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ORIGINAL PAPER

Detection of Argan Oil Adulteration Using Quantitative Campesterol GC-Analysis

Miloudi Hilali · Zoubida Charrouf · Abd El Aziz Soulhi · Larbi Hachimi · Dominique Guillaume

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Abstract Detection of edible oil adulteration is of utmost important to ensure product quality and customer protection. Campesterol, a sterol found in seed oils, represents less than 0.4% of argan oil total sterol content. Quantitative analysis of campesterol by gas chromatography of argan oil and of a mixture of argan oil and readily commercially available vegetable oils, consecutively with sterol separation, was carried out. Our study clearly demonstrated that determination of the campesterol level in argan oil (or oil presented as argan oil) can be proposed as the major analysis method to assess unambiguously argan oil purity up to 98%.

Keywords Argan oil · *Argania spinosa* · Argan tree · Campesterol · Adulteration · Morocco

Introduction

Alimentary argan oil is an oil of high dietetic value obtained by crushing the briefly roasted kernels from the argan tree (*Argania spinosa* (L.) Skeels) fruits [1]. This oil is exclusively produced in Morocco, where argan trees grow naturally. In this Northwestern African country, argan oil is traditionally consumed fresh or used for cooking. Because of its high level of unsaturated fatty acids and antioxidants [2], both types of compounds are known to reduce the risks of cardiovascular diseases [3, 4], argan oil popularity has recently crossed the Moroccan boundaries and reached most of the industrialized countries. This tendency is nowadays strongly reinforced by the scientific confirmation of argan oil's potential pharmacological properties [5–7] and the continuous discovery of anticancer substances in argan oil [8–11]. Since 1997, argan oil has been widely sold in Western-Europe, North-America and Japan. In 2002, the French Agency of the Sanitary Safety of Food (AFSSA) officially allowed the marketing of argan oil in France [12].

Argan oil is produced in low yield and its preparation is time consuming. An average value of 34 kg of dried fruits (the production of seven to eight trees), and 20 h of work are necessary to obtain 1 L of oil [1]. Consequently, argan oil is expensive. In 2005, in developed countries, the average price of a 100 mL bottle of argan oil was around US$20. Such a price is likely to incite unscrupulous behavior and, consequently, there is an urgent need to control and ascertain the quality of argan oil.

Quality control of food, and specifically vegetable oils, is not a new problem [13] and two kinds of evaluation should be envisioned depending on the suspected adulteration. Either the marketed product has really been prepared from the right plant but its preparation did not follow the correct procedure, leading to a low quality product, or the oil sold results from the blending of a cheap oil with the valuable one.

M. Hilali · Z. Charrouf (✉)
Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculté des Sciences,
Université Mohamed V-Agdal,
B.P. 1014 R.P, Rabat, Morocco
e-mail: zcharrouf@menara.ma

A. E. A. Soulhi · L. Hachimi
Laboratoire Officiel d'Analyses et de Recherches Chimiques,
25 rue Rahal Nichakra, Casablanca, Morocco

D. Guillaume (✉)
FRE2715-CNRS, 51096 Reims cedex, France
e-mail: dominique.guillaume@univ-reims.fr

So far, only two studies aimed at discriminating argan from other edible oils are available. One specifically concerns the discrimination from olive oil [14] while the second is more general [15]. Recently, we have reported a list of argan oil physico-chemical parameters and shown that some of them can be used as a marker to detect argan oil of unsatisfactory quality [16]. We herein demonstrate that because of its unusual campesterol ((24*R*)-24-methyl-Δ-5-cholestene-3β-ol) content, argan oil adulteration with commonly found cheap oils can be easily detected.

