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Oxidative stability of edible argan oil: A two year study

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ABSTRACT

The present study investigated the oxidative stability of the three marketed types of edible argan oil. Edible argan oil is prepared by pressing the slightly roasted kernels of peeled argan fruit. High quality edible argan oil is exclusively prepared using mechanical presses. However, hand-extracted argan oil is still artisanally produced and can be found in local markets. In this latter case, goat-peeled fruit is still sometimes introduced in the oil production chain even though the resulting oil is notoriously of unsatisfactory quality. The oxidative stability of press-extracted, hand-extracted, and goat-peeled fruit derived argan oil was analyzed using as physicochemical metrics: fatty acid composition, β-carotene level, phosphorus level, tocopherol level, iodine index, saponification, peroxide and acid values, specific extinction, and Rancimat induction time. The variations of these parameters were evaluated over a period of 2 years at 5°C, 25°C (protected or exposed to sunlight), or 40°C. After this period of time, mechanically pressed argan oil still presents an excellent physicochemical profile. Domestic and traditionally prepared argan oil presents much less satisfactory properties after the same period.

Keywords: *Argania spinosa*, edible argan oil, long-term oil preservation, long-term oil quality

1. Introduction

The main consequence of the drastic improvements recently brought to argan oil preparative process (Charrouf, Guillaume & Driouich, 2002) was its entry as a major actor in the edible-and-expensive-oil closed circle. Indeed, argan oil that was almost unknown out of the limits of the argan forest twenty years ago is now sold in virtually every gourmet-stores around the world. Edible argan oil is the basis of the Amazigh diet (Charrouf & Guillaume, 2010). It is prepared from the roasted kernels of the fruit of the argan tree (*Argania spinosa* (L.) Skeels) that is exclusively endemic in Southern Morocco (Morton & Voss, 1987). Recent attempts to sustainably develop this region (Charrouf & Guillaume, 2009) threatened by desertification have been consecutive to its designation as a Biosphere Reserve by UNESCO in 1998 and to our early intensive chemical work principally carried out on argan tree metabolites (Charrouf & Guillaume, 2002; Charrouf & Guillaume, 2005) and its fruit-derived oil (Charrouf & Guillaume, 1999). Some of these efforts have been fruitful and have led to the implantation in the argan forest of several argan oil-producing woman cooperatives where high quality argan oil is now prepared using a strictly controlled process. Particularly, new rules include 1) the banishment of goat-peeled fruit, and therefore the necessary use of scratching machines to peel the argan fruit, and 2) the use of screw-presses to extract the oil in place of water-requiring hand malaxing of argan dough. Consequently, low-grade argan oil has been gradually replaced by high quality argan oil. The combination of high levels of unsaturated fatty acids and antioxidants, and its unique taste and pharmacological properties (Charrouf & Guillaume, 2008), have ultimately boosted high quality argan oil market share.

If the negative influence of hand-malaxing, uncontrolled kernel-roasting time, or "goat-peeling" on argan oil quality is now particularly well documented (Hilali, Charrouf, El Aziz Soulhi, Hachimi & Guillaume, 2005; Charrouf, El Hamchi, Mallia, Licitra, & Guillaume, 2006), the real improvement in terms of oil preservation time has not been precisely studied and quantified, yet. To fill this gap, we decided to evaluate over a two-year period the oxidative stability of argan oil prepared 1) traditionally (hand malaxed), 2) mechanically (screw-pressed), and 3) using goat-peeled fruit (hand malaxed and animal-peeled). Such a study is highly desirable to determine an accurate shelf life for each type of oil on the domestic or international market. During our study, oil samples were kept at 5°C, 25°C, or 40°C. Because oil samples refrigerated at 5°C or heated at 40°C were necessarily sunlight protected (fridge or oven; respectively), and since light is well-known to possibly influence edible oil oxidation (Cinquinta, Esti & La Notte, 1997), we also decided to evaluate the influence of light on our oil samples kept at 25°C by use of clear-glass or dark-glass bottles. Therefore, twelve oil samples were periodically analyzed over a two-year period. To be able to eventually link possible characteristic variations to a preparative process, all our studied oil samples were prepared in the same cooperative. Consequently, to ascertain the general character of our results, before the beginning of our study, we first demonstrated the lack of influence of the geographic origin on argan oil initial physicochemical parameters by comparing the oxidative stability of argan oil produced at three different locations of the argan forest.

2. Materials and methods

2.1 Chemicals and materials

All the reagents were of analytical or HPLC grade. Isooctane and isopropanol used as HPLC mobile phase and cyclohexane used for extinction coefficient determination were purchased from Professional Labo (Casablanca, Morocco). Clear- and brown-glass-bottles were purchased from Cfimu sarl (Casablanca, Morocco).

2.2 Sample collection

Argan oil samples analyzed to determine the initial oxidative parameters were prepared in 2006 in the woman cooperatives of Ait Baha (Chtouka-Ait Baha county, Morocco), Tidzi (Essaouira county, Morocco), and Tiout (Taroudant county, Morocco) following our previously reported protocole (Hilali, Charrouf, El Aziz Soulhi, Hachimi & Guillaume, 2005). For the two-year study, argan oil samples were those prepared in the woman cooperative of Tiout.

2.3 Sample distribution

For the determination of the initial physicochemical parameters of argan oil, three types of oil were prepared: artisanal argan oil (AAO), mechanically-pressed argan oil (MAO), and traditional artisanal argan oil obtained from goat-peeled fruit (GPAO). Oil samples prepared from Ait Baha, Tidzi, and Tiout are indexed AB, TZ, and TT; respectively.

Time-dependent oxidative stability was studied by comparing the physicochemical properties of twelve samples. Ten liters of AAO, MAO, and GPAO were prepared. Each oil type was distributed in 360 60mL-glass bottles: 270 clear- and 90 brown-glass bottles. The remaining oil was used to determine initial values. For a given oil type, thirty clear-glass bottles were stored at 5°C, 25°C, and 40°C. Additionally, thirty brown-

glass bottles were stored at 25°C. Headspace volume (bottleneck volume) for each bottle was 3.5 (±0.5) mL.

