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Thermal stability and long-chain fatty acid positional distribution on glycerol of argan oil

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Abstract

The primary aim of this study was to determine the oxidative stability of argan oils by using peroxides and conjugated diene hydroperoxides measurements as analytical indicators. Both food and cosmetic argan oils were investigated. Their oxidative stability was also determined by monitoring the relative changes of their fatty acid profiles by ^{1}H NMR. In addition, valuable information regarding minor components as well as the acyl positional distribution, were obtained for both grades by high field ^{1}H and ^{13}C NMR, respectively. Given that the cosmetic and food grades have a similar profile and content of phenolic antioxidants, vitamers, and squalene, it appears that the ratio of fatty acid aliphatic to bisallylic CH_2 groups, much higher in argan oils than in other vegetable oils, is responsible for their higher thermal stability.

Keywords: Argan oil; Thermal stability; Regiospecificity; High field; ^{1}H and ^{13}C NMR

1. Introduction

Lipid oxidation is not only responsible for unpleasant flavours in foods but can also produce harmful reactive oxygen species (ROS) that may lead to carcinogenesis, mutagenesis and ageing in humans (Ferrari & Torres, 2003, chapter 7). Increasing evidence shows that antioxidant compounds may prevent cardiovascular diseases and cancers generated by ROS (Bartsch, Nair, & Owen, 2002; Gutteridge & Halliwell, 1994). Vegetable oils are important sources of antioxidants and contain essential long-chain fatty acids necessary for the proper development of human tissue (FAO, 1978).

Recent studies (Charrouf & Guillaume, 1999; Drissi et al., 2004; Khallouki, Spiegelhalder, Bartsch, & Owen, 2005) suggest that the dietary argan oil, an endemic Moroccan seed oil ecologically and socioeconomically important in the South–West of Morocco, plays a relevant role in disease prevention.

Traditionally, argan oil is mostly used for nutritional and cosmetic purposes (Charrouf & Guillaume, 1999). The unsaponifiable fraction of argan oil contains a large range of bioactive substances including tocopherols, terpenes, alcohols, sterols, as well as traces of phenolic compounds. (Farines, Charrouf, & Soulier, 1981; Khallouki et al., 2003). Moreover, with respect to other seasoning oils, argan oils contain a higher amount of squalene, which can be as high as 3.2 g kg^{-1} (Khallouki et al., 2003).

Typically, rancidity generated in oils during oxidation processes is commonly evaluated by measuring the amount of peroxides (AOAC, 1990) and diene hydroperoxides (Chan & Levett, 1977) formed over time. Vegetable oils have also been characterized by ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy (Gunstone, 1990; Mannina, Luchinat, Carmela Emanuele, & Segre, 1999; Mannina et al., 2000); in particular, this technique has been applied to estimate the

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thermal stability of canola and soybean oils (Wanasundara, Shahidi, & Jablonski, 1995).

In this paper, we have investigated two argan oil grades (food and cosmetic). Firstly, by using both peroxide measurements and ^{1}H NMR, we have studied the impact of changing the storage condition of argan oils. Secondly, using high field ^{1}H and ^{13}C NMR, respectively, we have obtained valuable information on minor components and determined the fatty acid distribution of triglycerides on the glycerol moiety of the oils.

2. Materials and methods

2.1. Samples

Food and cosmetic grades of argan oil were kindly donated by the argan Oil Women's Cooperative (Targanine), Tamanar (Essaouira region), Morocco. Cosmetic and food argan oils were extracted from kernels by mechanically cold press. For the food grade the extraction was performed on roasted kernels.

Oil samples (25 mL) were placed in sealed clear Pyrex containers (20 mm diameter and 50 mm height) wrapped with aluminium foil and kept in the dark in an oven at 60 °C for a period of 30 days. To estimate the oil thermal stability, samples were periodically removed after a period of 0, 5, 10, 20, 25, and 30 days. After removal and prior to the chemical analysis, all samples were flushed with nitrogen.

2.2. Peroxides and dienes hydroperoxides analysis

The peroxide values were determined iodometrically (AOAC, 1990). Specifically, 5.0 g of oil were introduced into a 250 mL Erlenmeyer glass with 50 mL of an acetic acid–chloroform solution (3/2, v/v). The flask was vortexed until dissolution and then 0.5 mL of a saturated KI solution was added. This solution was allowed to stand for one minute, and then 30 mL of distilled water were added. Titration was performed by adding exactly 0.010 N of $Na_2S_20_3$ until the yellow colour disappeared: a starch indicator (0.5 mL) solution was used. Peroxide (P) values are expressed in milliequivalents of active oxygen per kilogram of oil (meq O_2 kg^{-1}).

