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Determination of Coenzyme Q_{10}, Coenzyme Q_9, and Melatonin Contents in Virgin Argan Oils: Comparison with Other Edible Vegetable Oils

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Supporting Information

ABSTRACT: Virgin argan oil possesses high antioxidant capacity (AC), which may be partially explained by its high content in antioxidant molecules such as polyphenols and tocopherols. However, the content in other antioxidant molecules, for example, coenzyme Q10 (CoQ_{10}), coenzyme Q9 (CoQ_9), and melatonin (Mel), which have been identified in other edible vegetable oils, have not been evaluated in virgin argan oil. Consequently, it was decided to evaluate the contents of CoQ_{10}, CoQ_9, and Mel in virgin argan oils and compare the results to those obtained in extra virgin olive oils and some varieties of seed oils. By the use of sensitive HPLC-EC/F methods, the results showed that virgin argan oil is a rich source of CoQ_{10} and Mel, but no CoQ_9 was detected. Extra virgin olive oil showed higher levels of CoQ_{10} and lower levels of Mel than virgin argan oil. Between the seed oil samples, only virgin soybean oil showed higher CoQ_{10} and Mel levels than virgin argan oil. The results may be relevant for the contribution of CoQ_{10} and Mel to the biological activities of virgin argan oil.

KEYWORDS: bioactive compounds, argan oil, health properties, extra virgin olive oil, seed oils, antioxidants

INTRODUCTION

The argan tree [*Argania spinosa* (L.) Skeels] is an endemic species from southwestern Morocco protected by UNESCO in 2007 because of its irreplaceable socioeconomical value. The oil product of this tree is obtained from its kernels by two extraction methods: the traditional method that is usually carried out by indigenous women for home self-consumption, and a half-industrialized or semiautomatic method applied in recently developed cooperatives to produce and commercialize virgin argan oil of certified quality. In the traditional method, the roasted kernels are crushed and kneaded into a paste or dough with hot water and then hand-pressed; then the oil/water mixture is separated by decantation. Unfortunately, this method is very slow, leading to oil batches having variable organoleptic properties and important differences in chemical composition mainly due to nonreproducible roasting. In addition, and because of the extraction conditions, the oils obtained by this method have frequently unsatisfactory sanitary conditions. The main difference between two methods is that in the half-industrialized method the oil is extracted with a mechanical cold press without water addition.[1,2] Therefore, the half-industrialized methodology increases the oil quality and preserves its components, flavor, and nutritional properties better than the traditional method.[3,4]

Recent studies have suggested that virgin argan oil may have a relevant role in disease prevention due to its antioxidant potential, its hypolipidemic, hypocholesterolemic, and antihypertensive effects, and its relation to cancer prevention.[5−9] It has been described that argan oil consumption ensures a proper supply of essential polyunsaturated fatty acids, thereby improving the lipid profile associated with conditions related to oxidative damage, such as cardiovascular disease and diabetes.[10]

The therapeutic effects of virgin argan oil are due to its minor components.[9] Virgin argan oil is characterized by high levels of linoleic and oleic acids (mean contents of 38 and 45%, respectively), polyphenols, and tocopherols.[4,8,11] Minor compounds such as sterols, triterpenic alcohols, carotenoids, xanthophylls, and squalene also contribute to its nutritional value and health properties.[2,5,11,12] Some of these components, for example, polyphenols and tocopherols, contribute to the total antioxidant capacity of virgin argan oil, which has been reported to be higher than those of other edible vegetable oils.[13] However, it has been suggested that only the presence of polyphenols and tocopherols in virgin argan oil does not totally justify its high AC.[13] Consequently, virgin argan oil may contain other antioxidants that contribute to its high AC. In this regard, some of the most potent and important antioxidants for human health, for example, coenzyme Q_{10} (CoQ_{10}), coenzyme Q_9 (CoQ_9), and melatonin (Mel), have been found in high levels in other edible vegetable oils, mainly in extra virgin olive oils.[14,15] Soybean, corn, and rapeseed oils are