2.4 Analytical methods

Samples stored at 5°C were analyzed after 5, 11, 17, and 23 months of storage. Samples stored at 25°C or 40°C were analyzed after 1 month of storage then every two months until month 23.

Acid value, peroxide value , saponification value, iodine index, and UV-light absorption (K_{270} and K_{232}) were determined as previously described (Hilali, Charrouf, El Aziz Soulhi, Hachimi, & Guillaume, 2005).

For the fatty acid composition determination, the methyl esters were analyzed on a CP-Wax 52CB column (30m x 0.25 mm i.d.) using helium (flow rate 1mL/mn) as a carrior gas. Initial oven temperature was set at 170°C; injector temperature 200°C; detector temperature 230°C. Injected quantity was 1µL for each analysis.

The oxidative stability of each sample was determined as the induction period (IP, hours) recorded by a Rancimat 743 (Metrohm) apparatus using 3 g of oil sample with an air flow of 20 L/h. To identify the initial oxidative parameters, oxidative stability was successively determined at 90°C, 100°C, 110 °C, 120°C, 130°C, and 140°C. For the two-year study, IP was determined at 110°C.

Sterol composition was determined after trimethylsilylation of the crude sterol fraction. Trimethylsilylated derivatives were analyzed by gas chromatography using a Varian 3800 instrument equipped with a VF-1ms column (30m x 0.25 mm i.d.) using helium (flow rate 1.6 mL/mn) as carrier gas. Column temperature was isothermal at 270°C,

injector and detector temperature was 300°C. Injected quantity was 1µL for each analysis.

Individual tocopherol content was determined on the basis of the AOCS Official method Ce 8-89 (American Oil Chemists' Society, 1993). Tocopherols were analyzed by HPLC using Shimadzu instruments equipped with a C18-Varian column (25cm x 4mm). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). Eluent used was a 99:1 isooctane/isopropanol (V/V) mixture, flow rate 1.2 mL/mn.

Phosphorus content was determined using the NF T60-227 recommendation (Paquot & Hautfenne, 1987).

β-Carotene content was determined using a PFX-995 lovibond tintometer (cell length 10 mm).

Statistical Analysis. Values reported in tables and figures are the means ± SE of three to five replications. The significance level was set at P=0.05. Separation of means was performed by Turkey's test at the 0.05 significance level.

3. Results and discussion

Genuine edible argan oil is exclusively prepared in Morocco since argan trees are only endemic in this country. Three types of edible argan oil can be found on the market: "certified", "artisanal", and "family". Those denominations reflect the oil preparative process. Certified argan oil is exclusively prepared in woman cooperatives by use of mechanical presses; it is sold on both domestic and international markets. Artisanal

argan oil is prepared by decanting the liquid resulting from the prolonged mixture of argan dough and water; it is mainly sold on Morocco domestic market but can also be purchased on the internet. Family argan oil is generally prepared under rudimentary conditions with the risk of bacteriologically unsafe water and use of goat-peeled fruit; it is used in the family circle but surplus of oil is sometimes sold on the local market. Each type of oil presents its own physicochemical (Hilali, Charrouf, El Aziz Soulhi, Hachimi & Guillaume, 2005) and organoleptic profile (Matthäus, Guillaume, Gharby, Haddad, Harhar & Charrouf, 2010). The aim of our study was to evaluate the influence of a prolonged storage on argan oil physicochemical properties and oxidative stability. Consumption of argan oil usually occurs within 18 to 24 months. Accordingly, we chose a maximum storage time of two years for our study. Variations in oil processing can influence the initial oxidation of edible oil (Tatum & Chow, 1992). Therefore, we decided to determine the oxidative properties of the three common types of edible argan oil: mechanically-extracted (MAO), artisanally-extracted (AAO), and hand-extracted using goat-peeled fruit (GPAO). Four different storage conditions: refrigerated at 5°C, 25°C light-unprotected, 25°C light-protected, and 40°C (oven) were considered. Our study began with the careful determination of the initial parameters of our samples. Due to the large area covered by the argan forest, the question of the incorporation of the oil geographic origin as a to-be-considered parameter came out rapidly. Consequently, we first decided to carry out an oxidative stability study/physicochemical analysis of argan oil samples coming from the three main locations, in terms of argan oil production, of the argan forest.

3.1. Determination of the initial physicochemical parameters of the oil samples

Some physicochemical parameters of freshly prepared argan oil have already been reported to be either poorly dependent or independent on the nut harvest location (Charrouf, El Hamchi, Mallia, Licitra, Guillaume, 2006; Cayuela, Rada, Pérez-Camino, Benaissa, Abdelaziz & Guinda, 2008). Because 1) short term autoxidation of argan oil has been only partially studied (Chimi, Cillard & Cillard, 1994; Chimi, 2005), 2) the aspect of preservation has never been investigated in previous studies, 3) equipments used to obtain mechanically prepared argan oil are regularly upgraded, and 4) minute traces of metals can modify oil quality (Marfil, Cabrera-Vique, Giménez, Bouzas, Martínez & Sánchez, 2008), we decided to examine the physicochemical parameters of argan oil samples coming from Ait Baha (AB), Tidzi (TZ), and Tiout (TT), the three largest argan oil woman cooperatives in the argan forest. Table 1 lists the results of the physicochemical parameters analyzed.

