data show that the predominant tocopherol is actually  $\gamma$ -tocopherol, representing on average 75% of total tocopherols, compared with  $\delta$ -tocopherol (18%) and  $\alpha$ -tocopherol (7%). This is clearly shown by HPLC (Figure 2) of the diluted and by GC-MS (Figure 3c,d) data of the unsaponifiable fraction (KOH (10%) in distilled water) of the oils. As previously reported, the major tocopherol in olive and sunflower oils is  $\alpha$ -tocopherol (Figure 2) and the total values determined are very close to those previously published (Table 2).

### Squalene

Squalene content of the argan oils was determined by both GLC and GC-MS. Dilution of the oils in dichloromethane and analysis by GLC gave reasonable data but required continual replacement of the column packing material (Owen *et al.*, 2000c). Therefore a GC-MS method was developed. This entails saponification of the fat with KOH (10%) in methanol leaving an unsaponifiable fraction containing predominantly squalene and sterols (Figure 4). Although the squalene content (Table 3) of the three argan oils was very similar (mean 313  $\pm$  4 mg/100 g), a slightly higher content (321  $\pm$  6 mg/100 g) was detected in the aesthetic variety. The squalene content of the argan oils was somewhat lower than that of the extra virgin olive oil (499 mg/100 g) but significantly higher than in the sunflower oil (6 mg/100 g) sample.

### Sterols

The sterol content (Table 4) of the unsaponifiable fraction was also determined by GC-MS (Figure 4). The major sterols detected in argan oils were, on average, schottenol (48%) and spinasterol (40%) with minor amounts of  $\delta^{8,22}$ -stigmastadiene-3 $\beta$ -ol. This contrasts with the major sterol in olive and sunflower oil, which is  $\beta$ -sitosterol. Olive oil also contains lesser amounts of the dimethyl sterols methylenecycloartenol and cycloeucaenol, while campesterol and stigmasterol are also detected in sunflower oil.

### Phenolic compounds

The detection of phenolic compounds in argan oil is a challenge. Using techniques developed for the comprehensive evaluation of phenolic compounds in olive oil

