Effects of Growing Region and Maturity Stages on Oil Yield and Fatty Acid Profile of Pistacia Atlantica Desf. Fruit and Their Implications on Resulting Biodiesel

Ziyad Ben Ahmed, Toufik Hadj Mahammed, Mohamed Yousfi and Yvan Vander Heyden
Effects of growing region and maturity stages on oil yield and fatty acid profile of *Pistacia atlantica* Desf. fruit and their implications on resulting biodiesel

Ziyad Ben Ahmed  
Laboratory of Fundamental Science  
University Amar Telidji  
Laghout, Algeria  
benahmed_ziyad@yahoo.fr

Toufik Hadi Mahammed  
Laboratory of Fundamental Science  
University Amar Telidji  
Laghout, Algeria  
t.hadjmahammed@lagh-univ.dz

Mohamed Youssi  
Laboratory of Fundamental Science  
University Amar Telidji  
Laghout, Algeria  
yousfi.m8@gmail.com

Yvan Vander Heyden  
Department of Analytical Chemistry, Applied Chemometrics and Molecular Modelling  
Vrije Universiteit Brussel (VUB)  
Brussels, Belgium  
yvan.vander.heyden@vub.be

Abstract—In Algeria, the fruits of *Pistacia atlantica* Desf. (family Anacardiaceae) are commonly called "El khodiri" and their oil is used by natives in traditional medicine and as food additive. Hence, the present study was undertaken to determine the influence of maturity stages on oil content, fatty acid composition, tocopherol profile and on oil aptitude for biodiesel production. Fatty acids of the fruits samples were extracted with hexane, esterified, and then analyzed with gas chromatography (GC). Oil contents of the samples varied between 11.4-26.3%. A total of 8 fatty acids was identified. The C18:1 (oleic acid) was the major component, followed by C18:2 (linoleic acid), and C16:0 (palmitic acid). The oil amount, and the stearic, oleic, palmitoleic and linoleic acid contents increased with maturity, whereas palmitic, linoleic and linolenic acid contents decreased during the ripening of the fruits. The accumulation patterns of each fatty acid in oils of *P. atlantica* fruits were often more influenced by harvest time than by growing region. The biodiesel properties of the *P. atlantica* oil methyl esters, such as saponification value, iodine value, cetane number, long chain saturation factor, cold filter plugging point, cloud point, degree of unsaturation, oxidative stability, kinematic viscosity, density and higher heating value were determined and compared to the ASTM D6751-2010, EN 14214-2008 and GB/T 20828-2007 organization standards. Overall, the immature and mature fruit oils show potential as a new source of biodiesel within Algeria. It was concluded, based on the present findings, that the *P. atlantica* fruits at the immature stage have a healthy nutritional value (high oleic acid and tocopherol isomer contents), while the mature stage (high oil contents) is economically more important for biodiesel production.

Keywords—*Pistacia atlantica* Desf, fruit ripening stages, oil content, fatty acid profile, tocopherol isomers, biodiesel properties.

I. INTRODUCTION

The major part of all energy created worldwide comes from fossil sources (petroleum, coal and natural gas) [1]. However, these sources are limited, and will be exhausted in the near future [2]. Consequently, identification of diverse, renewable, and clean energy sources is essential for continuing advancement of human society, and this has resulted in an emerging global industry of biomass energy [3]. The use of plant biomass as a renewable energy source has recently received increased attention in response to climate change and the increasing global demands for energy [4]. Biodiesel has been gaining great importance worldwide as high quality, non-toxic, biodegradable fuel that is free from sulphur, aromatic hydrocarbons, metals and crude oil residues, and which can be used in compression ignition engines without any major modification [5]. Hence, it is a renewable energy resource as opposed to conventional diesel.

According to the American Society for Testing and Materials (ASTM), monoalkyl esters of long-chain fatty acids, produced through a transesterification process and resulting from edible, non-edible, and waste oils, are called biodiesel and assigned as B100 [6]. In a transesterification or alcoholsysis reaction, one mole of triglyceride reacts with three moles of alcohol to form one mole of glycerol and three moles of the respective fatty acid alkyl esters [7]. Biodiesel properties depend on its constituent methyl esters; in the other words, its properties depend on the composition of the fatty acids used [6]. The fatty acid composition of oil from vegetable sources varies depending on the plant origin, genetic factors and varieties [8]. It is thus of great interest to understand the biochemical pathways leading to the formation of fatty acids.