Satisfactorily, all fresh argan oil samples displayed the physicochemical properties necessary to access the edible grade as defined by the recommendations of the official argan oil norm (Service de normalisation industrielle, 2003). Nevertheless, the high acid value of $GPAO_{TZ}$ (>0.8%) was incompatible with an "extra virgin" label, even though it was acceptable for a "pure virgin" grade (Service de normalisation industrielle, 2003). Comparison between the determined parameters indicated that MAO consistently contained a significantly higher level of phospholipid/phosphorus than AAO and GPAO. It is likely that the development of heat at the press head during mechanical extraction results in a transfer of phospholipids into the oil and hence to a high amount of phospholipids in MAO. The low amount of phospholipids in AAO and GPAO results from a poor phospholipid extraction at room temperature. Phospholipids can trigger

technical problems during oil degumming or refining. In our case, such a consideration is only of limited importance since, oppositely to cosmetic argan oil, edible (extra) virgin argan oil is not refined. More importantly, phospholipids can act as antioxidants or prooxidants (Koprivnjak, Skevin, Valic, Majetic, Petricevic & Ljubenkov, 2008; Choe & Min, 2006) depending on their concentration and the presence of metal ions (Choe & Min, 2006) or tocopherols (Koga & Terao, 1995; Judde, Villeneuve, Rossignol-Castera & Le Guillou, 2003). Since argan oil is notoriously rich in tocopherols, the high content in phospholipids in MAO, compared to AAO and GPAO should merit further attention. Other studied parameters ((un)saturated fatty acid, β-carotene level, UV absorption) were remarkably constant, any of them significantly varying as a function of the oil geographic origin.

3.2. Determination of the initial oxidative stability of the oil samples

Conversely to argan oil physicochemical parameters, its oxidative stability has never been studied as a function of the oil geographic origin. To get a complete picture of argan oil oxidative stability, we decided to determine the induction period by Rancimat test at 90, 100, 110, 120, 130, and 140°C of our oil samples prepared in the three different locations. Results obtained at 110°C are presented Table 2.

Interestingly, homogenous Rancimat induction periods were observed within each group. For every given temperature, MAO consistently and independently of the geographic origin displayed much longer rancimat induction periods than AAO or

GPAO. Oxidative stability of argan oil has been attributed to its high content in tocopherols (Rahmani, 2005), and carotenes (Collier & Lemaire, 1974). Since these families of components are in a similar quantity in the three types of oil (Hilali, Charrouf, El Aziz Soulhi, Hachimi & Guillaume, 2005), our results show that in fresh argan oil, phospholipids do not act as prooxidants, and presumably act as antioxidants, reinforcing the strong preservation activity of tocopherols. Notably large induction period differences between the MAO group and the AAO and GPAO groups were observed between 90 and 110°C. At 90°C, MAO average rancimat induction period was found to be 110±6 hrs, whereas it was only 76±6 hrs, and 70 ±2 hrs for AAO, and GPAO, respectively. At 110°C, the difference between the mean rancimat induction period of MAO and AAO or GPAO was 11±2 hrs. Unexpectedly, AAO and GPAO displayed similar rancimat induction periods even though GPAO preservation time is commonly said to be low. This apparent contradiction can be explained since in our study AAO and GPAO samples were prepared using bacteriologically safe water, a parameter never controlled when argan oil is prepared in the family circle. Therefore, it is highly likely that the short preservation time attributed to GPAO, compared to AAO, principally results from microbiologically-induced damage, rather than a chemically-assisted process.

The homogenous results observed within each group during this preliminary study evidenced that consideration of the geographic origin was unnecessary for our study. Incidentally, we also decided to select 110°C as the optimum temperature to evaluate the oxidative stability of our oil samples since at this specific temperature afforded well reproducible results as already observed with olive oil (Mateos, Uceda, Aguilera, Escuderos & Beltran Maza, 2006).

246

247 *3.3 Preservation of argan oil, a two-year study*

248 Initial fatty acid, sterol and tocopherol composition was carried out prior the beginning
249 of our study. Values are listed Table 3-5.

250 *3.3.1 Acid value analysis*

251 Acid value of MAO, AAO, and GPAO stored at 5°C did not significantly changed over
252 two years. Initial acid values were 0.2 for MAO and AAO, and 0.9 for GPAO. After two
253 years at 5°C, acid value was 0.2, 0.4, and 1 for MAO, AAO, and GPAO; respectively.
254 Acid value of MAO also remained remarkably stable over two years independently on
255 the storage temperature and glass color (Figure 1). After two years at 25°C, acid value of
256 MAO was 0.3. It was 0.4 after two years of storage at 40°C.

257 Acid value of AAO stored in dark bottles at 25°C increased only very slightly (average
258 0.02 acid value unit/month) during the first 17 months of storage to reach the value of
259 0.5. After 17 months of storage, acid value increased 5-fold faster reflecting accelerated
260 triacylglycerol degradation (Figure 1). After 21 months at 25°C in colored glass bottles,
261 acid value of AAO reached the 0.8 limit, loosing its extra virgin label (Service de
262 normalisation industrielle, 2003). When AAO was stored unprotected from sunlight at
263 25°C, increase in acid value began two months sooner and the 0.8 limit was reached
264 after 19 months (Figure 1). However, after two years, final acid value of AAO stored in
265 clear or dark bottles was similar: 1.2±0.2. Stored at 40°C, AAO lost its extra virgin label
266 after 18 months.

267 Initial acid value of GPAO was much higher than that of MAO and AAO and already
268 above the 0.8 limit (Table 1). When stored at 25°C in clear glass bottles, acid value of
269 GPAO significantly and continuously increased (Figure 1) whereas that of samples

stored at the same temperature in dark bottles remained stable during 5 months (Figure 1). At 40°C, GPAO acid value increased continously, but less rapidly than the acid value of AAO at the same temperature (Figure 1). Interestingly, the 2-year acid value profiles of MAO, AAO, and GPAO stored at 40°C or at 25°C and unprotected from sunlight were almost similar (Figure 1). This result suggests that between 25 and 40°C, light is much more important than temperature to induce triacylglyceride oxidation in argan oil.