Detection of oil adulteration is a complex problem. Indeed, blending of two oils of similar composition can be hard to detect as witnessed by the difficulties of detecting adulteration of olive oil with hazelnut oil. Authentication methods should allow a rapid screening, allow a simple sample preparation, and require easily available reagents. Authentication methods applied to oils are generally classified as physical (non-separative) or chemical (separative). The former technique requires a combination of measurements carried out on the sample whereas the later 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 its removal from the adulterant make the adulteration unlikely. With all these considerations in mind, we started to analyze the results of the argan oil composition that we had recently carried out [16]. Although argan oil contains high levels of linoleic acid, this fatty acid could not be used as a marker for peanut oil or sesame oil, two common and cheap oils, contain similar levels [17]. Sterols are also good candidates for adulteration detection [18, 19]. Increasing the depth of our analysis, we observed that five sterols are commonly found in argan oil: campesterol ((24*R*)-24-methyl-cholestane-3β-ol), spinasterol ((24*R*)-24-ethyl-5α-cholesta-7,22-dien-3β-ol), stigma-8,22-dien-3-ol, schottenol ((24*R*)-24-ethyl-5α-cholesta-7-en-3β-ol or (24*S*)-Δ^7-stigmasterol), stigmasta-7, 24-dien-3-ol [16]; sterol concentration being less than 0.4, 34–42, 4–7, 42–49, 2–7%, respectively, [16]. Hence, campesterol, a sterol found in high concentration in most marketed oils, is only present as traces in argan oil. Beta-sitosterol is also absent in argan oil, however in our GC-conditions its retention time was very close to that of spinasterol. Since campesterol retention time (RT = 24.17 min) was clearly distinct from that of other sterols in the GC sterol chromatogram (stigma-8,22-dien-3-ol: RT = 26.42 min, spinasterol: RT = 28.16 min, schottenol: RT = 31.10 min, stigmasta-7,24-dien-3-ol: RT = 31.94 min), its precise quantification could be easily unambiguously achieved (Fig. 1). So we selected campesterol as the adulteration marker. To validate our method, we used GC to determine the camposterol level of mixtures of argan oil and common oils, and consequently putative adulterated oils, at a concentration ratio of 99/1, 98/2, and 95/5.

Experimental Procedures

Argan Oil Preparation

Argan oil was extracted by pressing in the cooperative of Tidzi (Essaouira county, Morocco) according to our previously reported procedure [16].

Other Oils

Other oils (presented as being the highest commercially available grade) were purchased from retail stores in Rabat and Casablanca (Morocco).

Analytical Methods

Campesterol quantitative analysis was carried out by GLC-FID/capillary column following the NP EN ISO 12228:1999 method [20], see also [21] for the detailed procedure). Sterols purified from unsaponifiable matter by HPLC (HP 1,100, Agilent Tech./Sicotel, Morocco equipped with column Microsorb Si60, Varian/Boyer, Morocco) were transformed into their trimethylsilyl ether counterparts using pyridine, hexamethyldisilazane, and trimethylchlorosilane 9:3:1 (V/V/V) [16, 17, 21]. The sterol profile was analyzed using a gas

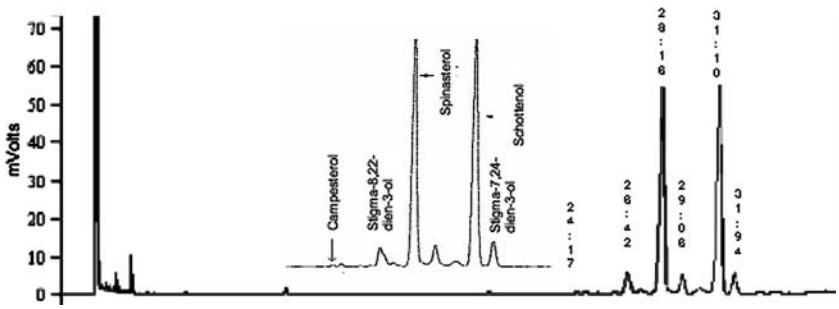

Fig. 1 Full GC-sterol profile of argan oil. (*Insert*: Chemical labeling of the peaks)

Table 1 Range of percentage of campesterol and of total sterol content in studied vegetable oils

Oil	Percentage of campesterol in total sterols (observed in our studied oil sample)	Total sterols in mg/100 g (observed in our studied oil sample)
Argan	<0.4 (0.29 ± 0.04)	142–223 (154.3 ± 3.1)
Soyabean	15.8–24.2 (19.8 ± 0.1)	180–410 (381.5 ± 4.5)
Rapeseed	24.7–38.6 (27.9 ± 0.1)	480–1130 (482.4 ± 4.6)
Sunflower	6.5–13 (10.5 ± 0.2)	240–460 (345.1 ± 3.6)
Apricot	2.2–5.6 (3.8 ± 0.07)	325–515 (342.9 ± 3.7)
Arachis	12.0–19.8 (17.3 ± 0.2)	90–290 (279.4 ± 3.6)
Hazelnut	4–7 (4.31 ± 0.2)	75–195 (168.2 ± 2.3)
Sesame	10–20 (18.5 ± 0.2)	450–1900 (540 ± 5.4)
Olive	2–4 (2.85 ± 0.1)	98–184 (182.7 ± 4.4)

Range values are taken from [19, 22–24] and precise values indicated between brackets represent the percentage found in the oil samples used in this present study

[a] Values given are the average of five analyses

phase chromatograph (HP 6,890, Agilent Tech./Sicotel, Morocco), fitted with a chroma pack CP Sil 8 C B column (30 m × 0.32-mm i.d.) (Agilent Tech./Sicotel, Morocco), and a flame ionization detector (Agilent Tech./Sicotel, Morocco). The temperature of the injector and detector were both 300 °C. The column temperature was 200 °C and programmed to increase at the rate of 10 °C/min to 270 °C. The carrier gas was dry oxygen-free nitrogen with an internal pressure of 8.6 bar. Sterol identification was achieved using reference samples and co-injection when necessary. Sterol quantification was achieved by use of an internal 0.2% chloroform solution of α-cholestanol. In these conditions, campesterol had a retention time of 24.19 min.

