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Study of the Traditional Argan Oil Oxidation under Different Storage Conditions

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Abstract:
The autoxidation of argan oil extracted traditionally was followed during a long-term storage, using plastic bottles in presence of air, under nitrogen atmosphere or at 4 °C, in comparison with transparent glass bottles. The autoxidation was evaluated by the simultaneous monitoring of four parameters for a period of 18 months: tocopherols content, oleic and linoleic acids, conjugated dienes and peroxide value.The results showed that the storage in plastic bottles in the light at room temperature, delayed the oxidative degradation of the oil relatively to the transparent glass. This degradation was mitigated by the presence of nitrogen. The maximum content of hydroperoxides, resulting from the degradation of unsaturated fatty acids, was reached after 306 days in the presence of air and 321 days in the presence of nitrogen in the plastic bottles. This effect was recorded only after 273 days in the glass vials. Conjugated dienes changed in the same way as hydroperoxides. The degradation of unsaturated fatty acids taked place after the destruction of tocopherols including γ-tocopherol, which was the major form in the argan oil. The shelf life of the oil was longer (over 18 months) when plastic bottles were placed at 4 °C. The results showed that argan oil extracted traditionally had high stability against the oxidation due to its richness in tocopherols which are powerful antioxidants.
Keywords: Argan oil, glass, nitrogen, oxidation, plastic, storage, temperature

1. Introduction

The autoxidation is the major cause of deterioration of edible oils during storage. It depends on several factors such as the initial composition of the oil, the presence and the content of minor components which have pro- or anti- oxidant activity (minerals, tocopherols, carotenoids, chlorophylls, etc.) and storage conditions including material storage, lighting, oxygen and temperature (Crapiste et al., 1999; Kondratowicz and Ostasz, 2000). The autoxidation alters edible oils by degrading essential fatty acids whose consequences are a decrease in the nutritional value and the formation of decomposition products. These are responsible for disagreeable odor and flavor and may even cause some toxicity (Pascaud et al., 1985; Pokorny, 2003).

Argan oil, extracted traditionally from the fruit of the Argan tree, constitutes an important source of dietary fat in the south of Morocco. It owns nutritional and pharmacological properties due to its chemical composition which is characterized by richness in antioxidants and unsaturated fatty acids (Khallouki et al., 2003; Guillaume and Charrouf, 2011). However, argan oil extracted traditionally is known to be not well preserved under normal storage conditions (Chimi, 2005; Cayuela et al., 2008. Marfil et al., 2008), even if its chemical composition allows suggesting the opposite. It is rich in tocopherols which are powerful antioxidants and do not contain linolenic acid as fatty acid particularly susceptible to oxidation. This rapid deterioration of the traditional oil would be a limiting factor for marketing and consumption in other parts of Morocco and even in other countries.

The origin of this instability is rarely studied. The works undertaken to evaluate the autoxidation of traditional oil based on long-term storage conditions, which reflect the actual conditions of conservation, are fragmentary (Chimi et al., 1994; Gharby et al., 2011). Faced with this situation, we undertook this work to study the effect of conditioning in bottles of translucent plastic on the alteration of the traditional argan oil retained in light at room temperature for a period of 18 months, in comparison with the transparent glass bottles which are frequently used in domestic or commercial conservation. Storage under nitrogen and low temperature (4 °C) was also studied. To determine the oxidation of the oil, we followed the evolution of four different parameters: peroxide value, rate of conjugated dienes, tocopherols and unsaturated fatty acids (oleic and linoleic acids).

2. Materials and methods

2.1 Sample of oil used

All operations were undertaken on the same argan oil sample extracted traditionally under our control. In fact, seeds of the argan tree from Admine region, located at 20 km east of Agadir city, were manually crushed and resulting almonds were roasted over low heat and then grounded using artisanal stone. The oil extraction was conducted by mixing the slurry obtained and on which drops of warm water are added from time to time. The oil rising to the surface was collected by decantation. A sample was immediately analyzed to determine the initial

composition of the oil.