3.3.2 Peroxide value analysis

Peroxides are the primary oxidation products that lead to rancidity. Therefore, their formation dramatically impacts oil shelf life and consumer acceptance. High temperature and light are two well-known factors generally promoting peroxide formation. In argan oil, the respective impact of these two factors is presently unknown. Initial peroxide value of MAO, AAO, and GPAO was found to be below 2 meq of O_2/kg oil, well below the maximum peroxide value of 15 meq O_2/kg oil defined for the extra virgin argan oil label (Service de normalisation industrielle, 2003). For MAO, AAO, as well as GPAO, storage at 5°C for two years led only to a very slight increase of the peroxide value (data not shown); the highest peroxide value of 3 meq O_2/kg oil was observed for AAO.

Peroxide value of MAO, AAO, and GPAO stored at 25°C or 40°C behaved differently. When stored at 25°C in dark or clear glass bottles, MAO peroxide value remained below the 15 meq O_2/kg oil limit for two years. Accurate examination of the changes indicated that MAO peroxide value increased permanently over two years to reach the almost similar maximum values of 10.7 meq O_2/kg oil for MAO protected from sunlight and 12 meq O_2/kg oil for MAO exposed to sunlight (Figure 2). However, the increase rate seems to be light-dependent since the oxidation kinetic observed between light protected

294 and unprotected samples was different (Figure 2). Argan oil natural antioxidants are also
295 likely to influence this kinetic. Storage of MAO in an oven at 40°C for two years led to
296 peroxide values permanently higher than that observed when stored at 25°C. The final
297 peroxide value was 16.3 meq O_2/kg oil, whereas the limit of 15 meq O_2/kg oil barrier
298 was crossed after 21 months. Interestingly, the profiles of the peroxide values at 40°C
299 and 25°C in dark bottles were similar. This likely means that a process identical, but
300 amplified at 40°C, occurs in MAO protected from sunlight at 25°C or 40°C, and
301 confirms that degradation and oxidative processes occurring in MAO are greatly
302 accelarated under sunlight.
303 AAO stored at 25°C in clear glass bottles crossed the 15 meq O_2/kg oil limit after 13
304 months. At the same temperature, 19 months were necessary when AAO was protected
305 from sunlight. When AAO was stored at 25°C and exposed to sunlight, the peroxide
306 value increased quite consistently over two years to reach 25.8 meq O_2/kg oil after two
307 years (Figure 3). Sunlight protection led to lower peroxide values (14.4 meq O_2/kg oil,
308 and 18.3 meq O_2/kg when stored at 25°C and 40°C; respectively) that were similar to
309 that observed for MAO for corresponding storage conditions. However those values
310 should be carefully handled since peroxide value underwent large fluctuations over two
311 years. Such phenomenon was not observed for MAO possibly suggesting the occurrence
312 for AAO of multiple secondary oxidation processes that did not occur in MAO and
313 hence that could be related to the different content in minor components.
314 With regards to the peroxide value, GPAO satisfied the virgin label requirements for 13
315 and 15 months when stored at 25°C in clear or dark glass bottles; respectively. When
316 GPAO was stored at 25°C in clear glass bottles, peroxide value was at its highest (19.8
317 meq O_2/kg oil) after 15 months. Then, it decreased to reach the low value of 6.9 meq

318 O_2/kg oil after two years. This phenomenon was amplified at 40°C (Figure 4). At 25°C
319 and protected from sunlight, AAO and GPAO peroxide values behaved globally
320 similarly. Therefore, as already observed from the acid value study, over two years, light
321 also appears as the major parameter promoting hydroperoxide formation in all types of
322 argan oil, elevated temperature favoring only secondary oxidation product formation.
323
324 *3.3.3 K_{232} analysis*
325 Primary oxidation product formation can also be monitored by measuring specific
326 extinction at 232 nm (K_{232}). High quality argan oil should present a K_{232} lower than 2.5
327 (Service de normalisation industrielle, 2003). During two years of storage at 5°C, K_{232} of
328 MAO, AAO and GPAO remained practically constant (initial value 1.06, 1.24, and 1.28
329 vs final value 1.27, 1.29, and 1.52 for MAO, AAO, and GPAO; respectively) as
330 expected from the results of the peroxide value study.
331 When MAO was stored at higher temperature, K_{232} was observed between 1.6 and 2 after
332 2 years. When stored at 25°C in clear or dark glass bottles, K_{232} and peroxide value
333 evolved in a similar way (Figure 2), suggesting the low incidence of secondary oxidative
334 product formation. During storage at 40°C, although peroxide value increased swiftly
335 between months 11 and 17, K_{232} absorption remained quite stable during this period.
336 This strongly suggests the occurrence at 40°C of multiple, complex, and not fully
337 identified oxidative processes for which the involvement of phospholipids can be
338 eliminated since a similar behavior was also observed for AAO and GPAO (Figure 3, 4).
339
340 Whereas MAO had K_{232} between 1.6 and 2 after two years, AAO K_{232} was between 1.8
341 and 3 after the same period of time. AAO K_{232} crossed the 2.5 barrier after 11 and 17