Statistical Analysis

The reported results are the average value of at least five independent measurements. The results are shown as tables of mean values. The differences between the values on different oil samples were analyzed using the analysis of variance, after the homogeneity of variance had been tested (Tukey test).

Results and Discussion

Table 1 shows the percentage range of campesterol and total sterol in the nine oils studied. Since our previous study had revealed a maximum level of campesterol of 0.39% in mechanically prepared argan oil, we considered the value of 0.4% as the highest percentage of campesterol acceptable in pure argan oil. Four of the possibly adulterated oils studied contained a high percentage of campesterol (above 10% of the total sterols) whereas three oils (olive, hazelnut, and apricot oil) have low percentage between 2 and 7%, sunflower oil being intermediate (6.5–13%).

To be able to validate our method of adulteration detection, we determined the camposterol level of all the studied oil samples. Since we were deliberately "adulter-

Table 2 Calculated (calc.) and observed (obs.) percentage of campesterol in mixtures of argan oil and other oil at concentrations of 100, 99, 98, 95% (argan vs. other oil)

Argan oil (%)	Campesterol (% of total sterols)							
	Soyabean oil		Rapeseed oil		Sunflower oil		Apricot oil	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
100	0.29 (±0.04)		0.29 (±0.04)		0.29 (±0.04)		0.29 (±0.04)	
99	0.64 (±0.12)	0.76	1.15 (±0.23)	1.25	0.58 (±0.03)	0.51	0.38 (±0.05)	0.37
98	1.07 (±0.08)	1.23	2.06 (±0.14)	2.18	0.75 (±0.07)	0.73	0.45 (±0.09)	0.44
95	2.35 (±0.19)	2.53	4.47 (±0.06)	4.75	1.45 (±0.06)	1.36	0.78 (±0.08)	0.65
0	19.8 (±0.1)		31.9 (±0.1)		10.5 (±0.2)		3.8 (±0.07)	

	Olive		Sesame		Hazelnut		Arachis	
	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.	Obs.	Calc.
100	0.29 (±0.04)		0.29 (±0.04)		0.29 (±0.04)		0.29 (±0.04)	
99	0.29 (±0.03)	0.32	0.88 (±0.01)	0.90	0.25 (±0.08)	0.33	0.51 (±0.06)	0.59
98	0.32 (±0.06)	0.35	1.41 (±0.07)	1.48	0.34 (±0.06)	0.38	0.97 (±0.05)	0.89
95	0.43 (±0.06)	0.44	3.35 (±0.11)	3.08	0.45 (±0.09)	0.51	1.67 (±0.09)	1.77
0	2.85 (±0.1)		18.25 (±0.2))		4.31 (±0.2)		17.3 (±0.2)	

[a] Values given are the average of five analyses

ating" our argan oil samples using oils of known campesterol level, the expected level of campesterol in the mixture could be calculated to certify the accuracy of our technique. Table 2 lists campesterol levels (observed and calculated) for argan oil and blends of argan oil with 1, 2, and 5% of the potential adulterating oils.

Our results clearly show a good correlation between calculated and measured campesterol levels validating the accuracy and reliability of our GC method to quantify edible oil sterols. The argan oil sample we studied contained 0.29% of campesterol, a value slightly above the average value (0.22%) calculated from the 16 mechanically prepared oil samples [16]. For all the blending carried out using campesterol rich oils, campesterol levels above 0.4% were indeed detected at adulteration levels of 1% as anticipated from the campesterol calculated values. Similar results were obtained with sunflower oil. For the low campesterol containing oils (olive, hazelnut, and apricot oils) our method allows the establishment of a 95% purity label in the case of olive and hazelnut oil and 98% purity label for apricot oil. Should more precise quality labels be needed, the campesterol level determination could be combined with the oleic acid level analysis since the three oils have levels of this kind of fatty acid much higher than that of argan oil.

Our study suggests that GC campesterol level determination is 95% precise, reliable, and easy to use method for the ascertainment of the quality of argan oil. Possibly complemented with a fatty acid analysis, this method should be very useful for guaranteeing argan oil purity.

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