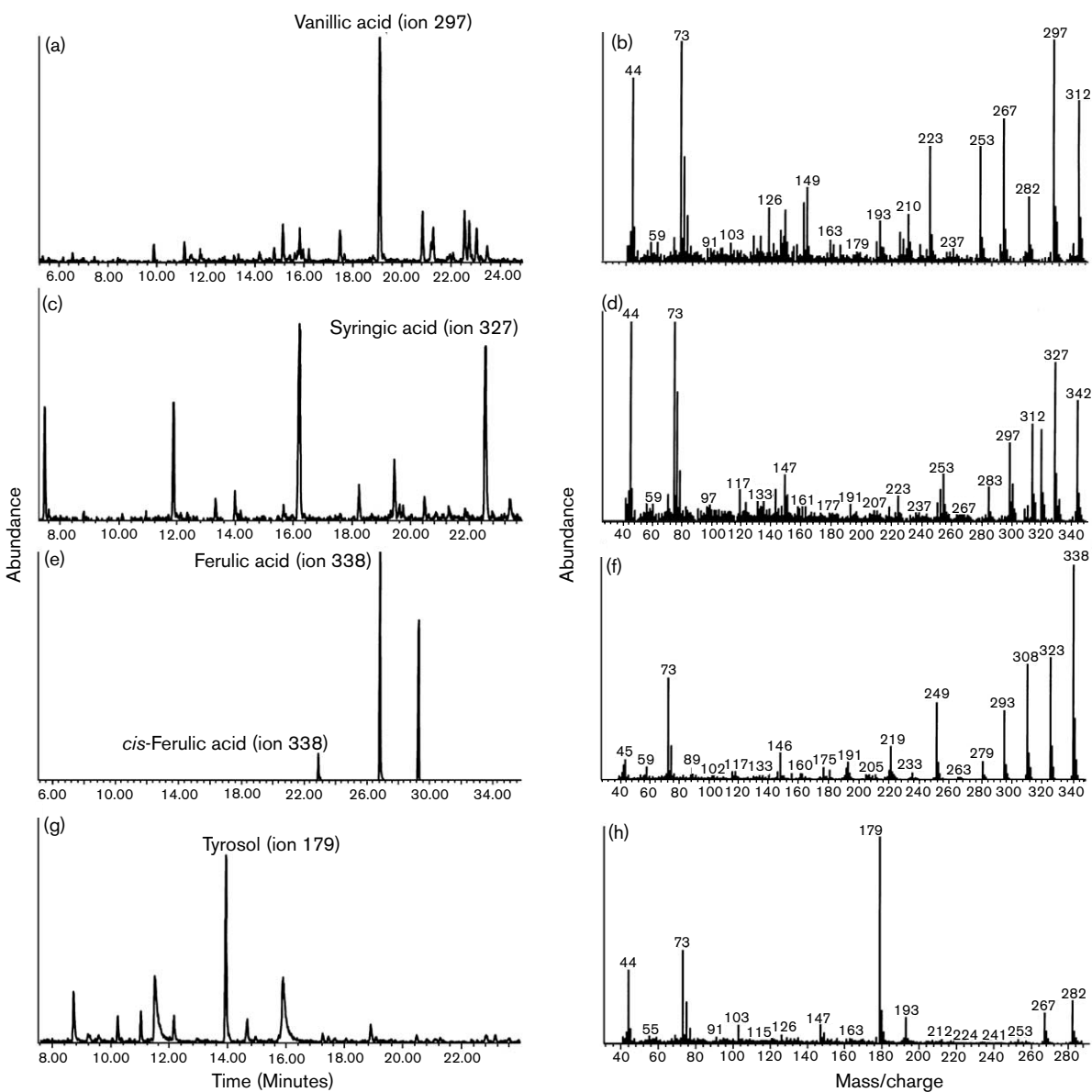
Table 4 Content of sterols in the oils

Sterol	Oil type				
	Argan (food)	Argan (aesthetic)	Argan (commercial)	Olive (extra virgin)	Sunflower
Schottenol	142 ± 11	137 ± 7	163 ± 11	nd	nd
Spinasterol	115 ± 7	100 ± 1	151 ± 9	nd	nd
δ <sup>8-22</sup> -Stigmastadiene-3β-ol	9 ± 1	11	15 ± 1	nd	nd
β-Sitosterol	nd	nd	nd	156 ± 3	312 ± 18
Campesterol	nd	nd	nd	12 ± 1	51 ± 3
Stigmasterol	nd	nd	nd	nd	37 ± 2
Others	29 ± 1	24 ± 2	28 ± 2	151 ± 10 <sup>a</sup>	77 ± 2
Total	295 ± 20	272 ± 10	357 ± 23	319 ± 14	477 ± 23

Data expressed in mg/100 g ± SEM from duplicate analyses by GC-MS.

<sup>a</sup>These are the dimethyl sterols 24-methylenecycloartenol (47 ± 3 mg/100 g) and cycloeucatenol (104 ± 7 mg/100 g).  
nd, not detected.

Fig. 5



GC-MS (TMS ethers) of the phenolic compounds detected in the food variety of argan oil. (a) Selected ion ( $m/z$  297) of vanillic acid and (b) its mass spectrum. (c) Selected ion ( $m/z$  327) of syringic acid and (d) its mass spectrum. (e) Selected ion ( $m/z$  338) of ferulic acid\* and (f) its mass spectrum. (g) Selected ion ( $m/z$  179) of tyrosol and (h) its mass spectrum. \*Only detected after acid hydrolysis of the methanolic extracts.