2.2 Storage conditions

The oil was distributed into translucent plastic bottles of high density polyethylene (HDPE), filled to three-fourths in a proportion of 30 g per vial. These were then sealed and stored under the following conditions:

3 bottles in light (250-lux intensity at the surface of the recipients), at room temperature in the presence of air,

3 bottles in light (250-lux intensity at the surface of the recipients), at room temperature under nitrogen,

3 bottles at 4 °C, in the presence of air.

The control sample was stored in three transparent clear glass bottles in the presence of air in the light and at room temperature.

2.3 Measurement of oil composition

2.3.1 Acidity (AFNOR, 1978a)

In an Erlenmeyer flask were mixed 50 ml of ethanol and 50 ml of diethylether. Two drops of phenolphthalein are added to this mixture which was then neutralized using a potassium hydroxide solution (0.1 N) until the color shift. This mixture was added to 10 ml sample of the oil which was immediately decolorized because of the presence of free fatty acids in the oil. Titration of the oil sample, dissolved in the mixture of diethylether/ethanol was carried out with an ethanolic solution of potassium hydroxide (0.1 N). The acidity was expressed as percent of oleic acid.

2.3.2. Evaluation of the oil degradation

The samples were taken periodically (range of 1 to 4 weeks) for more than 18 months. Four parameters were measured simultaneously:

2.3.2.1 Conjugated Dienes (CD)

The oil was diluted in cyclohexane at the concentration of 1% (v/v). The CD content was evaluated by the value of the absorbance at 233 nm using a Varian DMS 80 spectrophotometer.

2.3.2.2 Peroxide Value (PV) according to AFNOR (1978b)

To a mixture of 2 g of oil and 10 ml of chloroform was added 15 ml of acetic acid and 1 ml of potassium iodide. After stirring, the mixture was placed in the dark for 5 min. Then 75 ml of distilled water and the starch paste as a colored indicator, were added. The titration was carried out with sodium thiosulfate adition. PV was expressed in milli-equivalent of O2 per Kg of oil (meq O2/Kg).

2.3.2.3 Tocopherols content

Tocopherols composition of argan oil was performed with the HPLC method. The chromatographer comprised a Jasco 880-PU pump, a Jasco 875-UV spectrophotometer detector and a Varian 4400 integrator. Argan oil was diluted with 100% methanol and injected into a C18-grafted silica column (length 25 cm; diameter 3 µm). The tocopherol content in argan oil was determined in comparison to a standard mixture.

2.3.2.4 Content of unsaturated fatty acids

After its extraction, argan oil was methylated with Boron trifluoride (BF3). Three extractions with hexane were then made to obtain fatty acid methylic esters. These esters were evaporated under nitrogen conditions and 50 µg were directly injected into a Cpcil-88 column (length 50 m; diameter 0.25 mm) of a Carlo Erba gas chromatograph whose temperature increased from 150 to 225 °C (5 °C/min). After their separation in the column, fatty acids were detected using a flame ionization detector at 250 °C. The identification of fatty acids was made by comparing their equivalent chain length with a standard mixture of fatty acids. The pressure of vector gas (H2) was 1 bar.

III. Results and discussion

The analysis of the initial composition of argan oil is shown in table 1. The values of the chemical composition (fatty acids and tocopherols) agree with previous works (Hilali et al., 2005; Belcadi et al., 2008; Matthäus et al., 2010; Harhar et al., 2014). In the initial state, the oil had an acidity of 0.35% (expressed as percentage of oleic acid), a PV of 2.5 meq O2/Kg oil and conjugated dienes (CD) of 0.41. These values were lower than the upper limit set by Moroccan Standards which are 0.8% for acidity and 15 meq O2/Kg for PV (Rahmani 2005). These values are important parameters in assessing the quality of the oil. Indeed, the acidity can determine the content of free fatty acids that are considered pro-oxidants and their presence in the oil would initiate oxidation during storage. Similarly the PV and CD permitted to measure the oxidation primary products and thus to assess the early stages of autoxidation.