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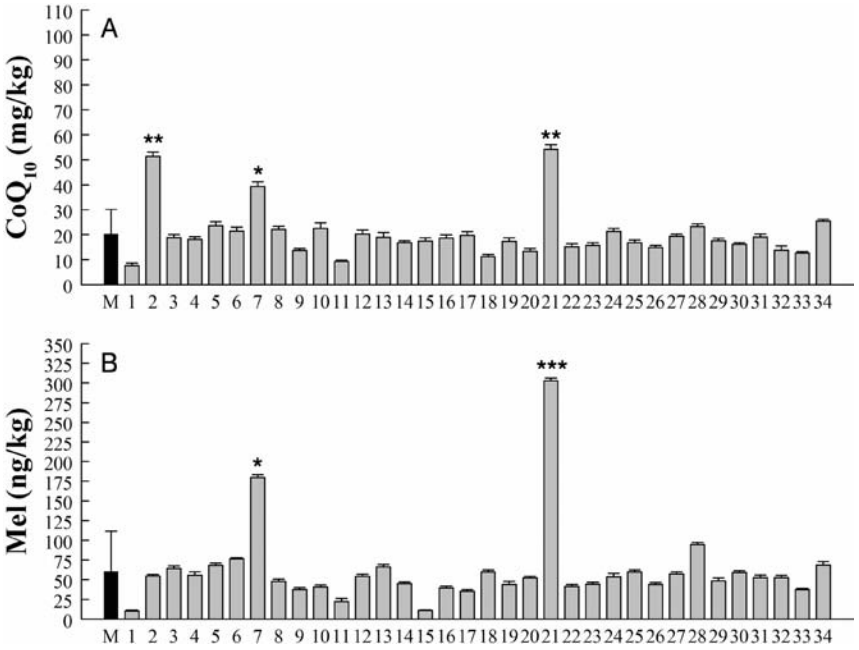

Figure 1. CoQ_{10} (A) and Mel (B) levels in 34 samples of virgin argan oil. M (black bar) represents the mean ± SD of the 34 samples in duplicate. The value of each individual sample represents the mean ± SD of two experimental measurements. ∗, $P < 0.05$ versus mean (M) value; ∗∗, $P < 0.01$ versus mean (M) value; ∗∗∗, $P < 0.001$ versus mean (M) value.

also very rich sources of CoQ_{10}, whereas CoQ_9 has been found in high concentrations in corn oil.[14] However, it is completely unknown whether virgin argan oil contains CoQ_{10}, CoQ_9, and Mel, and no data are currently available in the literature.

In addition to their antioxidant properties, CoQ_{10} and CoQ_9 are components of the mitochondrial respiratory chain in mammals and can regulate some mitochondrial proteins/functions.[16] Consequently, human CoQ_{10} deficiency from genetic origin is a cause of mitochondrial disorders with severe clinical phenotypes.[17] CoQ_{10} deficiency has been also described in myopathies caused by statin treatment as well as in aging, cardiomyopathy, muscle degeneration, and liver cancer.[16] In all of these disorders, CoQ_{10} supplementation is recommended. The benefits of CoQ_{10} supplementation have also been evaluated in Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease, Freidriech's ataxia, and Alzheimer's disease.[18]

Melatonin has also an important antioxidant capacity by directly scavenging free radicals and also by stimulating other endogenous antioxidant systems such as the glutathione system or superoxide dismutase. Moreover, Mel has an important anti-inflammatory action inhibiting the inducible isoform of nitric oxide synthase.[19,20] Because of these properties, Mel treatment has been proposed in diseases coursing with oxidative stress and inflammation, such as mitochondrial disorders, neurodegenerative diseases, and sepsis. Oncostatic actions of melatonin have been also reported.[21] Moreover, because the endogenous Mel is reduced during aging, exogenous supplementation of Mel is recommended.[19]

Due to the high antioxidant capacity of virgin argan oil, because other edible oils contain CoQ_{10}, CoQ_9, and Mel,[14,15] and because of the relevance of these compounds for human health, we decided to determine and quantify the CoQ_{10}, CoQ_9, and Mel levels in virgin argan oils obtained by the traditional and half-industrialized methods. We also compared the results with those obtained in extra virgin olive oils and other edible vegetable oils widely consumed.