Table 5 Content of phenolic compounds detected in the oils

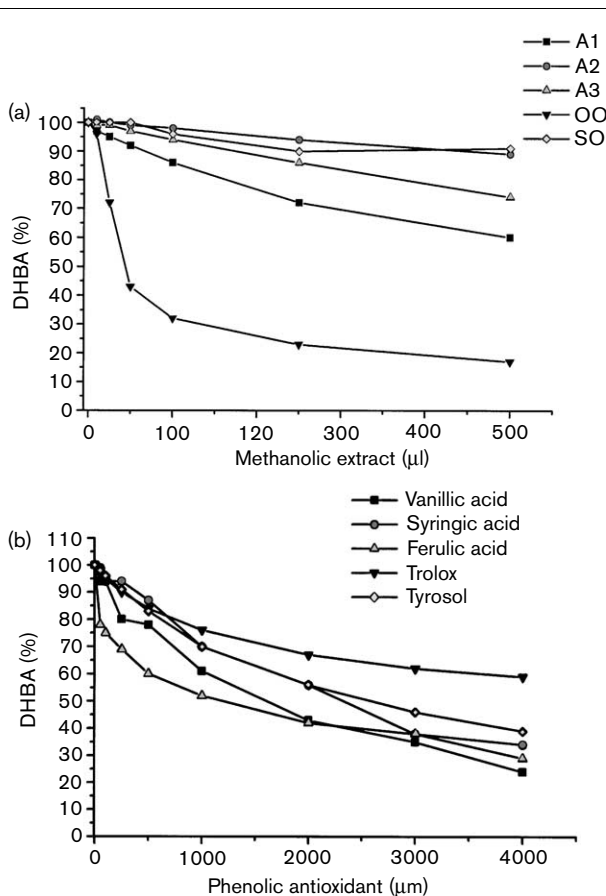
Oil type	Phenolic compound					
	Vanillic acid	Syringic acid	Ferulic acid <sup>a</sup>	Tyrosol	Others	Total
Argan (food)	67±3	37±5	3147±20	12±1	0	3263±29
Argan (aesthetic)	6±1	0	3211±5	3	0	3220±6
Argan (commercial)	123±12	68±4	3470±13	52±2	0	3713±31
Olive (extra virgin)	359±7	0	51±2	19 573±37	773 000±53 <sup>b</sup>	792 983±99
Sunflower	0	0	0	0	0	0

Data expressed in  $\mu\text{g}/\text{kg} \pm \text{SEM}$  as determined in duplicate by GC-MS relative to standard curves of the authentic compounds in the range 0–50  $\mu\text{g}/\text{ml}$ .

<sup>a</sup>Detected in methanolic extracts only after acid hydrolysis and includes the *cis*-isomer.

<sup>b</sup>Includes hydroxytyrosol, secoiridoids, lignans and flavonoids determined by HPLC as described by Owen *et al.* (2000c,d).

Fig. 6



Attenuation of reactive oxygen species detection. (a) By methanolic extracts of the oils in the hypoxanthine/xanthine oxidase assay. A-1 = food argan oil ( $\text{IC}_{50} = > 2.0 \text{ ml}$ ), A-2 = aesthetic argan oil ( $\text{IC}_{50} = > 2.0 \text{ ml}$ ), A-3 = French commercial argan oil ( $\text{IC}_{50} = 1.90 \text{ ml}$ ), OO = extra virgin olive oil ( $\text{IC}_{50} = 44 \mu\text{l}$ ), SO = sunflower oil ( $\text{IC}_{50} = > 2.0 \text{ ml}$ ). (b) By authentic phenolic compounds detected in the oils in comparison to Trolox. Vanillic acid ( $\text{IC}_{50} = 1.63 \text{ mM}$ ), syringic acid ( $\text{IC}_{50} = 2.20 \text{ mM}$ ), ferulic acid ( $\text{IC}_{50} = 1.17 \text{ mM}$ ), Trolox ( $\text{IC}_{50} = 12.24 \text{ mM}$ ), tyrosol ( $\text{IC}_{50} = 2.51 \text{ mM}$ ).