Table 1: Initial composition of traditional argan oil

Acidity (%)	0.35
Peroxyde value (meq O_2/Kg)	2.5
Conjugated dienes (E 1% 1cm at 232 nm):	0.41
Tocopherols (mg/Kg of oil)	
α-Tocopherol	62
γ-Tocopherol	250
δ-Tocopherol	80
Total Tocopherols	392
Fatty acids (%)	
C16:0	13
C18:0	5
C18:1 (n-9)	47
C18:2 (n-6)	34
C18:3 (n-3)	0,1

Regarding the fatty acid composition, table 1 showed that argan oil contained more than 80% of unsaturated fatty acids essentially represented by oleic acid (47%) and linoleic acid (34%). The latter is an essential fatty acid because it is not synthesized by the body and must be supplied in the diet. As for oleic acid, it helps prevent certain disorders such as cardiovascular disease and cancer (Khallouki et al., 2003). The oil was low in α-linolenic acid (0.1%), essential fatty acid containing three double bounds and thereby constitutes a privileged target for oxidation reactions. Nevertheless, the percentage of saturated fatty acids was around 18%. It was mainly represented by palmitic and stearic acids (13% and 5% respectively).

The content of total tocopherols in the traditional argan oil was 392 mg/Kg of oil (Table 1). The literature showed major variations, ranging from 390 mg/Kg to over 1400 mg/Kg, depending on the extraction method, the region of origin and phenotypic variability of the fruit (Belcadi et al., 2008; Cayuela et al., 2008; Matthäus et al., 2010;. Gharby et al., 2011, Belcadi et al., 2014). Moreover; the most important tocopherol in argan oil was the γ-tocopherol, followed by δ- tocopherol and α- tocopherol. This result was in agreement with literature data (Hilali et al., 2005: Cayuela et al., 2008, Khallouki et al., 2003 ; El Abassi et al., 2014).

The evolution of the chemical composition under light and room temperature according to the container (glass or plastic) showed that the oil remained stable during a period exceeding 200 days (figures 1 to 4). Our study showed that after 200 days of storage, the oil components were degraded by oxidation. It was noted that the degradation was more rapid in samples stored in clear glass bottles than those kept in translucent plastic containers. However, obscurity and low temperature (refrigerator), and storage under nitrogen in plastic bottles preserved the chemical composition of the oil (tocopherols and fatty acids) for a period over 18 months. The oil profile remained identical to that of the initial state (Figures 1-4).