342 months of storage at 25°C in dark and clear glass bottles; respectively (Figure 3).
343 Surprinsingly, during storage 40°C, K_{232} of AAO never reached the 2.5 value even
344 though observed peroxide value reflected the occurrence of intense oxidative processes.
345 Consequently, in that case, a direct correlation between peroxide value and K_{232} was
346 uneasy to establish, likely due to multiple and concommittant oxidation processes
347 favored by temperature.
348 Finally, for GPAO, K_{232} after two years was between 1.6 and 2.4. K_{232} absorption
349 remained surprisingly stable when GPAO was stored at 25°C in dark bottles. When
350 stored at 25°C in clear bottles, a good correlation was observed between K_{232} and
351 peroxide value, both indexes decreasing after 15 or 17 months (Figure 4). During storage
352 at 40°C, K_{232} and peroxide value increased simultaneously until month 17 but K_{232}
353 remained stable although peroxide value dramatically plummeted after this month.
354
355 *3.3.4. K_{270} study*
356 Carbonyl (aldehyde and ketone) compounds are the most abondant secondary oxidation
357 products formed in edible oils. Their formation is known to be accelerated by elevated
358 temperature and metal traces (Choe & Min, 2006). UV absorption at λ 270 nm (K_{270}) is
359 one of the markers used to follow secondary oxidation formation. Moroccan regulation
360 has set the maximum value for K_{270} at 0.35 (Service de normalisation industrielle, 2003).
361 Overall, K_{270} values did not significantly changed over the 2 years. Initial values are
362 given in Table 1. That argan oil samples stored at 5°C over 2 years displayed stable K_{270}
363 was not surprising. That this trend also occurred for oil samples stored at higher
364 temeperature was unexpected. Final K_{270} values for MAO were 0.24, and 0.31 when

samples were stored at 25°C, and 40°C, respectively. For GPAO, final values were in the same range for the three storage conditions. Only K_{270} of AAO stored at 40°C crossed the limit value of 0.35 after 17 months to end up at 0.39±0.05. Independently of the sunlight protection, the final value of AAO stored at 25°C was 0.21±0.05.

In summary, K_{232} and peroxide value depict the formation of primary oxidation products. The apparent sample-dependent correlation observed between K_{232} and peroxide value supports the idea of different ratio of hydroperoxides depending on the type of argan oil. Decomposition of these hydroperoxides into secondary oxidation products can be monitored by K_{270} examination. Our results show that hydroperoxides formed in the three types of argan oil decompose to unsaturated secondary oxidation products, and that MAO presents the slowliest decomposition rate. In GPAO, the oxidative profile is more complex. Keeping in mind that MAO presents a highly homogeneous chemical composition, likely induced by its highly homogeneous geographical origin, these observations are of the utmost importance, considering the negative influence of secondary oxidation products on oil taste and smell, from an organoleptic standpoint.

3.3.5. Rancimat study.

Then we investigated the oil oxidative stability by measuring every 6 months the rancimat induction period at 110°C of MAO, AAO and GPAO stored in our evaluated conditions. Results are reported Table 6. When oil samples were stored at 5°C, rancimat induction period did not significantly vary over two years. Amazingly, at storage temperatures above 5°C, rancimat induction period of each type of oil decreased during the first 6 months then remained almost unchanged during the last eighteen months.

MAO displayed by far the longest induction period confirming the good preservation properties of this type of oil. Rancimat induction time of oil samples stored at 25°C and exposed to sunlight was found to be slightly but significantly shorter than that of oil samples stored at the same temperature but protected from sunlight. This result is consistent with our previous observations that indicate the occurrence of a slower oxidative process in argan oil samples protected from sunlight. Storage of argan oil at 40°C for 2 years led to a reduction of the rancimat induction period almost similar to that observed for argan oil stored at 25°C and exposed from sunlight.

3.3.6. Miscealenous analyses

Finally, we also decided to analyze some of the physicochemical parameters of our oil samples after two years in order to possibly detect variations affecting its pharmacologically essential components. Because most of argan oil therapeutic properties are linked to its high unsaturated fatty acid content, we determined several parameters including the iodine index, saponification value, and fatty acid composition of every oil samples after 2 years (Tables 3-5, 7). Concerning the saponification value, the largest variation was observed for samples stored at 40°C but the saponification value calculated after 2 years was still satisfying the official norm (Service de normalisation industrielle, 2003). Over two years, iodine index underwent a minor reduction due to primary oxidation but, for all samples it was consistently found between 91 and 110 as required by the official norm (Service de normalisation industrielle, 2003). Additionnally, we also analyzed the β-carotene content of our oil samples since β-carotene actively participates in oleic-rich oil protection under

autooxidative and photooxidative processes (Goulson & Warthesen, 1999). While β-carotene level in MAO remained stable over two years, a strong decrease was observed for AAO stored at 25°C in clear glass bottles likely due to intense oxidative reactions. Measurements for GPAO were inconclusive due to the color variation of the oil samples after two years.

Concerning the fatty acid and sterol distribution in each oil samples, no significant changes were observed over two years. Results are listed Tables 3 and 4. Tocopherols possess antioxidative and anti free-radical properties. Therefore oil oxidative stability depends on changes occurring in tocopherol content during storage (Okogeri & Tasioula-Margari, 2002). Tocopherol high concentration in argan oil is not only essential for its preservation but also for its pharmacological activity (Khallouki *et al.,* 2003). Storage of argan oil for two years at 25°C in clear glass bottles resulted in a dramatic decrease in tocopherol level for the three types of oil. Sunlight protection resulted in a reduced tocopherol lost that was nevertheless consequent for oil samples stored at 40°C. Individually considered, α-, β-, and δ-tocopherol levels were almost divided by two after two years of storage in clear glass bottles or at 40°C. Only storage in dark-glass bottles allowed the preservation of a high γ-tocopherol level (Table 5).

Conclusions

Combined all together, our results designate light as the major factor involved in argan oil oxidation. After two years of storage at 25°C, MAO protected from sunlight displays several physicochemical properties and an oxidative induction period that remained similar to freshly prepared argan oil. MAO is the type of argan oil that is sold on the

435 international market and a shelf life of two years can reasonably be recommended for
436 such oil as long as it is protected from sunlight. Edible argan oil has a characteristic
437 copper color that helps consumers to distinguish it rapidly from other oils. Therefore the
438 use of colored glass bottles is unlikely to be easily accepted by a majority of consumers.
439 Argan bottles are generally packed in cardboard box, such practice should be preserved
440 since to help argan oil preservation.
441

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447

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Table 1. Physicochemical parameters of argan oil prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO). Oil samples prepared from Ait Baha, Tidzi, and Tiout are indexed AB, TZ, and TT; respectively. Mean ± standard deviation of the values (five replicates) are presented.