MATERIAL AND METHODS

Oil Samples. A total of 34 samples of virgin argan oil from southwestern Morocco were analyzed. Samples were taken randomly at different locations. Among them, 20 samples were obtained by the half-industrialized method and are commercially available (Table S1 of the Supporting Information). The other 14 samples were obtained by the traditional method and were purchased in local markets (Table S1 of the Supporting Information). We have previously reported the metal content and physicochemical parameters of 24 (14 obtained by the traditional method and 10 obtained by the half-industrialized method) of the samples used in this study.[4] In addition, to compare virgin argan oil with other edible vegetable oils, we analyzed 11 samples of extra virgin olive oil from the Picual variety of the same region of Granada, Spain (Protected Designation of Origin Montes de Granada) (Table S1 of the Supporting Information). The Picual variety is the most abundant variety of olive oil in Spain, and it possess very high levels of tocopherols, polyphenols, and antioxidant capacity. We also analyzed 12 samples of other edible and commercial oils (refined sunflower, refined grape seed, refined and virgin walnuts, virgin linseed, virgin sesame, wheat germ, primrose, and virgin soybean) that were available in Granada's market at the time of the study (Table S1 in the Supporting Information). Appropriate quality assurance procedures and precautions were carried out to ensure the reliability of the results. Acidity, peroxide value, humidity, volatile sludges, insoluble sludges in petroleum ether, and K_{270} and K_{232} extinction coefficients were determined according to the analytical methods described in Regulation EEC/2568/91 of the European Union Commission for olive oil.[4] All samples were stored away from light in amber-colored glass bottles at 4 °C until analysis in an inert nitrogen atmosphere. Preliminary assays established the appropriate amount of sample for analysis to ensure homogeneity between samples, and they were representative.

Quantification of CoQ_9 and CoQ_{10} Levels. Lipid components of the oils were extracted by mixing 990 μL of 1-propanol with 10 μL of the oil (note that a proportion of 900 μL of 1-propanol and 100 μL of the oil was tried in virgin argan oil samples to try to detect CoQ_9). After 2 min of vortexing at 1400 rpm at room temperature, the mixed solution

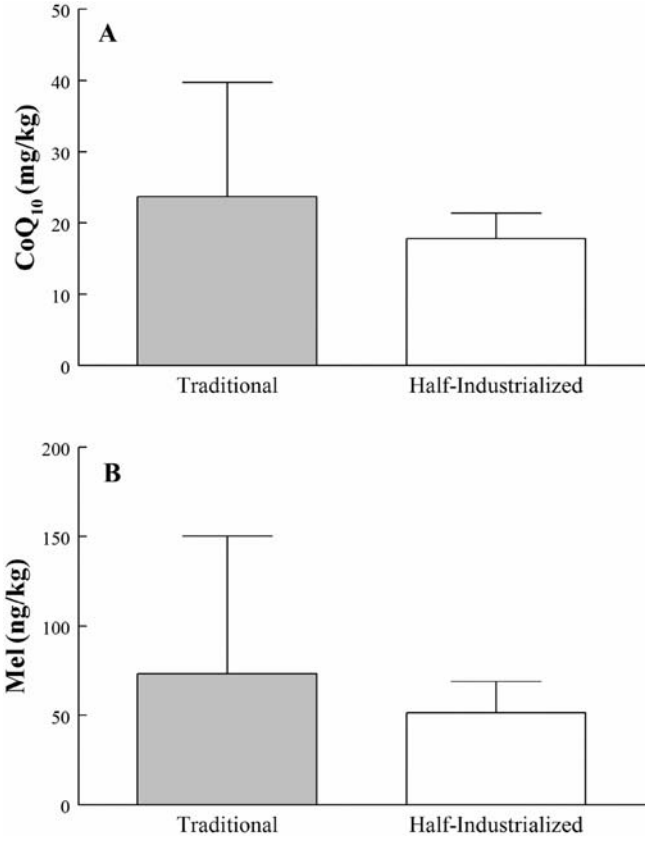

Figure 2. Comparison of CoQ_{10} (A) and Mel (B) levels in virgin argan oils extracted by traditional and half-industrialized methods. The values represent the mean ± SD of 14 (traditional) and 20 (half-industrialized) samples in duplicate.

was centrifuged at 11300*g* for 5 min at room temperature. The subsequent supernatant was diluted 1/5 or 1/10 in 1-propanol prior to HPLC injection.