The conjugated diene hydroperoxide values were determined spectrophotometrically with a Kontron UNVIKON 930 spectrophotometer by using an absorptivity of 26,000 ($\lambda_{max} = 234$ nm), as previously reported by others (Chan & Levett, 1977). Specifically, every 5 days, the oil samples were diluted in isooctane and the absorbance of the resulting solutions were measured. Conjugated diene (CD) values are expressed in millimoles per kilogram of oil (mmol kg^{-1}).

2.3. NMR spectroscopy

Low field ^{1}H NMR spectra were recorded on a BRUKER AC250 NMR spectrometer operating at a ^{1}H Larmor frequency of 250 MHz. Argan oil samples (35 mg) were dissolved in $CDCl_3$ (0.7 mL). The ^{1}H spectra were monitored every five days during the thermal treatment.

In addition, high field ^{1}H and ^{13}C NMR spectra were recorded on a BRUKER AVANCE 600 NMR spectrometer operating at a Larmor frequency of 600 and 150 MHz for ^{1}H and ^{13}C, respectively. To record the ^{1}H spectrum, 0.02 mL of the argan oil samples were dissolved in a mixture composed of DMSO-d_6 (0.02 mL) and $CDCl_3$ (0.7 mL). For the ^{13}C spectrum, 0.1 mL of the samples were dissolved in 0.7 mL of $CDCl_3$.

In all cases the ^{1}H and ^{13}C chemical shifts were referenced to tetramethylsilane, used as an internal standard.

The experimental procedure required for calculating the aliphatic/diallylmethylene ratio (R_{ad}) and the aliphatic/oleic ratio (R_{ao}) as well as the full experimental methodology required for estimating the percentage of fatty acids on the glycerol positions, have been described elsewhere (Wanasundara et al., 1995; Mannina et al., 1999). Specifically, in the case of the acyl positional distribution, it is sufficient to measure the intensity of each of the six ^{13}C NMR signals between 172 ppm and 173 ppm in the spectrum (Fig. 4). The results are reported as molar percentages calculated by measuring the intensity of each carbonyl resonance with respect to the sum of the intensities of all carbonyl resonances taken as 100%.

2.4. Statistical analysis

Peroxide (P) and conjugated diene (CD) measurements as well as ^{1}H and ^{13}C NMR experiments were run in duplicate. Differences were evaluated using Student's test at the 5% significance level.

3. Results and discussion

3.1. Thermal stability

To evaluate their thermal stability, both argan oil grades were kept in the dark at 60 °C for 30 days, and the peroxide (P) and conjugated diene (CD) values were monitored for both argan oil grades. Note that, although the P value may not entirely reflect the actual extent of oil deterioration, it is typically used for monitoring the storage condition of oils (AOAC, 1990). Accordingly, the evolutions as a function of time of the P and CD values are reported in Figs. 1, 2, respectively. In both cases, the extremely low P and CD values observed at the very beginning of the thermal treatment, probably due to the oil storage at the Cooperative, suggest the good quality of the oils.

Clearly, hydroperoxides are formed in both grades, and the P value observed for the cosmetic grade is higher after 30 days.

On one hand, it is important to observe that in argan oils the P value does not show any initial stability, often present in other vegetable oils, thus not giving any indication on an induction period. This result suggests that the

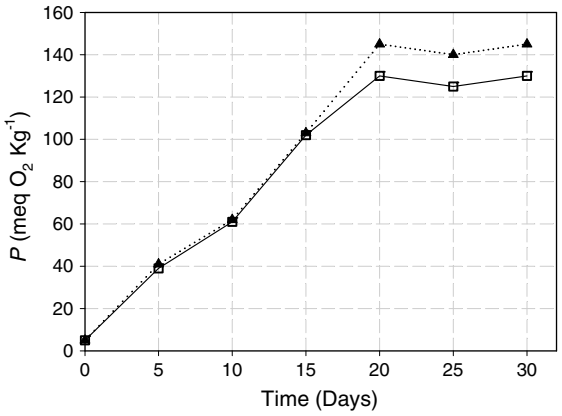

Fig. 1. Evolution of the peroxide (P) value as a function of the thermal treatment time at 60 °C for the food (□) and cosmetic (▲) argan oil grades.