(Owen *et al.*, 2000a,b,c,d), such compounds are not readily detected by HPLC in the methanolic extracts. To detect the phenolic compounds from this matrix it is necessary to subject the methanolic extracts to GC-MS.

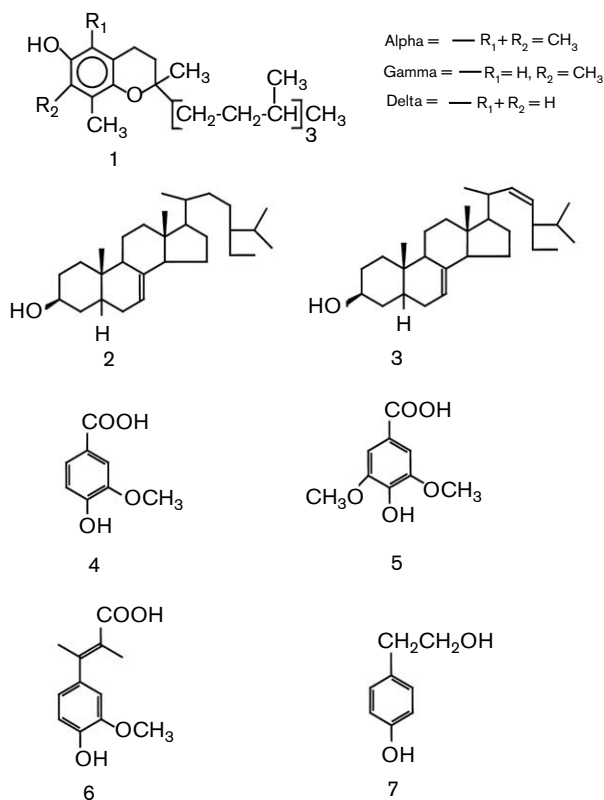
Using this technique the major phenolics were identified (Figure 5) as vanillic, syringic and ferulic acids plus tyrosol. Of these, ferulic acid was detected only in acid hydrolysates of the methanolic extracts suggesting conjugation to a sugar. The amounts determined were extremely low (Table 5) and were similar in crude extracts and after fractionation by silicic acid chromatography. *p*-Hydroxybenzoic acid, 3,4-dihydroxybenzoic acid and vanillin were also identified in trace amounts in these enriched fractions. In addition, a number of unidentified compounds with UV spectra similar to phenolics were also detected and warrant further investigation.

#### Antioxidant profiles of the oils

A major benefit, which can be derived from consumption of oils high in phenolic antioxidants is the ability of the latter to scavenge ROS. That the major simple phenols tyrosol and hydroxytyrosol are bioavailable in humans has been shown clearly by the studies of Covas *et al.* (2002), who demonstrated their presence in urine after daily challenges with olive oil. These data also demonstrate the ability of tyrosol to interact with ROS yielding higher than expected excretion rates of hydroxytyrosol based on the challenge given. This is consistent with the data of Owen *et al.* (2000a) demonstrating the scavenging by tyrosol of ROS generated by the faecal matrix yielding hydroxytyrosol.

Therefore, a basic evaluation of the antioxidant potential of methanolic extracts was conducted. The data show that of the three types of argan oil, the extracts of the food and French commercial oils are superior to the aesthetic variety. The antioxidant capacity of methanolic extracts of the food and commercial French varieties are clearly lower than that of extra virgin olive oil but moderately better than sunflower oil (Figure 6a). The antioxidant capacity of the four phenolic compounds definitively detected are superior to the soluble form of vitamin E, Trolox (Figure 6b) in the hypoxanthine/xanthine oxidase system, but their content in the oils did not correlate with the antioxidant capacity of the methanolic extracts.

Fig. 7



Structures of various compounds detected in argan oil. (1) Tocopherols, (2) schottenol, (3) spinasterol, (4) vanillic acid, (5) syringic acid, (6) ferulic acid, (7) tyrosol.