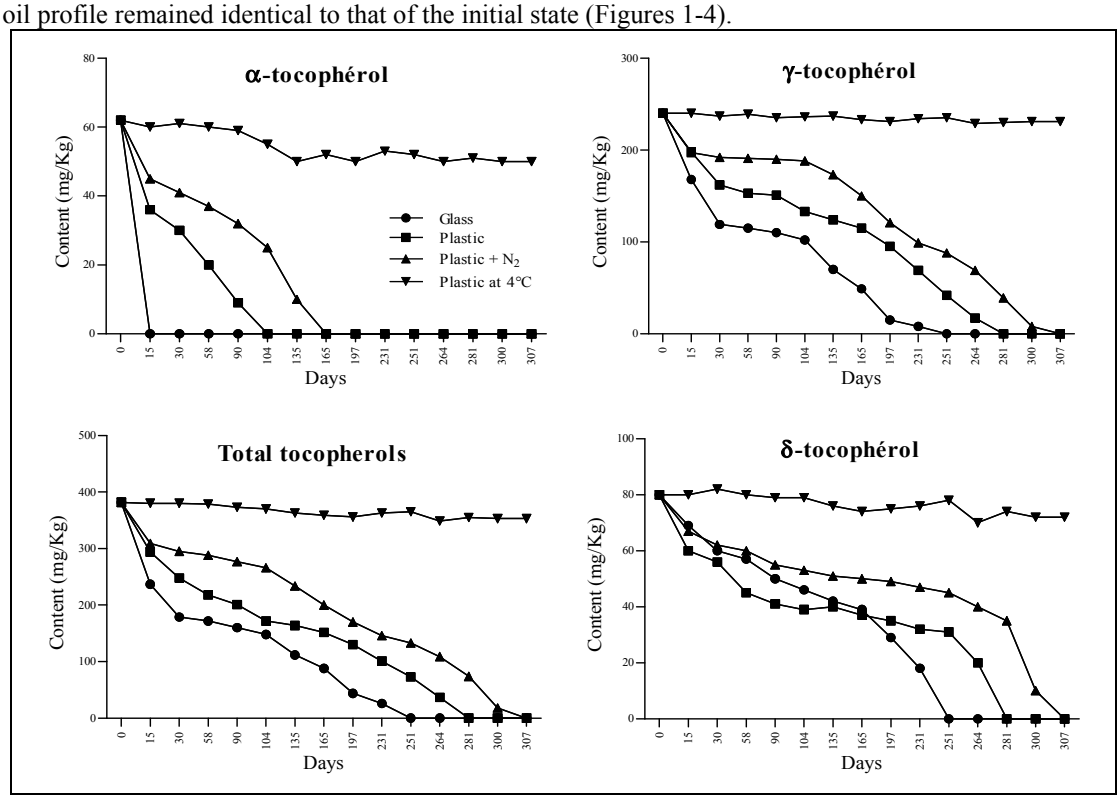

Figure 1: Evolution of tocopherols content under different conditions of storage

In the literature, the shelflife of the traditional argan oil during storage ranged from 200 days (Chimi et al., 1994; Chimi, 2005) to more than two years (Gharby et al., 2011;. Charrouf and Guillaume, 2014). This stability was maintained by the presence of tocopherols which are antioxidants that protect unsaturated fatty acids against oxidation (Chimi, 2005; Cayuela et al., 2008; Gharby et al., 2013.). Tocopherols are naturally present in vegetable oils. They act by blocking the chain reaction of free radicals production process from unsaturated fatty acids. They intervene as hydrogen donors to hydroperoxides radicals (Finley and Given, 1986; Perrin, 1992; Choe and Min, 2006; Elisia et al., 2013.). Our study showed that, during storage, the fatty acids could resist to oxidation during 200 days of storage. After this period, the degradation of important part of tocopherols caused subsequent oxidation of unsaturated fatty acids (Figures 1 and 2). The tocopherols content decreased until their total destruction that was reached at day 251 in glass bottles. In the plastic bottles, this disappearance was reached later, at days 281 and 307 respectively in the presence of air and nitrogen (Figure 1). Furthermore, our study demonstrated that the α-tocopherol deteriorated at first in light and the degradation kinetics was different depending on the nature of the flasks (Figure 1). Indeed, this molecule disappeared completely after only 15 days of storage in glass bottles, while in those of plastic the complete loss occurred after 104 days in the presence of air and 165 days in the presence of nitrogen. The two other tocopherols (γ- and δ-tocopherols), had a more lasting antioxidant effect, which exceeded 200 days, similarly for both bottles (Figure 1). Therefore, this study allowed attributing the antioxidant effect in the traditional argan oil to the latter two forms of tocopherol, more particularly to γ-tocopherol therein presented in the largest content. This result was already obtained for other oils especially olive, soybean and flax oils (Rastrelli et al., 2002; Player et al., 2006; Elisia et al., 2013.). In the literature, the antioxidant effect of tocopherols in vegetable oils depended of the tocopherols form (α, γ and δ), their concentrations and the origin of the oil (Jung and Min, 1990; Choe and Min, 2006; Braunrath et al., 2010). However, it was noted that the storage in translucent plastic kept vitamin E longer in the oil, and more preferably in the presence of nitrogen. This same result was described before for another lipophilic vitamin (vitamin A) showing that the storage of vegetable oils in non-transparent plastic bottles helped protecting against oxidative effect of light relatively to the glass (Piergiovannia and Limbo, 2009).

