

## Influence of maturity stage on chemical composition and antioxidant activity of *Pistacia lentiscus* seed oils leaves

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### Abstract

Quality plays a key role in the marketing of vegetable oils. This quality is influenced by a number of factors, including variety, crop management, date of harvest, place of cultivation and extraction procedure.

The objective of our study was to determine the ideal seed harvest period to reach their full potential of quality by analyzing the variation of chemical quality and antioxidant activity of *Pistacia lentiscus* seed oil in three different stages of maturation.

The results obtained in this study indicate that as maturity progressed, there was a decrease in acidity (From 7.13 to 2.61%), chlorophyll content (From 24.43 to 4.69 ppm), and the total phenol content (From 808.22 to 191.24 mg GAE / kg). Meanwhile there was an increase in the oil content (From 11.1 to 33.4%) and the composition of monounsaturated fatty acids increased from 44.36 to 54.43%), mainly oleic acid (From 43.11 to 52.01%). Oil extracted from first stage (Red-green seeds) showed the highest percentage of DPPH inhibition with around 57.56% followed by red seeds with a DPPH inhibition percentage of 49.99%. Earlier harvest would produce lentisk oils of good quality, stable and a composition rich in natural substances. The optimal stage of harvest is proposed by considering the compromise between the quantity and the quality of the studied parameters.

**Keywords:** *Pistacia lentiscus*, seed oil, Antioxidant activity, fatty acids, maturity.

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## Introduction:

The oleaginous forest plants of Morocco are renowned for the heterogeneous quality of the oils of their seeds. Oil seeds are important sources of food, industrial and pharmaceutical oils. It serves as an important source of energy in the absence of glucose (Ramadan and Morsel, 2003). Vegetable oils are used in industry according to their fatty acid composition, and this depends heavily on its natural origin, genetic factors, ripening grade of fruits and climatic conditions (Davis & Poneleit 1974; Velasco et al. 2005). In the food industry, there is a tendency to search for new sources of oil that may have potential nutritional value. *Pistacia lentiscus* seeds oil is an edible oil used by Moroccans local population in food specially for fish frying, egg and preparation of round bread. And in lighting as an oil to burn for light. It is effective treatment of several diseases such as stomach pain, stomach ulcers, injuries, diabetics and in case of circumcision (Hmimsa 2004). In addition, it is used as astringent, expectorant (Seigue 1985) and to treat burns or back pain (Bellakhdar 1997). Few studies are available about the biochemical and biological properties of this oil, which all are studied under the region and stage of maturity effect. Among these studies we note that the phenolic profile was determined by Mezni et al. (2018) of Tunisian seed oil. And the fatty acid composition was determined by (Charef et al. 2008; Dhifi et al. 2013; Akdemir et al. 2015). Also, fatty acid composition and antioxidant activity of *Pistacia lentiscus* seed oil from Algeria was studied (Belyagoubi Benhammou et al. 2018; Mezni et al. 2012). Finally the toxicity was tested on Algerian seed oils by Boukeloua et al. 2012 and Maameri et al. 2016. Our study aims to investigate the possible correlations among the quality index, antioxidant activity of oil and the maturation stage of seeds, in order to determine the real potential for quality at each of the three studied stages of maturity and to propose the best harvest period. This study is original due to the fact that total phenols, antioxidant activity and fatty acid composition of *Pistacia lentiscus* seeds growing in Morocco, have not been studied before.

## Materials and methods:

### 1. Plant material

The plant material of *P. lentiscus* L. was collected from the Azilal region located in the heights of the Atlas of Morocco (N 31°54'; E 6°35'). The plant of *Pistacia lentiscus* L was identified in Department of Biology, Faculty of Science, Semlalia, Cadi Ayyad University of Marrakech (Morocco) and a voucher specimen (MARK 10938) was deposited at the regional herbarium MARK of this faculty. The Samples were harvested in three maturation stages: Green-red (October 2018), Red (December 2018) and Black (January 2019), dried at room temperature, and kept separately.

### 2. Oil extraction

Oil was extracted by a Soxhlet apparatus for 6 hours using hexane as a solvent. The final extract was concentrated and solvent was removed by evaporation at 30°C. The oil was then stored at 4°C in darkness for chemical analysis. The oil content is calculated according to the NFV 03-907 method.