	MAO_{AB}	MAO_{TZ}	MAO_{TT}	AAO_{AB}	AAO_{TZ}	AAO_{TT}	$GPAO_{AB}$	$GPAO_{TZ}$	$GPAO_{TT}$
Acid Value (mg/g)	0.3±0.05	0.3±0.05	0.3±0.02	0.6±0.1	0.5±0.1	0.3±0.1	0.3±0.1	1.1±0.1	0.7±0.1
Peroxide value (Meq/kg)	0.7±0.1	1.2±0.1	0.6±0.1	1±0.1	1±0.1	1±0.2	1.1±0.1	1.3±0.1	1.5±0.2
Moisture (mg/100mg)	0.07±0.01	0.06±0.01	0.05±0.01	0.08±0.01	0.06±0.02	0.08±0.01	0.06±0.01	0.25±0.01	0.09±0.01
K_{232}	1.44±0.06	1.18±0.07	1.02±0.06	1.28±0.06	1.21±0.06	1.24±0.06	1.29±0.07	1.49±0.06	1.37±0.06
K_{270}	0.25±0.05	0.2±0.05	0.18±0.05	0.19±0.05	0.18±0.05	0.22±0.05	0.21±0.05	0.18±0.05	0.17±0.05
β-carotene (ppm)	20±0.5	20±0.5	21±0.5	11±0.5	13±0.5	18±0.5	15±0.3	16±0.5	17.5±0.5
Phosphorus ($mg/10^3g$)	42.8±0.2	61.5±0.8	80.2±0.8	7.8±0.1	5.3±0.1	3.9±0.1	3.6±0.2	9.1±0.1	6.1±0.1
Phospholipid (mg/100mg)	0.3	0.2	0.25	0.02	0.01	0.01	0.01	0.03	0.02
SFA[a] (mg/100mg)	19±0.7	17.7±0.5	19.3±0.3	19.1±0.3	18.2±0.1	19.6±0.1	20±0.4	17±0.2	18±0.2
UFA[a] (mg/100mg)	80±1	82±0.5	80±0.5	81±0.5	81±0.5	79±0.5	78.5±0.5	82±0.5	80.5±0.2

[a] SFA: saturated fatty acids, UFA: unsaturated fatty acids.

Table 2. Rancimat induction period (hrs) at 110°C of argan oil prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO). Oil samples prepared from Ait Baha, Tidzi, and Tiout are indexed AB, TZ, and TT; respectively. Mean ± standard deviation of the values (five replicates) are presented.

	MAO_{AB}	MAO_{TZ}	MAO_{TT}	AAO_{AB}	AAO_{TZ}	AAO_{TT}	$GPAO_{AB}$	$GPAO_{TZ}$	$GPAO_{TT}$
110°C	24±0.5	27±0.5	31±1	18±0.5	16±0.5	14±0.5	14±0.5	16±0.5	16±0.5

Table 3. Fatty acid distribution (initial and final) in argan oil samples prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO) and stored for 2 years at 5°C, 25°C in clear or dark glass, and at 40°C. Values are expressed in g/100g of total extracted fatty acids (±1) and result from four replicates.

	Initial	Stored at 25°C in clear glass	Stored at 25°C in dark glass	Stored at 40°C
MAO				
Palmitic acid	13	14	13	14
Stearic acid	5	5	5	5
Oleic acid	48	48	48	48
Linoleic acid	32	32	32	31
AAO				
Palmitic acid	13	15	14	14
Stearic acid	5	5	5	5
Oleic acid	47	47	47	47
Linoleic acid	33	33	33	33
GPAO				
Palmitic acid	14	15	14	15
Stearic acid	6	6	6	6
Oleic acid	48	47	48	48
Linoleic acid	31	30	31	30

Table 4. Sterol composition (initial and final) in argan oil samples prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO) and stored for 2 years at 5°C, 25°C in clear or dark glass, and at 40°C. Values are expressed in g/100g of total sterols (±2) and result from four replicates.

	Initial	Stored at 25°C in clear glass	Stored at 25°C in dark glass	Stored at 40°C
MAO				
Schottenol	46	46.5	46.5	45
Spinasterol	40	40	39	37
Δ-7-avenasterol	5.5	4	4	4
Stigmasta-8,22-dien-3β-ol	5	3.5	3.5	3
AAO				
Schottenol	44	44	44	44
Spinasterol	42	42	41	39
Δ-7-avenasterol	4	4	3.5	3
Stigmasta-8,22-dien-3β-ol	3.5	3	3	3
GPAO				
Schottenol	44	44	44	44
Spinasterol	43	42	41	40
Δ-7-avenasterol	6	3	4	3
Stigmasta-8,22-dien-3β-ol	4	3	4	3

Table 5. Tocopherol composition (initial and final) in argan oil samples prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO) and stored for 2 years at 5°C, 25°C in clear or dark glass, and at 40°C. Results are expressed in mg/kg and come from three replicates.

	Initial	Stored at 25°C in clear glass	Stored at 25°C in dark glass	Stored at 40°C
MAO				
Total	675±25	564±25	601±25	589±25
α-tocopherol	59±8	33±7	41.5±8	36.5±7
β-tocopherol	6±2	2±1	2±1	2±1
γ-tocopherol	531±25	479±25	503±25	495±25
δ-tocopherol	51±8	33±7	38.5±7	36±7
AAO				
Total	766±25	545±25	599±25	528±25
α-tocopherol	72±10	20±8	35±10	27±10
β-tocopherol	7±2	2±1	2±1	2±1
γ-tocopherol	585±25	471±25	491±25	445±25
δ-tocopherol	82±12	34±10	47±8	42±8
GPAO				
Total	660±25	462±25	559±25	518±25
α-tocopherol	70±10	35±10	43±10	40±10
β-tocopherol	5±2	2±1	3±1	2±1
γ-tocopherol	531±25	386±25	467±25	437±25
δ-tocopherol	39±7	20±6	31±8	25±7

Table 6. Rancimat induction period (hrs) at 110°C of argan oil prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO) and stored at 5°C, 25°C (clear or dark glass bottles), and 40°C for up to 2 years. Mean ± standard deviation of the values (five replicates) are presented.