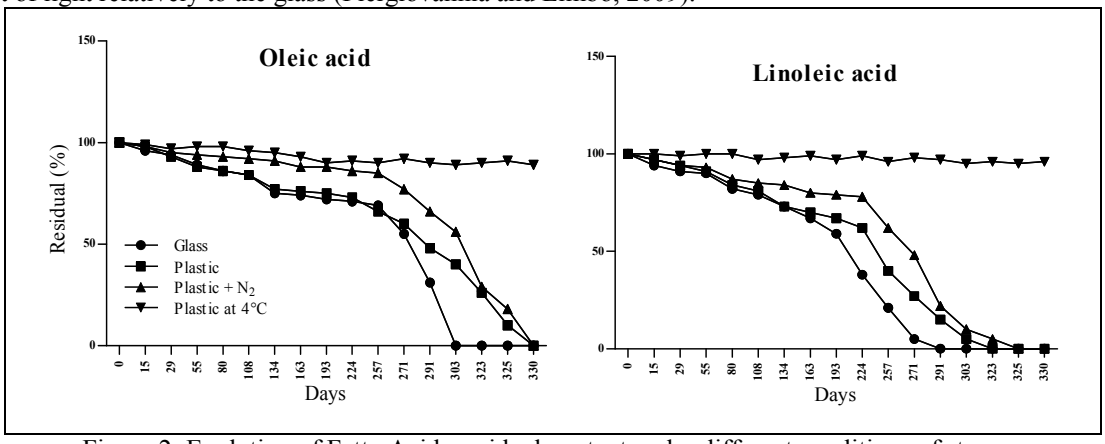

Figure 2: Evolution of Fatty Acids residual content under different conditions of storage

Furthermore, the total destruction of tocopherols instantly caused the acceleration of the degradation of unsaturated fatty acids (linoleic and oleic acids). The results obtained during the storage of the oil showed that the residual content of these acids was less than 5% after 291 days for linoleic acid and 303 days for oleic acid in the glass bottles, against 330 days (linoleic acid) and 325 days (oleic acid) in plastic flasks (Figure 2). Clearly, our experiment showed that linoeic acid oxidized faster than oleic acid. This result was already obtained by Chimi et al. (1994) and was explained by the fact that linoleic acid is more unsaturated than oleic acid and that the double bonds are less stable and therefore more vulnerable to free radicals attack during the oxidation of lipids (Martin-Polvillo et al., 2004; Choe and Min, 2006).

Oxidative degradation of unsaturated fatty acids, which followed that of the tocopherols was sequentially reflected by the production of hydroperoxides. These molecules, evaluated by peroxide value (PV) are the primary products of fatty acid oxidation and turned into conjugated dienes (CD) that are unstable. These are oxidized to form the corresponding secondary products of many molecules as oxidized acids, aldehydes and ketones, that are responsible for the deterioration of nutritional and sensory qualities of the oil (Choe and Min, 2006). In this study, the formation of primary oxidation products (hydroperoxides and CD) remained low during the first 200 days of storage and then sharply increased to reach the maximum value at different times depending on the storage material. Thus in transparent glass bottles, PV reached its maximum after 269 days and CD after 283 days (Figures 3 and 4) while storage in plastic bottles permitted delaying the formation of these products that attained the maximum value at days 303 and 325 respectively fort PV and CD. In the presence of nitrogen, the oxidation was slowed even more and the maximum registered for PV and CD was obtained respectively at

days 313 and 367. Our result also permitted to note that the oxidation of argan oil generated high levels of hydroperoxides and CD that could reach high levels in relation to initial fatty acid composition. Indeed, previous studies showed that the oxidation of rich oils in oleic and linoleic acids and poor in linolenic acid (soybean and sunflower), generated large amounts of hydroperoxides and CD (Kamal-Eldin, 2006 ; Elisia et al., 2013). Also, the evolution of the PV and CD showed that there was some correlation in the formation of these derivatives. This was in agreement with previous works on the oxidation of vegetable oils such as sunflower, olive and argan oils (Marmesat et al., 2009; Gharby et al., 2011, Elisia et al., 2013).