Nevertheless, the use of vegetable oils as alternative sources of diesel requires more efforts to either increase the production of oilseeds and or to develop more productive plant species with high oil yield. Only plants which are abundant or can be collected in large amounts and contain an appreciable quantity of oil are interesting to use for biodiesel preparation. Each country, in various regions, utilizes different types of plant oil feedstock, depending on the abundance or availability, for example, soybean oil in USA, rapeseed oil in many European countries, and coconut and palm oil in tropical countries [9].

Biodiesel is broadly classified in four generations, i.e. edible oils (first generation), non-edible oils (second generation), waste oils (third generation) and advanced solar biodiesel (fourth generation). On the other hand, more than 95% of the global biodiesel production originates from edible vegetable oils [10].

The genus *Pistacia* (Anacardiaceae), comprising more than 11 species, is widely distributed from south-west Asia to north-west Africa [11]. *Pistacia atlantica* Desf. is a tree,
which can reach over 15m in height and grows in arid and semi-arid areas [12]. Its fruits, which are called “El Khodirî” in Algeria, are round to oval, somewhat flat, and 0.5–0.7 cm in diameter. To the best of our knowledge, there are only few detailed studies on the chemical characteristics of *P. atlantica* fruit oil, from the southern region of Algeria, obtained at the final stage of fruit maturation [13, 14], and there is only one study on its composition from immature fruit [15]. Information on the biodiesel properties of *P. atlantica* fruits at different development stages is unavailable.

The purpose of this study was to investigate the effect of variation in fatty acid composition and oil accumulation in developing *P. atlantica* fruits on the resulting biodiesel properties. The results will provide an indication of the potential economic utility of *P. atlantica* as a raw material source for useful industrial oil components.

II. MATERIALS AND METHOD

A. Plant materials

The fruits were collected monthly from June until September 2010, at the middle of each month. This concerns two ripening stages: immature (June-July) and mature (August-September) (Fig. 1). Trees were sampled from two growing regions chosen along a transect of increasing aridity: Ain oussera (medium arid) and Laghouat (arid) located at 200 and 400 km, respectively, south of Algiers, Algeria.

B. Fatty acid methylester preparation and gas chromatographic (GC) analysis.

The fatty acids composition of the oil was determined by gas chromatography of the fatty acid methylesters (FAME) following the procedure described by Guenane et al [16].

C. Biodiesel fuel properties prediction.

Saponification number (SN), iodine value (IV), cetane number (CN), density (ρ), kinematic viscosity (KV), oxidative stability (OS), degree of unsaturation (DU), cloud point (CP), cold filter plugging point (CFPP), long chain saturated factor (LCSF) and higher heating value (HHV) of the FAMEs were calculated according to the following equations [17]:

\[ SN = \sum \frac{S_{n} x A_{i}}{MW_{i}} \]  
\[ IV = \sum \frac{254 \times DB 	imes A_{i}}{MW_{i}} \]  
\[ CN = 46.3 + \frac{5458}{SN} - 0.225 \times IV \]  
\[ f_{b} = \sum_{i=1}^{n} A_{i} \times f_{i} \]  
\[ DU = (\text{polyunsaturated } C_{n}: 1, \%) + 2 \times (\text{polyunsaturated } C_{n}: 2, 3, \%) \]  
\[ OS = -0.0384 \times DU + 7.7704 \]  
\[ LCSF = \frac{\Sigma (MP_{i} \times C_{n,i})}{100} \]  
\[ CFPP = 1.7556 \times LCSF - 14.772 \]  
\[ CP = \frac{CFPP + 2.5}{1.0191} \]  
\[ HHVs = 49.43 - [0.041 \times SN + 0.015 \times IV] \]

Where \( A_{i} \) is the percentage of the \( i \)th FAME, DB the number of double bonds, MW\(_{i}\) the molecular mass of each component, fb a function that represents any physical property (ρ or KV), fi a function of the individual \( i \)th FAME properties (ρ or KV), Cn is the percentage of the nth saturated fatty acid and MP\(_{n}\) the melting point of nth saturated fatty acid. Total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) FAs were quantified by summation of the percent quantity of corresponding fatty acids. All biodiesel property parameter analyses were replicated three times for each sample, and the value of the biodiesel properties parameters were reported as the mean ± standard deviation.

