Is geographic origin a good marker for cumin seed oil (*Cuminum cyminum* L.)?

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We evaluated the chemical variability of cumin oil produced in different regions of Morocco to verify if a geographic origin label represents useful information to depict cumin (*Cuminum cyminum*) fixed-oil quality oil composition, assessed by chromatographic analysis, revealed significant differences among regions for some investigated traits. Petroselinic acid was consistently shown to be the main fatty acid with amounts between 54.9% and 60.9%. The variation for linoleic acid was a little smaller ranging between 30.1% and 31.3%. The oil is characterised by high content of α-tocopherol (20.9-50.3 mg/100 g) and α-tocotrienol (37.2 – 72.4 mg/100 g). In addition, our results showed that oil content varies between 16.3% and 25.7%. The oil showed a high oxidative stability in the Rancimat test at 110°C varying from 25 – 34 h, depending on the region of harvest. Combining our results and literature data, we suggest that information on seed quality would be more useful for consumers or industrials than the geographic origin.

Keywords: seed-oil quality, geographic origin, fatty acid, petroselinic acid.

1. INTRODUCTION

To meet an ongoing consumer demand, new natural sources, beneficial to human health, are consistently searched for. Alongside, to meet an ongoing industrial demand, new economic crops supporting a sustainable and viable farming-model are also consistently searched for.
Cumin (*Cuminum cyminum* L.), belonging to the Apiaceae family, is one of the oldest spices [1]. It is a very important ingredient in the culinary world and is highly popular all around the world. Cumin seeds are also used in the folk therapy [2]. Therefore, cumin seeds have a strong nutraceutical potential [3]. Cumin seeds are essential - and fixed-oil rich [2]. More specifically, regarding its fixed-oil content, cumin seeds are rich in petroselinic acid [4] a relatively rare octadecenoic acid whose unsaturation occurs between C6 and C7. Therefore, petroselinic acid that is already used in the cosmetics and pharmaceutical industries [3-6] could be used as the precursor of a large variety of chemical compounds [7, 8] prepared following the green chemistry principles [9]. In animal diet domain, the use of a cumin seed meal has also been envisioned as an alternative to wheat bran [10]. For all these reasons, cumin oil has been recently listed in a list of 25 oils presenting implications for industrial applications [11]. Cultivation of cumin plants on a large scale, and consequently the cumin seed production, could undoubtedly open new opportunities in the human, animal-food and industrial markets, as well.
The country of origin of cumin seeds is frequently mentioned and commercially used as a tacit quality marker. Such terminology leads to the assumption that cumin oil content produced from seeds originating from the single country

would be a homogeneous and well-defined compound. Accordingly, the geographic origin has been shown to dramatically impact the chemical composition of cumin seed [2]. Nevertheless, no official regulation rules cumin oil composition preventing the setting of quality parameters and consequently the ascertainment of quality certification.
In this study, using cumin oil from 6 different origins in Morocco, we prove that a country-based denomination is irrelevant and misleading since variations, in terms of yield and chemical content, can be observed within the borders of a single country.
For this purpose, we selected 6 samples of Moroccan cumin, of different geographic origin (Bouarfa, Kalaat Sraghna, Rhamna, Chamaaia, Alnif and Tazarine) to describe the chemical composition of fatty acids and tocopherols of the lipid fraction of cumin seeds. Additionally, the oxidative stability of each oil sample was also determined.

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL AND CHEMICALS

Mature cumin seeds were harvested in June 2015 in six locations in Morocco: Bouarfa (32°31'51" N, 1°57'47" W; elevation: 1174 m), Tazarine (33°5'48" N, 4°10'48" W; elevation: 972 m), Alnif (31°06'42" N, 5°10'13" W; elevation: 882 m), Rhamna (32°46'48" N, 8°57' W; elevation: 482 m), Kalaat Sraghna (32°03' N, 7°24' W; elevation: 400 m), and Chemaïa (32°04'14" N, 8°39'12" W; elevation: 364 m). After the harvest, the seeds were sundried for 24h and stored at 4°C until processed.
All reagents were of analytical or HPLC grade. Iso-octane and iso-propanol used as HPLC mobile phase were purchased from Professional Labo (Casablanca, Morocco).

2.2 METHODS

2.2.1 Oil content

Twenty grams of finely ground seeds were extracted in a Soxhlet apparatus with 150 mL of boiling n-hexane for 8 h. The organic phase was collected, concentrated under vacuum, and dried until weight constancy at 105°C. Extraction yield was determined using the official method ISO 659:2009 [12], and the oil was used for the analyses.