On another hand, the evolution of argan oil showed three phases: a first phase (initiation) in which the concentration of PV and CD remained low and this for more than 200 days, a second phase corresponding to a rapid accumulation of these products, and a third phase where these molecules decomposed quickly to generate secondary products. This result did not agree with the study of Gharby et al. (2011) where the maximum PV and CD recorded are low and do not exceed 20 meq O2/Kg (PV) and 2.8 (CD) even after 2 years of storage. In contrast, our results are in agreement with the study of Chimi et al., (1994) who found that the maximum CD (60) reached after 200 days of storage and the total degradation of fatty acids (oleic and linoleic acids) was obtained before 250 days of storage.

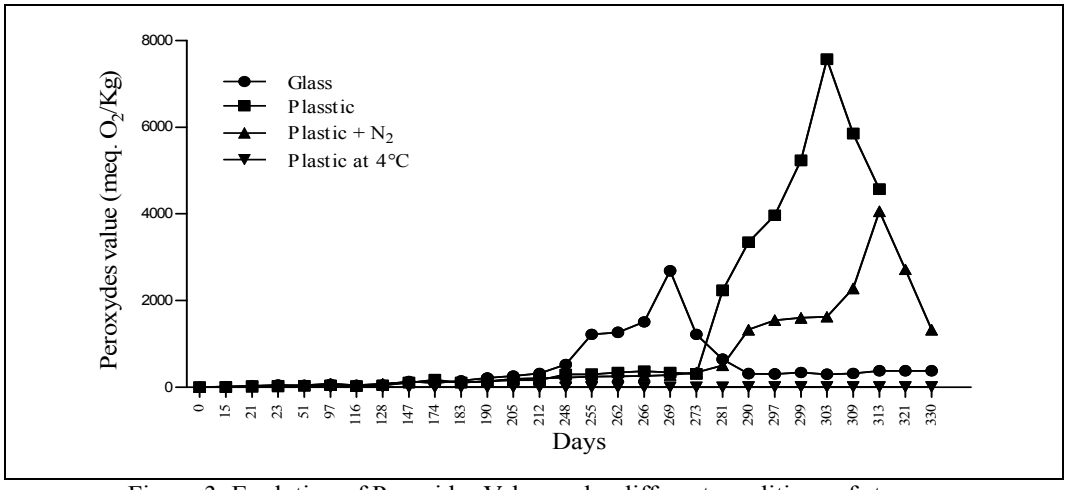

Figure 3: Evolution of Peroxides Value under different conditions of storage

However, we noted that the hydroperoxides rate was higher when the oil was stored in plastic, 7570 and 4060 meq O2/Kg respectively in the presence of air and nitrogen, relatively to the glass (2687 meq O2/Kg only). In fact, several works on the olive oil showed that storage in a plastic material led to faster deterioration than in the glass (Méndez and Falqué, 2007; Pristouri et al., 2010; Afaneh et al., 2013). This result was explained by the fact that the oxygen of the air crossed the plastic during storage, unlike glass, which is not permeable to this gas.