### 3. Oil analyses

The determination of the acidity and the specific extinction at 270 nm and 232 nm were achieved by the methods T60-204 and T60-223 respectively.

The viscosity of the oil is achieved by a viscometer according to the method described by IUPAC (KARLESKIND, 1992). The chlorophyll content of the oil is determined according to the method described by Wolff (1968).

### 4. Fatty acid composition

The total fatty acid composition of the lentisk oils was determined according to the analytical methods described by the International Olive Council standard (IOC, 2017). The methyl esters were then analyzed by gas chromatography (GC) using a Varian CP 3380 chromatograph with a flame ionization detector equipped with a capillary column packed with a stationary phase: CPWAX 52 CB , (Length: 25 m, inner diameter: 0.25 mm, outer diameter: 0.39 mm). Using injector split/splitless equipped with auto-sampler Varian CP-8400. The temperature of the oven: 180°C, the temperature of the injector: 200°C and the temperature of the detector: 210°C. The carrier gas is nitrogen.

### 5. Total phenols content

The Total phenol content was determined by the Folin–Ciocalteu method as fixed by Vazquez Roncero et al. (1973). Phenolic extracts were prepared by a triple extraction from a solution of 10 g of lentisk oil in 20 ml hexane with 30 ml of a methanol-water mixture (80/20, v/v). Extract (100 µl) was mixed with 3.9 ml of distilled water and 100 µl of Folin–Ciocalteu reagent and allowed to stand at ambient temperature for 3 min. A 1 ml of sodium carbonate solution (20%) was added to the mixture. The tubes were left for 60 min in the dark at room temperature. The absorbance was measured at 725 nm. Values are expressed in mg equivalent of gallic acid per kilogram of oil (mg GAE.kg<sup>-1</sup>).

### 6. DPPH radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured using the method described by Brand-Williams et al. (1995). A 50 µl of oil in ethanol (950 µl by volume) was added to 1 ml of ethanolic DPPH solution (60 µM). The decrease in absorbance at 517 nm was measured after one hour of incubation in darkness at room temperature. The radical scavenging activity was expressed as the inhibition percentage (IR) and monitored using the following equation:

$$IR = [(AC-AS) / AC] \times 100$$

AC: absorbance of control (ethanol) and AS: absorbance of sample solution.

### 7. Statistical analysis

Data analysis presented in tables and figures as (means and ± standard deviations) of three replicates, was performed using IBM SPSS statistic 20. The results were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlations between phenol content and antioxidant activity, with DPPH assay of seed oil at different stages of maturation were also investigated by Pearson's product moment correlation at P ≤ 0.05.

## Results and discussion:

### I. Biometrics indices

In Table 1 we reported the results of the biometric indices for each maturation stage. We have included the following details:

**Table 1: Results of the biometric indices of lentisk seeds**

	L (mm)	I (mm)	L.I <sup>-1</sup>	Form	100 seed weight (g)
<b>GL RV</b>	5,29±0,27	4,68±0,3	1,13	S	6,52±0,01
<b>GL R</b>	5,40±0,52	5,15±0,96	1,05	S	6,60±0,21
<b>GL N</b>	5,08±0,47	4,63±0,38	1,10	S	6,87±0,52

**S:** Spherical **L:** length **I:** width **GL RV:** red-green seeds lentisk **GL R:** red seeds seeds lentisk **GL N:** Black seeds lentisk

The dimensions of the seeds vary between 5.08 mm and 5.40 mm in length, likewise the width varies between 4.63 mm and 5.15 mm, which make the seeds wider and longer red. In addition the weight of 100 seeds varies between 6.52 g and 6.87 g and the black seeds are the heaviest. Seeds in all stages of maturity have the same spherical shape. The shrinkage of the seeds following the loss of water explains the drop in size and the increase in weight.

### II. Oil analysis

The oil content results of *Pistacia lentiscus* seed during maturation are showed in table 2. The oil content reached 11.07% for red-green seeds, 14.78% for red seeds, and 33.40% for Black seeds. This corresponds to previous results were reported by Trabelsi et al. (2012) who found that 11.95% in Green–red, 19.45% in Red, and 42.51% in Black and Charef et al. (2008) who found (11.7% in Red and 32.8% in Black. And lower values were denoted by Mezni et al. (2014) who found 4.37% in Green–red, 9.66% in Red, and 14.84% in Black.