D. Physico-chemical properties measure

The physicochemical properties of the biodiesel produced were determined experimentally by official methods established by the American Society for Testing and Materials (ASTM). The density was determined using ASTM D 97 specification. The d CP (cloud point) were tested based on ASTM D 2500 specification. The iodine value (IV) was determined using the Wijs method. The Saponification value (SV) was determined by saponifying the oil with a potassium hydroxide solution. The main details of each characterization are explained in the following sections.

The following sections were added to the Materials and method section.

III. RESULTS

A. Fatty acid profile

Eight fatty acids were identified and expressed as percentage of the total fatty acids (TFAs) of the fruit oil. Not all maturity stages showed all fatty acids (Table 1). Palmitic (C16:0), linoleic (C18:2), and oleic acid (C18:1) were measured as major fatty acids. Linolenic (C18:3), stearic (C18:0), and palmitoleic (C16:1) acid were less present in the fruit oil, while, myristic (C14:0) and arachidic (C20:0) acid were only present in trace amounts. Saturated fatty acids rather than unsaturated, seem to be metabolically active during fruit maturation.

The influences of the main factors, harvest month and region, were examined in the same way as on the oil content. In the Ain oussera region, one-way ANOVA showed a significant difference in the palmitic \( (p < 0.0005) \), palmitoleic \( (p = 0.001) \), stearic \( (p < 0.0005) \), oleic \( (p = 0.044) \) and linoleic \( (p < 0.009) \) acid contents between the different harvest months.

This difference was not statistically significant in the Laghouat region for the palmitic \( (p = 0.156) \), palmitoleic \( (p = 0.074) \), oleic \( (p = 0.471) \) and linolenic \( (p = 0.106) \) acid contents, while significant differences were found for stearic \( (p < 0.0005) \), and linoleic \( (p = 0.006) \) acids over the considered period.


**B. Biodiesel properties**

Fatty The fuel properties of the *P. atlantica* fruit methylesters were determined and compared with the relevant specifications from the American, European and Chinese biodiesel standards: ASTM D6751-10, EN 14214-08 and GB/T 20828-07 (Tables 2 and 3). The ideal vegetable oil for biodiesel production should contain a high percentage of monounsaturated fatty acids, a low fraction of polyunsaturated fatty acids, and a controlled amount of saturated fatty acids [16]. The CN values in the *P. atlantica* fruit methylesters, with higher oleic acid and lower linoleic acid contents are above 51, which satisfy all specifications. All examined samples have a degree of unsaturation below 137, which make them to meet the specifications for the cetane number (Table 2). Low cetane numbers have been correlated with more polyunsaturated fatty acids, such as linoleic (C18:2) and linolenic (C18:3) acid [16].

The standards for biodiesel state that the density should be 860–900 kg m⁻³ (EN 14214-08) or 820–900 kg m⁻³ (GB/T 20828-07) [20]. ASTM D6751-2010 does not include a specification for density [18]. The results obtained for Ain oussera show that the densities were within the limits of both standards. Those of the Laghouat region was around the minimum value prescribed in EN 14214-08 (860 kg m⁻³) but did not comply with the minimum values prescribed in the GB/T 20828-07 standards. Those of the Laghouat region was around the minimum value prescribed in EN 14214-08 (860 kg m⁻³) but did not comply with the minimum values prescribed in the GB/T 20828-07 standards. The oxidative stability of the methylesters produced in this work was within the range specified in ASTM D6751-10 but did not comply with the minimum values prescribed in the EN 14214-08 (6 h) and GB/T 20828-07 (6 h) standards. It is well known [16] that it is very difficult to meet this limit for biodiesel fuels derived from *P. atlantica* oils because the fatty numbers have been correlated with more polyunsaturated fatty acids, such as linoleic (C18:2) and linolenic (C18:3) acid [16].