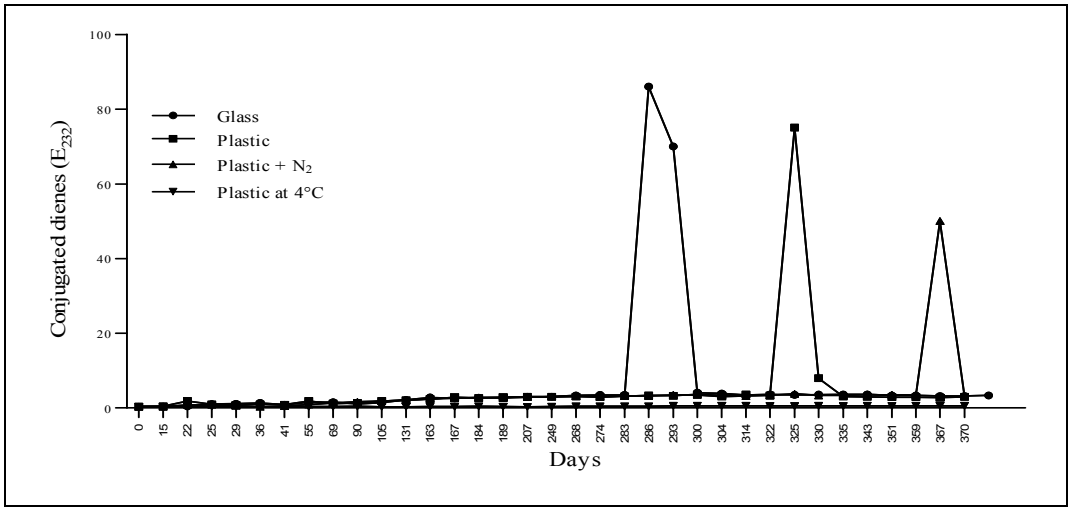

Figure 4: Evolution of Conjugated Dienes under different conditions of storage

Accordingly, this work demonstrated that traditional argan oil had a relatively high oxidative stability to light (200 days) and that this stability was more elongated if the oil was stored in containers made of transparent plastic (HDPE) compared with transparent clear glass. In this regard, numerous studies previously

demonstrated the impact of the storage material (plastic or glass) on the oxidative degradation induced by light on vegetable oils such as olive, rapeseed and soybean oils (Warner and Mounts 1984; Méndez and Falqué, 2007; Pristouri et al., 2009; Dabbou et al., 2011; Afaneh et al., 2012). Our results were consistent with those of the studies that showed that the non-transparent plastic (PET, HDPE or PVC) was more effective in the conservation of the oil in light as it formed a barrier against the passage of light with wavelengths less than 300 nm (Afaneh et al., 2012). Unlike, in the dark it was the storage in the glass that was more efficient because the plastic was permeable to oxygen (Méndez and Falqué, 2007).

Regarding the use of nitrogen as the storage environment, our experiment showed that this condition presented a limited impact against oxidation of the oil. This was probably due to the amount of oxygen dissolved in crude oils, as is the case of traditional argan oil, that contain more oxygen compared to refined oils (Choe and Min, 2006). In addition, the permeability of plastic bottles to the oxygen of the air could compromise the effect of nitrogen for storage (Warner and Mounts, 1984). However, storage in the dark and at low temperature (4 °C) remained the best conditions because it kept the oil quality for more than 18 months, regardless of the nature of the packaging bottles.

Conclusion

Our results led to the conclusion that the period of the argan oil storage in light, at room temperature and in the presence of air, is about 200 days. Under these conditions, generally corresponding to the usual conditions of domestic or commercial conservation, the use of translucent plastic HDPE as material storage is advantageous relatively to the clear glass. Tocopherols which are the main oil antioxidants are better protected from light, especially the α-tocopherol that disappears from the early days of storage (15 days) in the glass compared to plastic (104 days). Thus, in storage conditions, the use of non-transparent plastic during storage is preferable because it prevents the passage of light waves. The presence of nitrogen slightly increases this protection. However, at 4 °C (dark and low temperature), storage in plastic under nitrogen could confer long-term stability to the traditional argan oil.

However, additional studies are needed in which other storage containers should be tested, such as non-transparent glass, metal bottles or plastic bottles containing oxygen scavengers.

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