CoQ_9 and CoQ_{10} present in the oil extract were separated by reversed-phase high-performance liquid chromatography (HPLC; Gilson, WI) with a C18 symmetry column (3.5 μm, 4.6 × 150 mm) (Waters Chromatography, Barcelona, Spain) using a mobile phase consisting of methanol, ethanol, 2-propanol, acetic acid glacial (500:500:15:15), and 50 mM sodium acetate at a flow rate of 0.9 mL/min. The electrochemical detector consisted of an ESA Coulochem III with the following setting: guard cell (upstream of the injector) at +900 mV and conditioning cell at −600 mV (downstream of the column) followed by the analytical cell at +350 mV.[22] CoQ_9 and CoQ_{10} concentrations were estimated by comparison of the peak areas with those of standard solutions of known concentrations (0, 25, 100, 300, and 600 ng/mL). The use of CoQ_6 as an internal standard indicated a recovery of 90−100% of total CoQ_6 added to samples. The results were expressed in milligrams of CoQ per kilogram of oil using the density of the different oils (0.9−0.92 kg/L).

Quantification of Mel Levels. For each oil sample, 500 μL of oil was mixed with an equal volume of methanol and vortexed at 1400 rpm for 20 min at room temperature. The extract was centrifuged at 10000*g* for 20 min at 4 °C. The supernatant was collected into an eppendorf tube and evaporated to dryness for 30 min in a SPD2010 SpeedVac System (ThermoFisher Scientific, Madrid, Spain). The residue was resuspended in 500 μL of PBS (pH 7.4), mixed with 1 mL of chloroform, and vortexed at 1400 rpm for 20 min at room temperature. After centrifugation at 1000*g* for 5 min at 4 °C, the water-soluble phase was discarded and the organic phase was washed twice with 500 μL of 50 mM $NaHCO_3$ (pH 10.25). Finally, 500 μL of the organic phase was evaporated to dryness for 30 min in an SPD2010 SpeedVac system (ThermoFisher Scientific). The dry extract was resuspended in 100 μL of mobile phase consisting of 100 mM sodium phosphate, 0.1 mM EDTA, and acetonetrile 25% (pH 5.2).[15]

The oil content of melatonin was then measured by HPLC (Shimazdu Europe GmbH, Germany) with a C18 Waters Sunfire column (5 μm, 150 × 4.5 mm) (Waters Chromatography). After the column had been stabilized with the mobile phase, 20 μL samples were injected onto the HPLC system at a flow rate of 1 mL/min, and the fluorescence of melatonin was measured in a fluorescence detector (Shimadzu RF-10A XL fluorescence detector) with excitation and emission wavelengths of 285 and 345 nm, respectively. A standard curve for melatonin (Helsinn Chemicals SA, Switzerland) was constructed with 4.45, 8.9, 17.9, 35.9, 71.6, and 143.2 ng/L, and the concentration of melatonin in the samples was calculated according to the peak area. The use of 5-fluorotryptamine as an internal standard indicated a recovery of 90−100% of total 5-fluorotryptamine added to samples.[23]

Statistical Analysis. All measurements were done in duplicate. Individual values for each sample are represented as the mean ± SD of the duplicate determination. Additionally, the mean ± SD was calculated from the data obtained in virgin argan and extra virgin olive oils, and individual values were compared with the mean using Dunnett's test followed by one-way ANOVA post test analysis. *P* values of <0.05 were considered to be statistically significant.

RESULTS

CoQ_{10}, CoQ_9, and Mel Levels in Virgin Argan Oils. As shown in Figure 1, all virgin argan oil samples used in this study contained CoQ_{10} and Mel. Nonetheless, in our analytical conditions CoQ_9 was not detected. Therefore, we can conclude that if virgin argan oil contains CoQ_9, its concentration should be <0.05 μg CoQ_9/mL oil (~0.055 mg CoQ_9/kg oil). Nevertheless, our results show that argan oil contains CoQ_{10} in a concentration of 20.2 ± 9.9 mg CoQ_{10}/kg oil (mean ± SD). Three samples of argan oil (samples 2, 7, and 21) showed higher concentrations of CoQ_{10} ($P < 0.01$, $P < 0.05$, and $P < 0.01$, respectively) (Figure 1A). Our results also revealed that virgin argan oils contain Mel in a concentration of 60.5 ± 51.2 ng Mel/kg oil (mean ± SD). Moreover, two samples of virgin argan oil (samples 7 and 21) showed higher concentrations of Mel ($P < 0.05$ and $P < 0.001$, respectively) (Figure 1B). Additionally, when we compared the results on virgin argan oils extracted by traditional versus half-industrialized methods, we observed a trend toward higher levels of both CoQ_{10} (Figure 2A) and Mel (Figure 2B) on virgin argan oils extracted by the traditional method. However, virgin argan oils extracted by the traditional method showed highly variable results, whereas the values in virgin argan oils extracted by half-industrialized methods were very consistent (Figure 2).