	6 Months	12 Months	18 Months	Final
MAO				
Stored at 5°C	32±1	30±1	31±1	30±1
Stored at 25°C in clear glass	28±1	28±1	26±1	25±1
Stored at 25°C in dark glass	29±1	28±1	27±1	27±1
Stored at 40°C	28±1	27±1	26±1	24±1
AAO				
Stored at 5°C	13±0.5	12±0.5	13±0.5	14±0.5
Stored at 25°C in clear glass	11±0.5	11±0.5	10±0.5	9±0.5
Stored at 25°C in dark glass	13±0.5	12±0.5	11±0.5	10±0.5
Stored at 40°C	10±0.5	10±0.5	10±0.5	8±0.5
GPAO				
Stored at 5°C	15±0.5	14±0.5	15±0.5	15±0.5
Stored at 25°C in clear glass	13±0.5	12±0.5	11±0.5	10±0.5
Stored at 25°C in dark glass	15±0.5	13±0.5	12±0.5	12±0.5
Stored at 40°C	12±0.5	12±0.5	11±0.5	9±0.5

Table 7. Saponification value, iodine index, and β-carotene level (initial and final) of argan oil samples prepared from argan kernels pressed 1) mechanically (MAO), 2) artisanally pressed (AAO), or 3) artisanally and obtained from goat-peeled fruit (GPAO) stored for 2 years at 5°C, 25°C in clear or dark glass, and at 40°C. Mean ± standard deviation of the values (five replicates) are presented.

	Initial	Stored at 5°C	Stored at 25°C in clear glass	Stored at 25°C in dark glass	Stored at 40°C
			Saponification value (mg of KOH/g of oil)		
MAO	189.5±0.5	189.7±0.2	190.8±0.5	190.6±0.4	194±0.1
AAO	192.6±0.5	192.7±0.5	193±0.5	193.2±0.2	193.9±0.5
GPAO	190.6±0.4	192.5±0.5	191.7±0.6	191±0.5	193.4±0.6
			Iodine index (g of I_2/100g of oil)		
MAO	97.7±0.1	96.9±0.5	96.7±0.5	96.7±0.5	95.9±0.5
AAO	102.4±0.5	101.2±0.5	99.4±0.5	99.4±0.6	98.3±0.4
GPAO	96.8±0.5	95.7±0.5	94.5±0.5	96.4±0.5	95.6±0.5
			β-Carotene level (mg/kg)		
MAO	20.7±0.5	18.8±0.5	17±0.5	17.4±0.5	17±0.5
AAO	18±0.5	17.9±0.5	7.1±0.5	10.1±0.5	11.3±0.5
GPAO	17.5±0.5	15.4±0.5	6.6±4	10.2±5	15.4±5

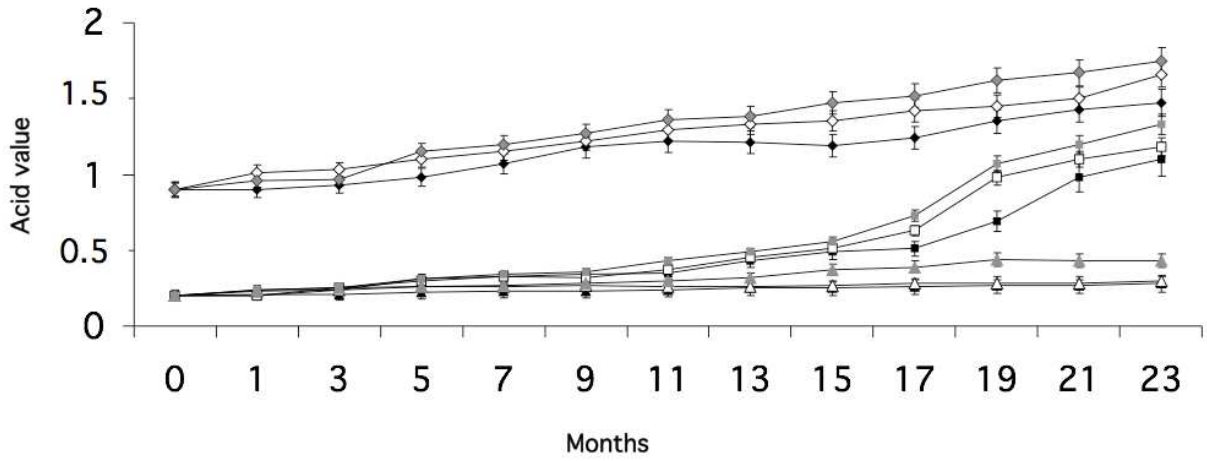

Fig. 1. Acid value of argan oil as a function of time (months). Oil samples were either sunlight-protected and stored at 25°C (black symbols), exposed to sunlight and stored at 25°C (white symbols), or sunlight-protected and stored at 40°C (grey symbols). Oil was prepared from fruits either peeled by goats (rhombs), mechanically peeled (triangles), or manually peeled (squares). Mean ± standard deviation of the values (three replicates) are presented.