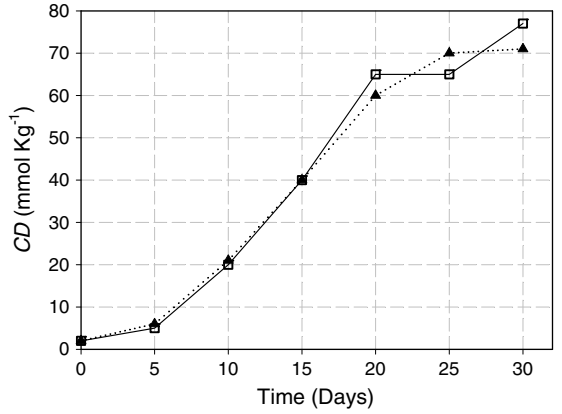

Fig. 2. Evolution of the conjugated dienes hydroperoxides (CD) value as a function of the thermal treatment time at 60 °C for the food (□) and cosmetic (▲) argan oil grades.

induction period reported in the literature (Yaghmur, Aserin, Mizrahi, Nerd, & Garti, 2001) is related to the presence of other natural antioxidants not decomposed or removed during the simple extraction process.

On the other hand, another interesting point to note is that, after a period of about 20 days, the P and CD values are more or less constant. This suggests that argan oils have a good stability (good shelf life).

Generally, lipid oxidation curves are typically characterized by a second oxidation step during which the metabolites are transformed into undesirable products such as aldehydes and ketones. This step leads to a decrease in the P and CD values. Because these decreases were not observed here, the hydroperoxides formed in the argan oil samples seem to be, at least in our experiments, stable.

Furthermore, to better elucidate the effects of thermal treatment on the argan oil samples, 250 MHz ^{1}H NMR experiments were performed at different thermal treatment time. The ^{1}H NMR spectra showed significant changes in the aliphatic, olefinic, and diallylmethylene resonances of the fatty acid chains. Accordingly, Adhvaryu et al. have reported that bisallylic protons are the most sensitive to oxidation and showed that a high aliphatic/diallylmethylene proton ratio (R_{ad}) indicates higher activation energy of oxidation, which in turn implies that the oil is more resistant to oxidation (Adhvaryu, Erha, Perez, & Liu, 2000). Conversely, these authors have shown that an increase in the aliphatic/olefinic proton ratio (R_{ao}) decreases the activation energy. The percentages of characteristic CH_2 groups (olefinic, aliphatic, bisallylic, α-CO, and allylic) for four distinct vegetable oils: canola, soybean, sunflower, and argan oils are summarized in Table 1. According to these data, the argan oil food grade has the highest level in aliphatic and the lowest in bisallylic CH_2 groups.

In addition, Fig. 3 shows the relative changes in the R_{ao} and R_{ad} ratios as a function of the thermal treatment time for the food grade argan oil. As evidenced in Fig. 3, until the 20th day of thermal oxidation, both ratios vary slightly and exhibit clearly a good stability, especially in comparison to canola and soybean oils (Wanasundara et al., 1995).

In contrast, after 20 days of thermal treatment, R_{ad} increases whereas R_{ao} remains almost constant. This trend is characteristic of an oxidation process. This oxidation gives rise to intermediary endoperoxides which eventually form carbonyl compounds through rearrangement reactions.

These results indicate that argan oil is less affected by oxidation processes than soybean and canola oils.

3.2. Minor components and fatty acid distribution by high field ^{1}H and ^{13}C NMR

High field ^{1}H NMR can be used to obtain interesting information about minor components in vegetable oils

Table 1
Relative amounts of different CH_2 groups (olefinic, aliphatic, bisallylic, α-CO, and allylic) determined by ^{1}H NMR for four distinct vegetable oils

Oils	Olefinic (%) [5.2–5.5 ppm]	Aliphatic (%) [1.2 ppm]	Bisallylic (%) [2.78 ppm]	α-CO (%) [2.25–2.35 ppm]	Allylic (%) [1.9–2.1 ppm]	Ratio aliphatic CH_2/bisallylic
Canola[a]	10.2	67.3	2.0	7.3	13.3	33.6
Soybean[a]	12.4	62.6	5.0	7.4	12.6	12.5
Sunflower[a]	12.6	63.5	4.8	7.3	11.9	23.2
Argan[b]	7.6	70.8	1.9	7.3	12.5	37.3

[a] Owen et al., 2000.
[b] This work.