**Table 2. Results of the physicochemical analysis of *Pistacia lentiscus* seeds oil**

	Oil content %	Acidity (%)	Viscosity (%)	Specific extinction			chlorophyll (ppm)
				E232	E270	$\Delta E (10^{-2})$	
<b>HL RV</b>	11,07±0,2	7,13±0,0	1,37	1,74 ±	1,30 ±	-4,36 ±	24,43 ±0,18
	0	2	±0,12	0,05	0,02	0,83	
<b>HL R</b>	14,78±0,4	7,59±0,0	2,57	1,75 ±	1,44 ±	-0,15 ±	19,60 ±0,14
	2	3	±0,12	0,08	0,02	0,75	
<b>HL N</b>	33,40±5,2	2,61±0,0	1,63	5,03 ±	0,45 ±	-8,51 ±	4,69 ±0,03
	4	1	±0,12	0,03	0,02	0,19	

**HL RV:** Oil extracted from green/red seeds    **HL R:** Oil extracted from red seeds    **HL N:** Oil extracted from black seeds

The gradual increase in oil content can be explained by the accumulation of lipids in the seeds along with the increased maturation period. Therefore, black seeds can be considered as equivalent to other oilseed as the seeds of groundnuts, sunflower and cotton (30–45%) (Karlenskind 1992).

The acidity values for the studied lentisk oil samples range from 2.61 for black seeds to 7.59% for red seeds. These results are in corresponds to that reported for Algerian mature black seeds reaching 2.27% (Boukeloua et al. 2012), whereas the value of the red seeds, not ripened, is more consistent with that found by Charef et al. ( 2008) reaching 7.7% for the same oil from another region of Algeria. The Mature green-red or red seeds contain higher levels of free fatty acids as compared to mature black seeds. This high level can be explained by the hydrolysis of triglycerides under the action of lipase contained in the fruit, resulting in the release of free fatty acids (Abaza et al. 2002), due to the bad conservation of the seeds before extraction and analysis or to the incomplete ripeness of the seeds (Charef et al. 2008).

Viscosity is not affected by the stage maturity, it is noted that at the three studied stages of oil has a low viscosity value, which is much lower than that found for Algeria lentisk reaching 126.05 mm<sup>2</sup>/s at 20°C (Alloune et al. 2012).

We can conclude for this parameter that the black stage is the most suitable.

The specific extinction coefficient at 232 nm ( $K_{232}$ ) is linked to the degree of primary oxidation of the oil and therefore directly correlated to the amount of hydroperoxide (Maskan and Bagci 2003; Ku and Mun 2008).  $K_{232}$  is also an indicator for the conjugation of polyunsaturated fatty acids, while  $K_{270}$  is linked to secondary oxidation products (unsaturated ketone, diketone) (Karleskind 1992). The specific extinction values at 270 nm are practically similar, reaching 1.3 and 1.4 for the green/red and red seeds respectively, whereas a slight decrease was recorded for the black seeds 0.45. The low value of  $K_{270}$  (0.45) indicates that black seed oil contains a smaller amount of secondary oxidation products than green-red (1.30) and red (1.44). However,  $K_{232}$  values show an increase with maturation time. The relatively high value of  $K_{232}$  of black seeds (5.05) confirms that this oil is much more oxidized than the oil from green-red (1.74) and red (1.75). We can conclude from this parameter that seeds from the green-red and red stages can be considered as the most suitable.

The turnover of chlorophyll content during the maturation of seeds (Table 2) continuously decreased. This agrees perfectly with other reported data for olives (Minguez- Mosquera et al. 1990). The chlorophyll content in the oil depends on the maturation stage of the seeds. The chlorophyll contents in the oils tested were 24.43 ppm for green-red seed and 19.60 ppm for red seeds. These values were in the range of crude canola oil 4–30 mg/kg (Ghazani & Marangoni 2013). While the content of black seed is 4.69 ppm which has a good quality (< 10 ppm) according to Perrin (1992). These low levels are desired in order to avoid the pro-oxidant action of the chlorophyll pigments and to ensure good preservation of the oils (Kiritsakis et al. 1987).

### III. Fatty acid composition

The results of fatty acid composition of seeds oils extracted at different maturation stages (expressed as % of total fatty acids, g/100 g) are reported in Table 3.