CoQ_{10}, CoQ_9, and Mel Levels in Extra Virgin Olive Oils. As shown in Figure 3, all extra virgin olive oil samples used in this study contained not only CoQ_{10} (Figure 3A) and Mel (Figure 3C) but also CoQ_9 (Figure 3B). The mean concentrations of the three molecules were 84.2 ± 12.5 mg CoQ_{10}/kg oil (mean ± SD), 6.4 ± 2.5 mg CoQ_9/kg oil (mean ± SD), and 30.8 ± 12.9 ng Mel/kg oil (mean ± SD).

CoQ_{10}, CoQ_9, and Mel Levels in Different Edible Vegetable Oils. Because of the different sources of the seed oils, a wide variability was detected in CoQ_{10} (Figure 4A), CoQ_9 (Figure 4B), and Mel (Figure 4C) levels of these oils. We can establish three groups of oils depending on the CoQ_{10} levels: oils with <10 mg CoQ_{10}/kg oil (IDs 48, 54, 55, and 59, corresponding to refined sunflower, virgin sesame, wheat germ, and primrose oils, respectively),

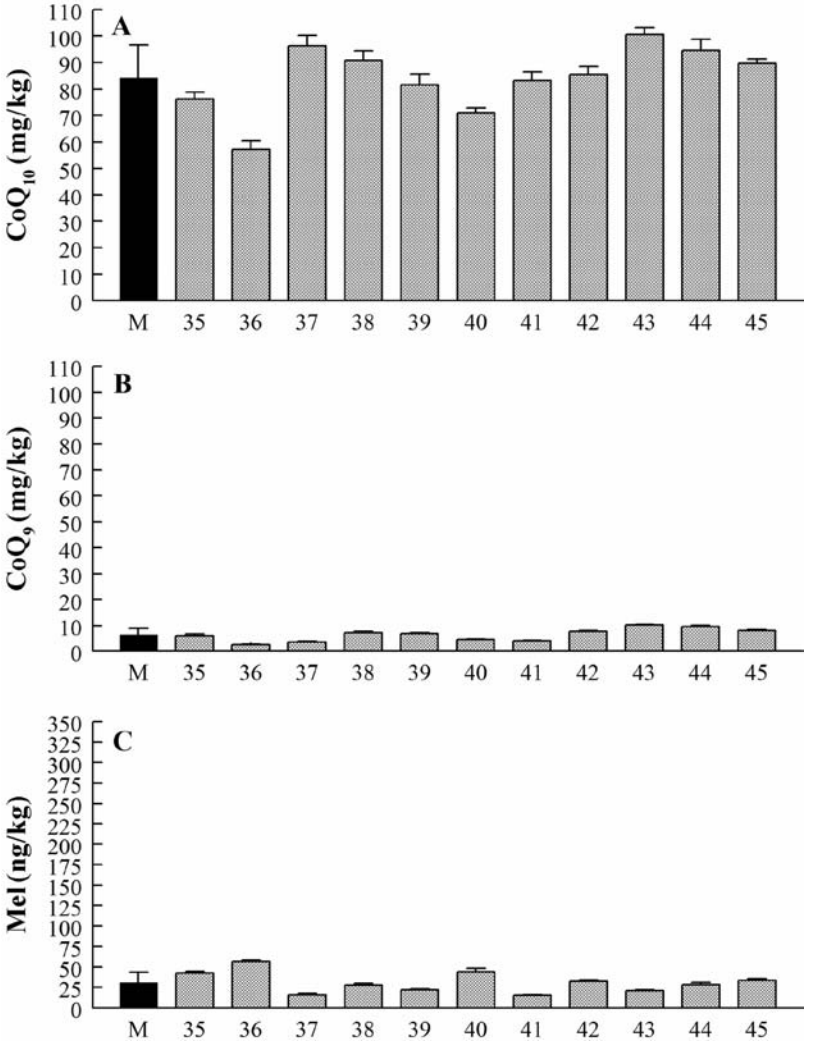

Figure 3. CoQ_{10} (A), CoQ_9 (B), and Mel (C) levels in 11 samples of extra virgin olive oil (Picual variety, Protected Designation of Origin Montes de Granada, Granada, Spain). M (black bar) represents the mean ± SD of the 11 samples in duplicate. The value of each individual sample represents the mean ± SD of two experimental measurements.

oils with a range of 10−50 mg CoQ_{10}/kg oil (IDs 49, 50, 51, 52, 53, 56, and 57, corresponding to refined grape seed, refined walnut, virgin walnut, virgin linseed, and linseed oils, respectively), and oils with >50 mg CoQ_{10}/kg oil (ID 60, corresponding to virgin soybean oil) (Figure 4A). On the other hand, CoQ_9 was detected in only four oil samples (IDs 48, 50, 55, and 60, corresponding to sunflower, refined grape seed, wheat germ, and soybean oils, respectively). Interestingly, the predominant CoQ form in sunflower (ID 48) and wheat germ (ID 55) oils was CoQ_9 rather than CoQ_{10} (Figure 4A,B), as it