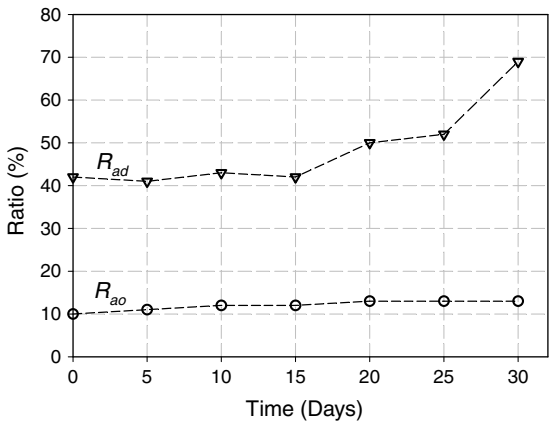

Fig. 3. Evolution of the aliphatic-to-diallylmethylene ratio, R_{ad}, and the aliphatic-to-olefinic ratio, R_{ao}, as a function of the thermal treatment time for the food argan oil grade at 60 °C.

(Mannina, Patumi, Proietti, Bassi, & Segre, 2001). Inspection of the 0.4–1.0 ppm spectral region of the 600 MHz ^{1}H spectrum of argan oils shows that the linolenic fatty acid concentration is extremely low (data not shown). Specifically, similarly to hazelnut oils, the linolenic concentration in argan oil is much lower than that commonly observed in other vegetable oils, such as canola, soybean, and olive oils. This may contribute to the argan oil oxidative stability evidenced in the previous section (Khallouki et al., 2003), although oils with the lowest linolenic acid contents do not systematically exhibit the highest stabilities.

Moreover, argan oils do not contain detectable amount of *β*-sitosterol, which is otherwise systematically present in olive oils; in fact, the ^{1}H spectrum of argan oils does not show the typical resonance at 0.62 ppm due to the 18-methyl of β-sitosterol. Other minor components such as squalene, an extremely important antioxidant, and *sn* 1,2 sn *1,3* diglycerides were easily identified in the ^{1}H spectrum by means of the diagnostic resonances at 1.62 ppm, 3.64 ppm and 4.00 ppm, respectively (Mannina et al., 2001). Food and cosmetic grades have the same amount of squalene, present in many vegetable oils, whereas the *sn*1,2/*sn*1,3 diglycerides ratio is around 2 in both argan grades suggesting a good conservation state. In fact, the *sn*1,2/*sn*1,3 diglycerides ratio is strongly related to the quality-freshness of oils; e.g. young and good quality olive oils usually contain mainly native *sn*1,2 diglycerides and only a small amount of *sn*1,3 diglycerides. The *sn*1,3 diglycerides (of lipolytic origin) increase in oils after several months of storage or when stored in unsuitable conditions, due to intramolecular transposition and/or lipolytic phenomena. No volatile compounds such as aldehydes and terpenes were detected, probably due to the extraction procedure.

High field ^{13}C NMR has proved to be a suitable technique to provide valuable information on the acyl positional distribution (1,3-acyl and 2-acyl) in tri-acyl-glycerols of different vegetable oils (Mannina et al., 1999). This distribution is a fine characteristic of triglycerides. In this context, the ^{13}C NMR spectrum of an argan oil sample is reported in Fig. 4. This spectrum shows resonances grouped in four sets of signals: carbonyls (from 172 to 174 ppm), unsaturated carbons (from 125 to 135 ppm), glycerol backbone carbons (from 60 to 80 ppm), and aliphatic carbons (from 15 to 35 ppm). Interestingly, the ^{13}C spectrum also indicates the presence of a significant amount of diglycerides (Fig. 4b).