**Table 3. The fatty acid profile of Pistacia lentiscus seeds oil**

	HL RV	H LR	H LN
<b>C15:0</b>	0,02	0,03	0,01
<b>C16:0</b>	23,71	21,00	23,64
<b>C16:1</b>	1,03	1,26	2,40
<b>C17:0</b>	0,06	0,07	0,02
<b>C17:1</b>	0,09	0,08	0,02
<b>C18:0</b>	1,79	2,08	0,54
<b>C18:1w9</b>	43,11	44,44	52,01
<b>C18:2</b>	28,72	29,41	21,88
<b>C18:3</b>	1,17	1,37	0,46
<b>C20:0</b>	0,15	0,12	ND
<b>C20:1</b>	0,13	0,14	ND
<b>AGMI</b>	44,36	45,92	54,43
<b>AGPI</b>	29,89	30,78	22,34
<b>AGS</b>	25,75	23,30	24,21
<b>AGMI/AGPI</b>	1,48	1,49	2,44
<b>AGS/AGI</b>	0,35	0,30	0,32
<b>C18:1/C18:2</b>	1,50	1.51	2.38

**HL RV:** Oil extracted from green/red seeds **HL R:** Oil extracted from red seeds **HL N:** Oil extracted from black seeds **AGS:** Saturated fatty acids **AGMI:** Monounsaturated fatty acids **AGPI:** Polyunsaturated fatty acids **AGI:** Unsaturated fatty acids.

The seed oil obtained from three stages of maturity showed the same three main fatty acids with difference in percentage of the total fatty acids. The majority fatty acids in all studied stages are oleic (C18:1), linoleic (C18:2) and palmitic (C16:0) acids whereas minority fatty acids are stearic (18:0) and linolenic (18:3). These results are concordant with previous study of Ait Mohand et al. 2020; Tej- Yakoubi and Dhaous 2007.

From our results, we noted that the first stage of maturity gives a palmitic acid content of 23.71%, the most important saturated acid, which decreased in the second harvest stage to reach 21.00%, while it increased in the third stage of maturity to reach 23.64%. On the other



hand, Oleic acid is the majority fatty acid in lentisk oil, increased with maturation from ripe and over-ripe samples (from 43.11 to 52.01%), in the same way linoleic acid increased from 28.72 to 29.41% between the two stages green-red and red. This increase can be explained by the activity of the enzyme oleate desaturase transforming oleic acid into linoleic (Baccouri et al. 2008). Then decreased to 21.88% in the black stage.

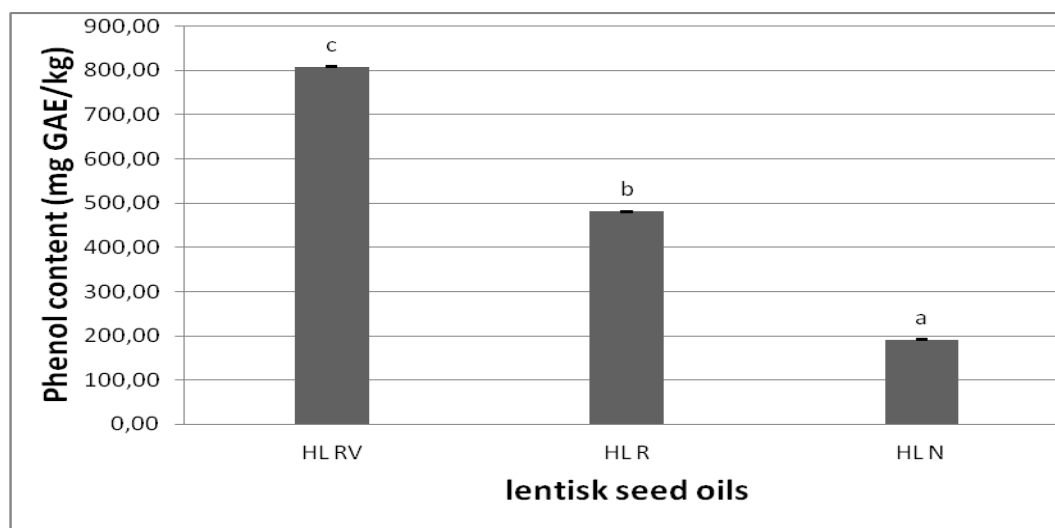
The content of unsaturated fatty acid (AGI) in the studied samples increased during maturation from 74.25% at green-red stage to 76.77% at the black seed oil. This was accompanied by a decrease in the saturated fatty acids from 25.75% in green-red stage to 23.30% in red stage then increased to 24.21% in the black stage.