2.2.2 Fatty acid (FA) composition

Total lipid extract was transesterified into fatty acid methyl esters (FAMEs) by shaking a solution of 60 mg oil and 3 mL of hexane with 0.3 mL of 2N methanolic potassium hydroxide. Fatty acid composition was determined using the ISO 5508:1990 [13] method. FAMEs were analysed by gas chromatography in a splitless mode using a Varian CP-3800 (Varian Inc.) chromatograph equipped with a FID. The column used was a CP-Wax 52CB column (30 m × 0.25 mm i.d., film thickness 1-2 µm; Varian Inc., Middelburg, The Netherlands). The carrier gas was helium and the total gas flow rate was 1 mL/min. The initial and final column temperature was 170°C and 230°C, respectively, and the temperature was increased in steps of 4°C/min. The injector and detector temperature were 230°C. Data were processed using a Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA). Results were expressed as the relative percentage of each individual FA present in the sample.

2.2.3 Tocopherol composition

High performance liquid chromatography (HPLC) was used to determine tocopherols with a solution of 250 mg of oil in 25 mL of n-heptane as recommended by the ISO 9936:2016 [14] method. Tocopherols were analysed by HPLC using Shimadzu CR8A instruments (Champ sur Marne, France) equipped with a C18-Varian column (25 cm × 4 mm; Varian Inc., Middelburg, The Netherlands). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). The eluent used was a 99:1 isooctane/isopropanol (V/V) mixture, flow rate of 1.2 mL/min.

2.2.4 Oxidative stability

The oxidative stability was evaluated by the Rancimat method. Stability was expressed as oxidative induction period (IP, hours) measured at 110°C, according to the method ISO 6886:2006 [15], on a Rancimat 743 (Metrohm Co, Basel) apparatus using 3 g of oil sample with an air flow of 20 L/h. Volatile oxidation products were stripped from the oil and dissolved in cold water and its conductivity increased progressively. The time taken to reach the turning point of the measurement curve was given as oxidative induction period.

2.2.5 Statistical analysis

Analyses were carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance (ANOVA). The level of significance for all analyses was $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. OIL CONTENT

Six cumin seed sites distributed in the Morocco's cumin production area were evaluated in this study (Figure 1). The oil content of the cumin seeds (Table I) significantly varied as a function of the harvest locality. Oil content ranged from 16.3 g/100 g (Tazarine) to 25.7 g/100 g (Kalaat Sraghna). A similar high oil

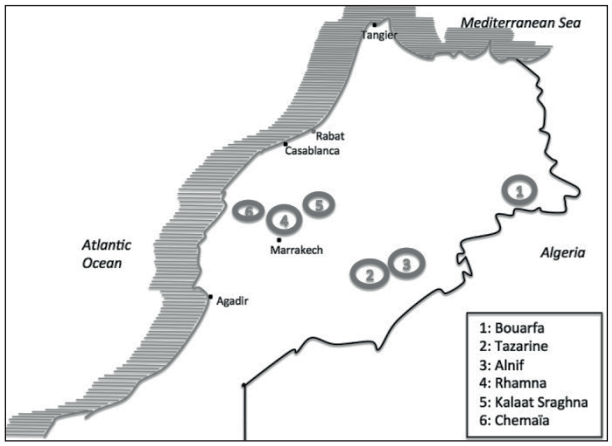

Figure 1 - Location of the evaluated cumin seed sites of production within Morocco.

examined our results in the light of reported cumin seed-oil content.

3.2. Fatty acid composition

Ten fatty acids were identified in our evaluated cumin oil samples. Major ones are listed in Table I. In all studied samples, petroselinic acid [(6*Z*-octadec-6-enoic acid)] was, by far as major fatty acid identified with an average content of 58.9% Such high level likely confirms the maturity of all cumin seeds without necessarily demonstrating an identical degree of maturity for all samples. However, petroselinic acid was found in cumin seeds grown in Chemaïa, with the relatively low content of 54.9%, a value significantly different from all other analyses. Petroselinic acid content in

Table I - Oil yield (g/100 g) and main fatty acid composition (%) of cumin seeds cultivated in different localities.