### Structures

The structures elucidated for some of the compounds detected in argan oil are depicted in Figure 7.

### Discussion

The major identifiable end-point of this comprehensive study of argan oils is that the predominant vitamer is  $\gamma$ -tocopherol not  $\alpha$ -tocopherol and is therefore clearly at odds with reports in the literature (Boukhobza and Pichon-Prum, 1988). The reasons for this discrepancy are probably methodological, because in our study the structures of the vitamers were confirmed by GC-MS and the assignments are unequivocal.

*In vivo* data (Jiang *et al.*, 2000) indicate that  $\gamma$ -tocopherol may be a far more important antioxidant than  $\alpha$ -tocopherol. Both  $\gamma$ -tocopherol and its metabolite 2,7,8-trimethyl-2-( $\beta$ -carboxyethyl)-6-hydroxychroman ( $\gamma$ -CEHC), which is mainly excreted in urine, unlike  $\alpha$ -tocopherol and its corresponding metabolite inhibit cyclooxygenase activity (Jiang *et al.*, 2000). In addition, it is noteworthy that supplementation with high doses of  $\alpha$ -tocopherol depletes both plasma and tissue  $\gamma$ -tocopherol

in contrast to supplementation with  $\gamma$ -tocopherol, which increases both (Jiang *et al.*, 2000). It is highly likely that diets providing substantial amounts of  $\gamma$ -tocopherol protect against ROS-mediated inflammation. *In vitro*, it is difficult to compare the relative efficacy of tocopherol vitamers with other phenolic antioxidants. *In vivo*, the tocopherol vitamers are incorporated into cell membranes and their antioxidant capacity is modulated by the concentration of vitamin C. In the case of phenolic antioxidants, a continuous supply is required to maintain an antioxidant environment, but in the case of the tocopherol vitamers, stable incorporation into cell membranes and regeneration of the quinone radical (produced after interaction with ROS) to the parent compound by vitamin C (Halliwell and Gutteridge, 1993) renders their pharmacology rather different in that their half-life is far longer. Therefore argan oil as a major dietary source of  $\gamma$ -tocopherol may confer health benefit effects on the Moroccan population in a manner somewhat different from that of countries within the Mediterranean basin, where a continuous supply of phenolic antioxidants is obtained from olives and olive oil in addition to those from fruits and vegetables.

Another point to note is that similar to olive oil, argan oil (compared to many other seasoning oils) contains relatively high contents of squalene which is suggested to be protective against skin cancer (Newmark, 1997) and enhances excretion of xenobiotics in rats and mice (Kamimura *et al.*, 1992).

The major sterols identified in argan oils were spinasterol and schottenol, in agreement with the literature data. These sterols are very rare in vegetable oils. In animal studies the effects of spinasterol are documented to be a reduction in plasma and liver cholesterol levels of mice, modulated by increasing faecal cholesterol excretion (Uchida *et al.*, 1983). Antitumorigenic potential of spinasterol has also been demonstrated by Villasenor and Domingo (2000), whereas schottenol exhibits an anticarcinogenic and cytotoxic potential (Arizawa *et al.*, 1985).

Although the content of phenolic antioxidants in argan oils is extremely low, vanillic, syringic and ferulic (probably glycosylated) acid, along with tyrosol were definitively identified and quantitated. This contrasts with a previous publication (Chimi *et al.*, 1988) which identified caffeic acid and oleuropein as the major phenolic constituents. In the current study these were not detected.

In conclusion, argan oil is shown to contain  $\gamma$ -tocopherol as the major vitamer. The oil is relatively rich in squalene but has very low quantities of phenolic constituents (<5 mg/kg), other than the tocopherol vitamers.



However the definitive presence and quantitation of vanillic, syringic, ferulic acid and tyrosol are described for the first time. The unidentified phenolic-like compounds warrant further investigation.

## Acknowledgements

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