The oleic acid / linoleic acid ratio varies according to the degree of maturity. It is used as a stability parameter and several studies have shown that no report has been indicated for high value has a significant oxidative stability (Matos et al. 2007). In the present study, there is an increase in oleic / linoleic ratio during maturation.

The studied lentisk seeds show that at the early stages there is a great advantage concerning the essential fatty acids and generally polyunsaturated fatty acids while at the last stage there is more advantage of oleic oil.

#### IV. Total Phenols Content

The results of total phenols content of oils extracted from seeds at different stages of maturation are presented in Figure 1.



**HL RV:** Oil extracted from green/red seeds **HL R:** Oil extracted from red seeds **HL N:** Oil extracted from black seeds

**Figure 1:** Phenol content in *Pistacia lentiscus* seed oils at different stages of maturity.

Values are expressed as mean  $\pm$  standard deviation (SD) of three replications. Different letters among columns designate significant differences ( $p < 0.05$ ) according to the Tukey test.

Phenols give plant products some of their main organoleptic properties; they have an important role in taste, especially the sensations of astringency and bitterness (Shahidi 2000). Also they play a role in cancer treatment and cardiovascular disease (Hallman 2001; Visioli & Galli 1998).

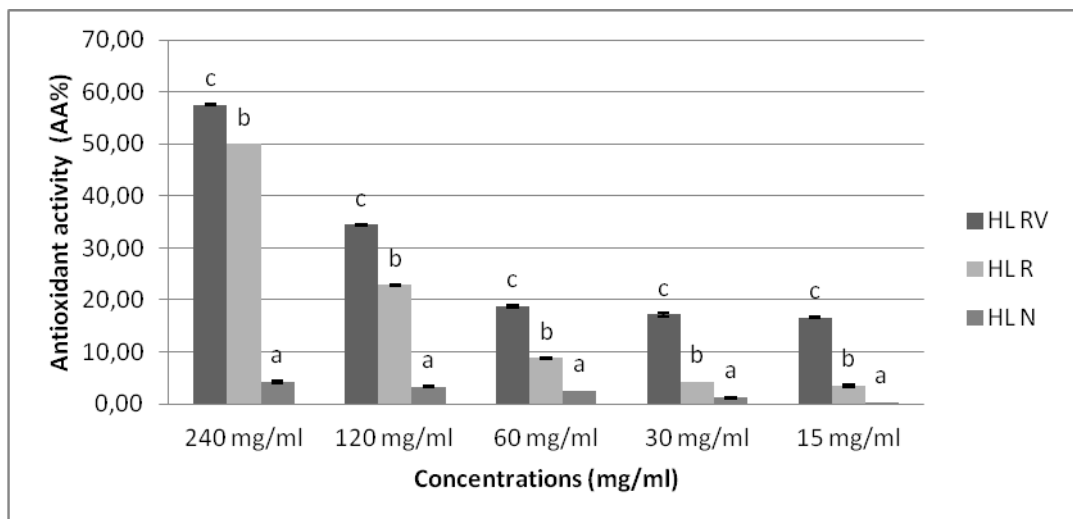
The results show a decrease in total phenol content during maturation, Total phenol content in oils obtained from Red-green seeds was  $808.22 \pm 0.61$  mg GAE.kg<sup>-1</sup> oil. This result is concordant with Belyagoubi Benhammou *et al.* (2018) who reported a value of 810 mg GAE.kg<sup>-1</sup> for lentisk mature fruit oils. Subsequently, a value of  $481.4 \pm 1.32$  mg GAE.kg<sup>-1</sup> was recorded from the oil extracted from red seed and this value is close to that found by Mezni *et al.* (2018) for lentisk seed oil in Jbel Masour (538.03 mg/kg oil). In the last stage, black seed oil contains few total phenols  $191.24 \pm 0.36$  mg GAE/kg. These results show higher phenols content than other edible vegetable oils, i.e. virgin argan oil (from 6.07 to 152.04 mg GAE/kg oil) (Marfil *et al.* 2011), sunflower oil (from 3 to 4 mg.kg<sup>-1</sup>) and soybean oil (from 60 to 80 mg.kg<sup>-1</sup>) (Valavanidis *et al.* 2004).