	Bouarfa	Tazarine	Alnif	Rhamna	Kalaat Sraghna	Chemaïa
Oil yield	18.6 ± 0.2^{a}	16.3 ± 0.8^{b}	22.0 ± 1.4^{c}	19.8 ± 0.5^{a}	25.7 ± 1.2^{d}	19.0 ± 1.1^{a}
Acids:						
Palmitic	3.0 ± 0.1^{a}	3.3 ± 0.1^{a}	3.4 ± 0.1^{a}	2.9 ± 0.1^{a}	3.3 ± 0.1^{a}	3.0 ± 0.1^{a}
Petroselinic	59.5 ± 1.0^{a}	60.5 ± 1.4^{a}	58.2 ± 0.5^{a}	59.5 ± 0.6^{a}	60.9 ± 1.1^{a}	54.9 ± 1.2^{b}
Oleic	2.3 ± 0.1^{a}	1.7 ± 0.2^{b}	1.3 ± 0.1^{b}	2.3 ± 0.2^{a}	1.4 ± 0.3^{b}	1.4 ± 0.4^{b}
Linoleic	30.7 ± 0.1^{a}	30.7 ± 1.1^{a}	30.3 ± 0.3^{a}	30.8 ± 1.2^{a}	31.3 ± 0.7^{a}	30.1 ± 0.5^{a}

$^{a-c}$: Similarly indexed values within a same line are not significantly different.

yield has already been reported for cumin seeds from Egypt [16]. Cumin seeds collected in Bouarfa and Tazarine, the two highest localities in our study, showed the lowest oil yield suggesting a possible influence of the elevation on cumin oil yield.

Fully mature cumin seeds have already been shown to result in a better oil yield than immature seeds [16]. Elevation could influence oil yield by generating a shorter warm season in the uplands areas compared to lowlands resulting in the predominant production of seeds not as mature as those harvested in other places. Implication of elevation on oil in low-yield production could appear in contradiction with a previously published study that indicated that Tunisian and Indian cumin seeds presented oil yield of 17.7 and 15.4 g/100 g, respectively [2], even though both had been grown at the sea level. However, it must be mentioned that:

1) the Indian origin of the cumin seeds used in this previous study is not guaranteed,
2) seed maturity is not considered,
3) cultivation occurred in a greenhouse with controlled, and quite far from natural Moroccan biotope, humidity, and temperature conditions.

To further evaluate if growing location could impact cumin seed-oil content, we evaluated the FA content of our samples and their oxidative stability. We also cumin seeds grown in other locations showed no significant variations. Therefore, geography seems to moderately influence petroselinic acid content in cumin oil.

Linoleic acid was found to be the second major fatty acid (30.1 to 31.3%). No significant variations were observed based on the location. Palmitic and oleic acid content in cumin seed oil was found to be around 3 and 2%, respectively. Such contents are very similar to those recently reported for Egyptian cumin oil but quite different from those reported for Tunisian or Indian cumin oil [2]. Linolenic, arachidic, behenic, lignoceric, palmitoleic, stearic, and hypogenic acids were only identified as traces (less than 1%).

At this stage, we decided to compare our results with those reported for other locations for cumin growth. Data regarding the main fatty acids are presented in Table II.

Data reported for Indian cumin oil indicate that large intergeographic variations can be observed but within a country. Possibly, these large variations can result from the seed maturation degree. Comparison also indicates that Moroccan cumin oil is very similar to Egyptian cumin oil. Even more noteworthy, linolenic acid was reported to be a very minor component of cumin oil from Morocco, Egypt, or India whereas it is an important component of cumin oil from Pakistan. Linoleic acid is also the subject of large content variations

Table II - Reported average oil yield (g/100 g) and fatty acid content (%) in cumin seed oil according to geographic origin.

	Pakistan [19]	Tunisia[a] [16]	India [2]	India [4]	Egypt [20]	Morocco[b]
Oil yield	20.4	8.2 to 16.9	15.4	14.5	23	16.3 to 25.7
Acids:						
Petroselinic	51.3	10.6 to 55.9	41.4	83.4	61.8	54.9 to 60.9
Palmitic	11.7	52.8 to 23.8	30.3	11.7	4.6	2.9 to 3.4
Oleic	14.6	8.1 to 0.3	0.4	0.2	1.4	1.3 to 2.3
Linoleic	0.8	3.9 to 12.4	19.4	4.7	30.4	30.3 to 31.3
Linolenic	18.7	3.8 to 0.2	0.1	-	0.6	0.4 to 0.6

[a]: Variations attributed to maturation, [b]: this study.

possibly due to the seed origin. This large range of values could confuse industrials and incite them to consider cumin seed oil as a poorly homogeneous product. It is also a real worry for the food industry that is generally eager to promote the nutritional value of its products.