is shown in the rest of sample oils. Finally, Mel levels were in the range of 25−75 ng Mel/kg oil, except in the case of one sample of refined linseed oil (ID 57) and another sample of virgin soybean oil (ID 60) that showed levels of 293.3 ± 2.2 and 192.9 ± 1.9 ng Mel/kg oil, respectively (Figure 4C).

DISCUSSION

This present study is the first of its kind to claim that the virgin argan oils contain CoQ_{10} and Mel, two important molecules for human health. For these determinations we have used sensitive and rapid methods for biological samples based on HPLC systems with electrochemical (EC) and fluorescent (F) detectors.[22,23] Compared to other food sources of CoQ_{10}, virgin argan oil represents a rich dietary source of CoQ_{10}.[14] However, extra virgin olive oil (Figure 3A) and virgin soybean oil (Figure 4A) have higher CoQ_{10} levels than virgin argan oil (Figure 1A), as they have >50 mg CoQ_{10}/kg oil, and they are considered to be very rich CoQ_{10} sources.[14] The Mel levels in virgin argan oil (Figure 1B) were higher than the Mel levels in extra virgin olive oil (Figure 3C). In discrepancy with these data, de la Puerta and colleagues[15] reported higher Mel levels (71−119 pg/mL) in extra virgin olive oil measured by commercial ELISA kit. These higher levels of Mel (compared to our data) previously reported in extra virgin olive oil[15] may be caused by cross-reactivity in the ELISA method or by the differences in olive oil origins. Mel levels in refined linseed oil and virgin soybean oil were higher than Mel levels in virgin argan oil, and all of them are comparable with the levels of Mel described in other Mediterranean foodstuffs.[24]

Virgin argan oil has an important AC. Marfil et al.[13] tested different methods to determine the total AC of virgin argan oil and concluded that this edible oil presents higher AC than other