Typically, the acyl positional distribution can be determined by analysing the carbonyl region: in fact, within this spectral region, the low and high field carbonyl resonances are due to chains esterified in the *sn*1,3 positions and the *sn*2 position, respectively (Fig. 4a). These two sets of carbonyl resonances are separated by a shift of about 0.41 ppm. This shift, which is consistently detected for all chains, was explained by noting that C=O groups of 2-position chains experience two γ-*gauche* interactions against just one interaction intervening for carbonyls of *sn*1,3-chains. As reported in the literature (Mannina et al., 1999), the

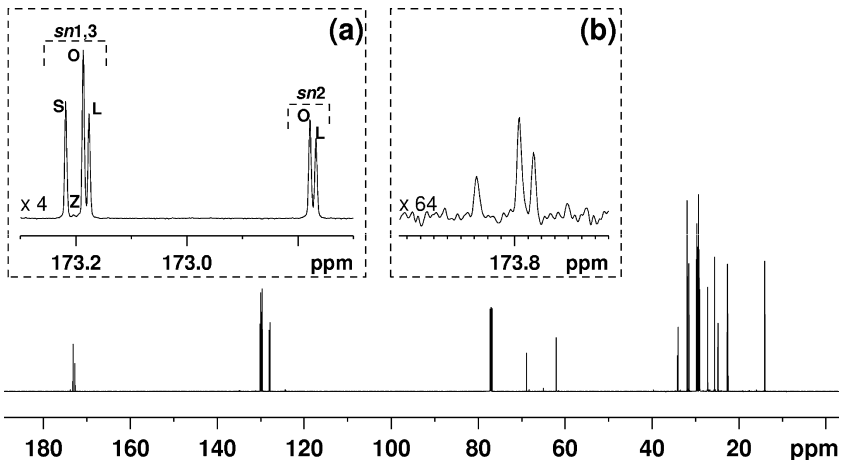

Fig. 4. 150 MHz ^{13}C NMR spectrum recorded in $CDCl_3$ at 300 K of an argan oil sample (food grade). Insets (a) and (b) are expanded views of the carbonyl region. In (a), the reported letters refer to different types of fatty acid chains according to: S = Saturated; Z = Eicosenoic and Vaccenic; O = Oleic; L = Linoleic. Inset (b) shows the resonances due to the diglycerides.

Table 2
Acyl positional (*sn*1,3 vs. *sn*2) distribution of saturated and unsaturated fatty acids in the food and cosmetic argan oil grades as determined by ^{13}C NMR

	Food grade (%)	Cosmetic grade (%)
*sn*1,3 fatty chains		
Saturated	15.6	16.1
Oleic	22.1	23.6
Eicosenoic + vaccenic	0.8	0.7
Linoleic	14.0	15.1
*sn*2 fatty chain		
Oleic	26.2	23.8
Linoleic	21.2	20.7

^{13}C NMR carbonyl resonances can be assigned as follows: signals at 173.22 and 173.21 ppm are due to saturated chains (stearic and palmitic acids) and eicosenoic and vaccenic fatty acid chains, respectively, in the *sn*1,3 position.

In addition, signals at 173.19 and 173.18 ppm are due to oleic acid and linoleic acid in the *sn*1,3 position, respectively, whereas signals at 172.78 and 172.72 ppm are due to oleic and linoleic acids in the *s*2 position, respectively.

In Table 2, the percentage of long-chain fatty chains on the glycerol moiety positions is reported. It is immediately clear that saturated fatty acids (palmitic and stearic acids) re totally distributed over the *sn*1,3 positions, the amount present in the *sn*2 position being undetectable. Conversely, unsaturated long-chain fatty acids are more abundant in the *sn*2 position. Interestingly, among unsaturated long-chain fatty acids, linoleic has a higher preference for position 2 than oleic acid which is, instead, equally distributed in the two positions.

4. Conclusions

Food and cosmetic grades of argan oil have been studied. Peroxide and hydroperoxide measurements as well as low field ^1H NMR experiments have shown that both grades offer a good resistance against autoxidation. The NMR data show a high ratio of fatty acid aliphatic to bisallylic CH_2 groups (much higher in argan oils than other vegetable oils), which may contribute to the argan oxidative stability. In addition, argan oils have been investigated by high field ^1H and ^{13}C NMR spectroscopy. On one hand, ^1H experiments have shown that in argan oils valuable minor components, such as linolenic fatty acid or β-sitosterol, are either not present at all or present in extremely low and undetectable amounts, whereas they are systematically detected in other vegetable oils, such as olive oils. On the other hand, ^{13}C NMR data have allowed the acyl positional distribution on the glycerol moiety of food and cosmetic argan oils to be determined.

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