3.3. Tocopherol composition

Tocopherols and tocotrienols are important components of the unsaponifiable fraction in vegetable oils. They are considered as highly active natural antioxidants [17, 18]. Therefore, their presence in cosmetic ingredients is well regarded. α-Tocopherol and α-tocotrienol are the main tocopherol and tocotrienol, respectively, of cumin seed oil. Other tocopherols and tocotrienols were detected as traces. Large variations were observed in terms of tocopherol and tocotrienol content (Table III). Cumin seeds harvested at the lowest elevation had the lowest tocopherol content (from 20.9 to 32.8 mg/100 g) whereas seeds collected at a higher elevation had a higher content. Seeds collected in Alnif had a level as high as 50.3 mg/100 g of oil. α-Tocotrienol presented also a quite large content variation. However, the Alnif-sample that had the highest α-tocopherol content, had the lowest α-tocotrienol content. On the contrary, the Rhamna-sample, that had the lowest α-tocopherol content, had the highest α-tocotrienol content. Therefore, the sum of α-tocopherol and α-tocotrienol contents for each sample was not significantly different.

3.4 Oxidative stability

The preservation of edible oil is an important economic parameter. Because of the observed variations in antioxidant molecule content, we determined the oxidative stability of the oil samples at 110°C using the Rancimat method (Table IV). Oil prepared from seeds collected in Rahmna and Kalaat Sraghna showed limited preservation properties (short induction time). Other samples had a similar oxidative behaviour.

Table III - Tocopherol content of cumin seeds from different region (mg/100 g)

	Bouarfa	Tazarine	Alnif	Rhamna	Kalaat Sraghna	Chemaïa
α-tocopherol	36.1 ± 2.3[a]	40.6 ± 0.5[b]	50.3 ± 1.5[c]	20.9 ± 0.5[d]	33.3 ± 0.2[a]	32.8 ± 0.3[a]
α-tocotrienol	52.6 ± 1.5[a]	53.3 ± 0.2[a]	37.2 ± 0.5[b]	72.4 ± 2.0[d]	56.5 ± 1.2[e]	53.7 ± 2.2[a]
β- tocopherol	-	0.4 ± 0.1[a]	-	0.3 ± 0.1 [a]	0.5 ± 0.1[a]	1.1 ± 0.1[b]
γ- tocopherol	2.6 ± 0.1[a]	1.0 ± 0.1[b]	6.5 ± 0.2[c]	1.9 ± 0.1[d]	2.5 ± 0.2[a]	3.7 ± 0.1[e]
β-tocotrienol	-	2.4 ± 0.1[a]	0.9 ± 0.1[b]	2.9 ± 0.1[a]	2.3 ± 0.1[a]	3.6 ± 0.1[c]
plastochromanol-8	2.5 ± 0.1[a]	1.8 ± 0.1[b]	2.0 ± 0.1[b]	1.5 ± 0.2[b]	2.4 ± 0.2[b]	2.6 ± 0.1[b]
γ-tocotrienol	3.6 ± 0.1[a]	-	2.8 ± 0.1[b]	-	-	-
δ- tocopherol	-	0.5 ± 0.1[a]	0.3 ± 0.1[a]	-	2.4 ± 0.2[b]	2.4 ± 0.1[b]
δ-tocotrienol	2.5 ± 0.1	-	-	-	-	-

[a-e] : Similarly indexed values within a same line are not significantly different.

Table IV - Oxidative stability (hour) of cumin seeds from different region measured by Rancimat test at 110°C.

Location	Bouarfa	Tazarine	Alnif	Rhamna	Kalaat Sraghna	Chemaïa
Rancimat	29 ± 1[a]	30 ± 2[a]	34 ± 2[a]	25 ± 1[b]	27 ± 1[b]	32 ± 2[a]

[a-b] : Similarly indexed values are not significantly different.

4. CONCLUSION

Our study underlined that large variations can be observed in a single country in terms of cumin oil yield or chemical content. Elevation, or other factors that are still unidentified, can be responsible for this variability. Therefore, variations initially attributed to the different geographic origin of the seeds could result

from different causes, the most obvious is just the elevation. Whereas the exact nature of all parameters influencing cumin seed oil content needs to be studied, the use of a nomenclature, as Indian or Tunisian or Moroccan seeds, suggesting that there is a geographic indication is not informative at all, not to say misleading. Indications regarding the degree of ripening would be more important to describe the quality of the seeds and their fatty acid content and we recommend their addition as a cumin seed quality marker.

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Received: September 18, 2017
Accepted: February 6, 2018