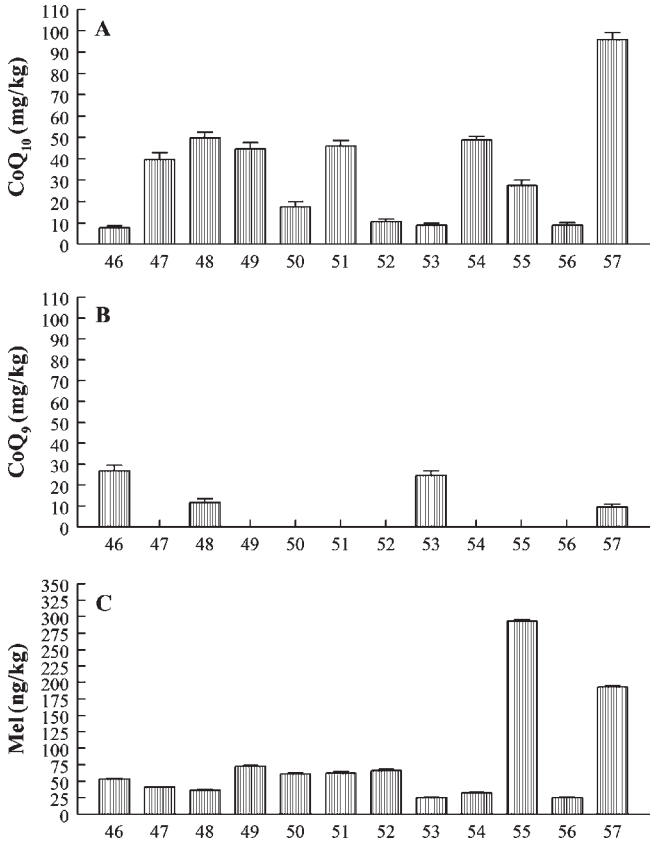

Figure 4. CoQ_{10} (A), CoQ_9 (B), and Mel (C) levels in 12 samples of a variety of seeds oils. The value of each individual sample represents the mean ± SD of two experimental measurements.

edible oils. Polyphenols and tocopherols have been classically considered as the main contributors to this high AC in virgin argan oil. Marfil et al.[13] reported data on polyphenol content in virgin argan oil ranging between 6.07 and 152 mg GAE/kg oil. Tocopherol content ranged from 427.0 to 654.0 mg/kg, γ-tocopherol being the major fraction.[13] However, the authors did not find a direct correlation between the AC and tocopherol levels, suggesting that other antioxidant molecules may participate in the AC of virgin argan oils. Similarly, Cayuela et al.[3] reported total tocopherol content ranging from 389 to 503 mg/kg, and the γ-tocopherol fraction represented between 84.4 and 86.4% of the total. These authors claimed that the low tocopherol content found in certain samples could be due to inadequate oil storage conditions or the extraction method because they found higher total tocopherol content in oils obtained by the traditional method than in oils obtained by the semi-industrial extraction method. Other authors have speculated that the wide variability in the antioxidant content could be due to genetic, environmental, and technological factors (e.g., seed roasting temperature and time, amount of water added during the oil extraction process, and storage conditions).[13] The detection of high CoQ_{10} and Mel levels in virgin argan oils may be now seriously considered for the contribution of these molecules to the total AC of this oil. Additionally, our results of CoQ_{10} and Mel levels in virgin argan oil show that two samples (IDs 7 and 21) (Figure 1) have significantly higher levels of both molecules. These two samples correspond with virgin argan oil from Essauira (Morocco), the main producing region of virgin argan oil, extracted by the traditional method. These two sample oils also showed high tocopherol levels.[13] Consequently, our results imply that extraction method could affect the content of antioxidant molecules (e.g., CoQ_{10}, Mel, and tocopherols) in virgin argan oil. However, the number of virgin argan oil samples extracted by the traditional method that have higher content of CoQ_{10} and Mel is limited. Consequently, we cannot exclude that uncontrolled factors during the extraction have influenced these results. Anyway, our results suggest that the study of the factors that influence the content of CoQ_{10} and Mel could be extended to other edible oils and, therefore, further research studies are needed to determine the factors that influence the content of antioxidant molecules in edible oils.