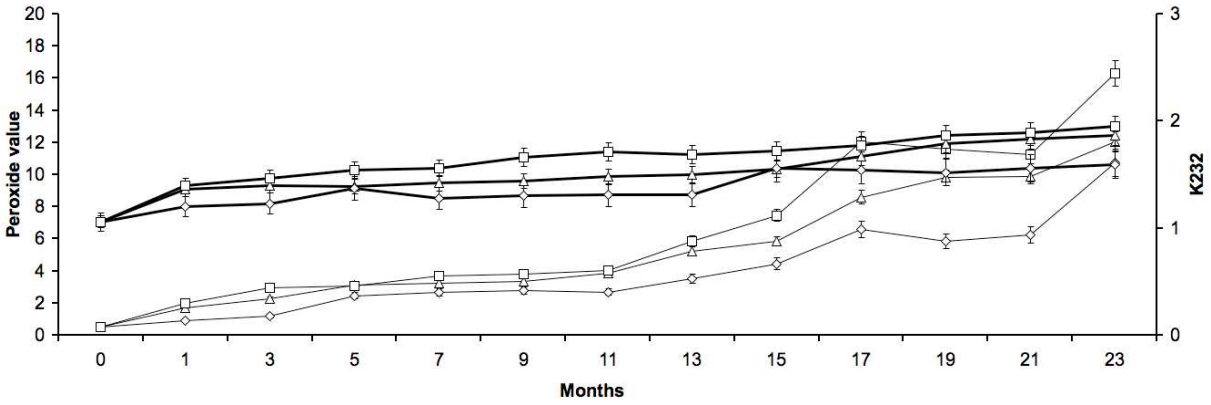

Fig. 2. Peroxide value and K_{232} (bold line) of mechanically prepared argan oil (MAO) as a function of time (months). Samples were either protected from sunlight and stored at 25°C (rhombs), exposed to sunlight and stored at 25°C (triangles), or protected from sunlight and stored at 40°C (squares). Mean ± standard deviation of the values (three replicates) are presented.

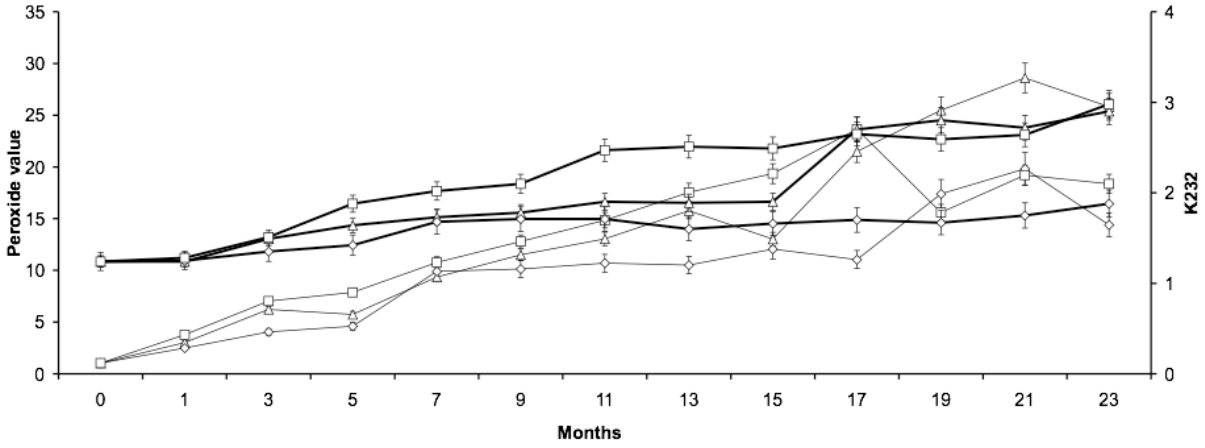

Fig. 3. Peroxide value and K_{232} (bold line) of artisanally prepared argan oil (AAO) as a function of time (months). Samples were either protected from sunlight and stored at 25°C (rhombs), exposed to sunlight and stored at 25°C (triangles), or protected from sunlight and stored at 40°C (squares). Mean ± standard deviation of the values (three replicates) are presented.

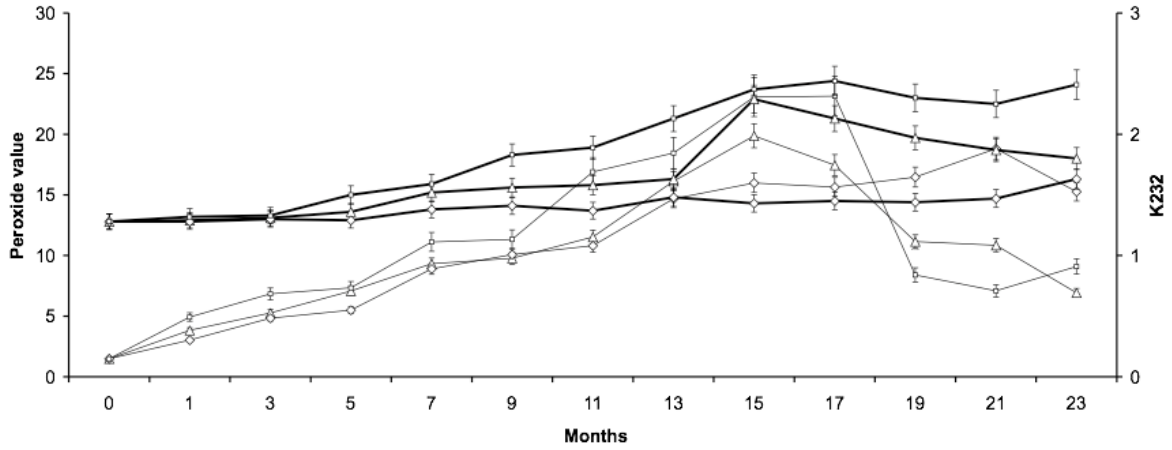

Fig. 4. Peroxide value and K_{232} (bold line) of argan oil prepared from goat-peeled fruit (GPAO) as a function of time (months). Samples were either protected from sunlight and stored at 25°C (rhombs), exposed to sunlight and stored at 25°C (triangles), or protected from sunlight and stored at 40°C (squares). Mean ± standard deviation of the values (three replicates) are presented.