It is well known that the phenol content gives vegetable oils a quality of oxidation resistance (Morello *et al.* 2004). The studied oils of different maturing stages contain a high total phenol content compared to other edible oils which make them more resistant to oxidation. The strong odor and bitter taste of lentisk oils and seeds (green-red and red) can be explained by the high levels of phenolic compounds (Shahidi 2000).

The impact of the stage of maturity on the phenol content has been clearly recorded. The oils studied had high levels of phenol content in the early stages of maturity, and then the phenol content decreased as the seeds ripened. Therefore a harvest in the early stages of maturity is recommended to obtain lentisk oil with high phenol content. However, the red seeds are also interesting; they offer consistent values of fairly high polyphenols, capable of offering better stability and nutritional value to the oils extracted at this stage.

### V. Antioxidant activity

Antioxidant activity determined with DPPH assay of the studied oils were illustrated in Figure 2.



**Figure 2.** Antioxidant activity of *Pistacia lentiscus* seed oils determined with DPPH assay. Values are expressed as mean  $\pm$  standard deviation (SD) of three replications. Different letters in the same column designate significant differences ( $p < 0.05$ ) according to the Tukey test.

The antioxidant potential determined by DPPH radical scavenging activity was significantly different between oils at different stages of ripening and at different concentrations. The results described in Figure 2 show that the oil extracted from green-red seeds, at concentrations between 25 and 240 mg/ml, has a percentage of inhibition ranging from  $16.57 \pm 0.14$  to  $57.56 \pm 0.21\%$  respectively. Followed by the oil obtained from red seeds which varied respectively for the same concentrations between  $3.49 \pm 0.32$  and  $49.99 \pm 0.04\%$ . On the other hand, the antioxidant activity was very low in the black seed oil which varied between  $4.30 \pm 0.25\%$  (for 240 mg / ml) and  $0.30 \pm 0.06\%$  (for 25 mg / ml).

According to the bibliographic data, lentisk seed oils studied present higher antioxidant activity than seed oils reported by Mezni et al. (2014), and lower than those reported by Belyagoubi Benhammou et al. (2018).

**Table 4. Pearson's correlation between Phenol content and Antioxidant activity with DPPH assay of seed oil**

	AA_120mg/ ml	AA_15mg/ ml	AA_240mg/ ml	AA_30mg/ ml	AA_60mg/ ml	Phenol content
AA_120mg/ml	1	,809**	,999**	,959**	1,000**	,935**
AA_15mg/ml		1	,784*	,889**	,810**	,958**
AA_240mg/ml			1	,950**	,999**	,920**
AA_30mg/ml				1	,959**	,947**
AA_60mg/ml					1	,936**
Phenol content						1

\*\*The correlation is significant at the 0.01 level (bilateral);

\*The correlation is significant at the 0.05 level (bilateral).

As mentioned in Table 4, a correlation is recorded between the values of the total phenol content and the antioxidant activity of the oils studied during maturation, showing that activity is the result of the presence of natural antioxidants as phenolic compounds having a hydroxyl group in their structure responsible for scavenge free radicals, rising antioxidant capacity. These results are consistent with several studies demonstrating a significant correlation between antioxidant activity and the phenol content (Stellman and Dufresne 2000; Alali et al. 2007; Zhang and Wang 2009; Grati-Kammoun et al. 1999). The difference in antioxidant activity between maturation stages may be related to variation in the nature of phenols, in fact during seed maturation some phenols increase while others decrease as reported by Grati-Kammoun et al. (1999).

## Conclusion

The evolution of the chemical composition and antioxidant activity of Moroccan lentisk seed oil can be divided into three phases. The first, which corresponds to the unripe stage where the seeds have a green-red color give less oil, which is rich in total phenols, chlorophyll, and it has significant high antioxidant activity. The second stage is marked by increase in the oil content and fatty acid composition as well as decrease in the content of chlorophyll, total phenols and antioxidant activity of the oil. At the third stage of maturity, corresponding to black and mature seeds, the seeds contain high proportions of oil, and a decrease in the

content of phenols, chlorophyll and antioxidant activity.

The variations can be noticed in chemical composition and antioxidant activity from year to year depending on climatic conditions, method of extraction, region, and variety.

From the obtained results, it can be deduced that the best seed harvest period is the unripe stage which gives a good quality of oil with high antioxidant activity enabling their application in pharmaceutical and nutritional fields.

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