The presence of a high content of CoQ_{10} in virgin argan oil, as well as in other edible oils (e.g., extra virgin olive oil, virgin soybean oil, refined grape seed oil, refined and virgin walnuts oils, and virgin linseed oils), meats, fishes and nuts,[14] is important because 25% of CoQ_{10} in the adult human body is replaced daily by endogenous synthesis and nourishment.[25,26] However, endogenous biosynthesis progressively declines during aging[16,26] and in some genetic defects;[17] statin treatment also decrease endogenous CoQ_{10} levels.[16,25] Consequently, exogenous intake of CoQ_{10} from rich CoQ_{10} nourishment and food supplements is important for human health. Additionally, beneficial effects of CoQ_{10} administration have been reported in different clinical presentations.[16,27,28] These beneficial effects of CoQ_{10} in human health are related to its lipophilicity and its redox properties.[29] In the cell, CoQ_{10} may be in oxidized (ubiquinona) or reduced (ubiquinol) forms, and both pools are continuously interchanged by the Q cycle. The antioxidant capacity of CoQ_{10} has been mainly attributed to ubiquinol (as a chain-breaking antioxidant), but when ubiquinone is exogenously administrated, it can be converted into ubiquinol by the Q cycle, and this ubiquinol would scavenge reactive oxygen species (ROS). Moreover, it has been demonstrated that ubiquinone itself reacts rapidly with the superoxide anion, contributing to the scavenging of this free radical.[30] Therefore, the total CoQ_{10} pool (ubiquinone + ubiquinol) must be considered for the antioxidant activity CoQ_{10} in the cell. In addition to its antioxidant activity, CoQ_{10} has other important and critical functions in the cell, including the transfer of electrons through the mitochondrial respiratory chain, stabilization of biological membranes, participation in the activity of the mitochondrial permeability transition pore and uncoupling proteins, and functioning as a cofactor in the reaction catalyzed by dihydrooratate dehydrogenase.[16] Besides CoQ_{10}, human cells have also a small proportion of CoQ_9. The reasons of the presence of these two ubiquinone forms are not understood but may be related with the different functions of CoQ and membrane compositions. Consequently, the maintenance of an adequate proportion of CoQ_9 may be also important. In this regard, CoQ_9 in virgin argan oil was not detectable in our system. Other edible oils, mainly sunflower and wheat germ oils but also extra virgin olive oil, refined grape seed oil, and virgin soybean oil, have CoQ_9. It is interesting to note that in the case of sunflower and wheat germ oils, CoQ_9 is the major form of ubiquinone (Figure 4A,B), as previously reported.[14]

Mel has been found in high concentrations in some edible plants, including feverfew and St. John's wort, in seeds of various plants, and in the cherry fruit.[31] Mel has been also detected in vegetable-derived products, for example, red wine, beer, and extra virgin olive oil.[15,24] Our results include now other edible vegetable oils, especially virgin argan, linseed, and soybean oils, in the list of nourishment sources of the indolamine. Whereas Mel

concentration was much lower than the concentrations of CoQ_{10}, polyphenols, and tocopherols (ng vs mg), it is important to note that Mel has nanomolar affinity for its membrane receptors,[32] and it is biologically active and effective in reducing oxidative stress at nanomolar concentrations.[33,34] Mel, consumed in the diet, is absorbed by the gut and significantly modifies circulating levels of the indoleamine. Because the concentration of Mel in the blood correlates positively with the total antioxidant status of this fluid, the implication is that consuming foodstuffs containing Mel would increase the antioxidant capacity of the organism. Mel has a direct antioxidant action by scavenging most ROS, but Mel has also an indirect antioxidant action by up-regulating other antioxidant systems in the cell.[19,35] Mel also increases the efficiency of the mitochondrial oxidative phosphorylation system, reducing electron leakage and, therefore, lowering free radical generation.[19,20] Another important action of Mel is the reduction of iNOS expression and activity, which is especially relevant for the therapeutic benefits of Mel in the treatment of diseases coursing with inflammation.[19] Analogous to CoQ_{10}, Mel content in peripheral organs is decreased during aging, to a similar extent as pineal melatonin production.[36,37] Therefore, exogenous intake of Mel from rich Mel nourishment and food supplements is important for human health.[19,31,38]

In conclusion, our study identifies for the first time that virgin argan oils possess two antioxidants, CoQ_{10} and Mel, with enormous importance for human health. Two virgin argan oil samples extracted by traditional method had higher CoQ_{10} and Mel levels, suggesting that the extraction method could affect the content of these molecules. The levels of CoQ_{10} and Mel found in the virgin argan oil samples are in the upper-medium range compared with other edible oils. Because virgin argan oil possesses an important AC, CoQ_{10} and Mel should be now added to the factors that contribute to this superb quality of the virgin argan oil. However, the presence of both CoQ_{10} and Mel in the argan oil is important not only for their antioxidant activities but also for other biological activities, for example, mitochondrial function/homeostasis, induction of endogenous antioxidants, and inflammatory regulation, carried out by these molecules. Consequently, the content of CoQ_{10} and Mel may be also essential for future applications of virgin argan oil in the food and cosmetic industries.

■ ASSOCIATED CONTENT

Supporting Information. Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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