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Immature</th>
<th>Mature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>July</td>
</tr>
<tr>
<td>Laghouat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td>20.79±4.16a</td>
<td>16.90±3.11a</td>
</tr>
<tr>
<td>C16:0</td>
<td>0.24±0.08a</td>
<td>0.47±0.17a</td>
</tr>
<tr>
<td>C18:0</td>
<td>0.58±0.13a</td>
<td>1.10±0.15a</td>
</tr>
<tr>
<td>C18:1</td>
<td>54.15±1.26a</td>
<td>56.86±0.44a</td>
</tr>
<tr>
<td>C18:2</td>
<td>21.59±1.51a</td>
<td>22.35±0.85ab</td>
</tr>
<tr>
<td>C18:3</td>
<td>0.63±0.08a</td>
<td>0.85±0.06a</td>
</tr>
<tr>
<td>C20:0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ΣSFA</td>
<td>23.37</td>
<td>18.00</td>
</tr>
<tr>
<td>ΣMUFA</td>
<td>54.39</td>
<td>57.33</td>
</tr>
<tr>
<td>ΣPUFA</td>
<td>22.22</td>
<td>23.20</td>
</tr>
<tr>
<td>ΣPUFA/ΣSFA</td>
<td>1.04</td>
<td>1.28</td>
</tr>
<tr>
<td>ΣTFA</td>
<td>97.98</td>
<td>98.53</td>
</tr>
</tbody>
</table>

**TABLE 1:** Monthly variation in the fatty acid composition (%) of *P. atlantica* fruits oils from the Laghouat and Ain oussera regions. ND: Not detected.

Average in one row followed by the same superscript do not differ significantly according to SNK post hoc test. Superscripts a → b → c: indicate increasing concentrations.
TABLE 2: Estimated biodiesel properties of *P. atlantica* oil methyl esters from the Laghouat and Ain oussera regions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Immature</th>
<th>Mature</th>
<th>Laghouat</th>
<th>Ain oussera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>June</td>
<td>July</td>
<td>Average</td>
<td>August</td>
</tr>
<tr>
<td>SN</td>
<td>189.22±6.21*</td>
<td>189.54±6.13*</td>
<td>189.42±6.42</td>
<td>187.01±6.25*</td>
</tr>
<tr>
<td>IV</td>
<td>85.54±1.39*</td>
<td>89.97±1.42*</td>
<td>87.75±2.73</td>
<td>90.68±1.62*</td>
</tr>
<tr>
<td>CN</td>
<td>56.01±1.01*</td>
<td>54.86±1.29*</td>
<td>55.43±1.18*</td>
<td>55.12±1.63*</td>
</tr>
<tr>
<td>LCSF</td>
<td>6.09±1.19*</td>
<td>5.14±0.87*</td>
<td>5.61±1.05*</td>
<td>5.23±1.23*</td>
</tr>
<tr>
<td>CFPP (°C)</td>
<td>-4.16±2.10*</td>
<td>-5.73±1.54*</td>
<td>-4.94±1.85*</td>
<td>-5.57±1.54*</td>
</tr>
<tr>
<td>CP (°C)</td>
<td>-1.63±2.06*</td>
<td>-3.17±1.53*</td>
<td>-2.40±1.81*</td>
<td>-3.02±1.93*</td>
</tr>
<tr>
<td>DU</td>
<td>98.86±4.24*</td>
<td>104.02±7.04*</td>
<td>101.44±4.06</td>
<td>104.67±1.71*</td>
</tr>
<tr>
<td>OS (h, 110°C)</td>
<td>3.97±0.16*</td>
<td>3.77±0.06*</td>
<td>3.87±0.35*</td>
<td>3.76±0.07*</td>
</tr>
<tr>
<td>HHVs(MJ kg⁻¹)</td>
<td>40.38±0.38*</td>
<td>40.30±0.25*</td>
<td>40.34±0.34</td>
<td>40.40±0.39*</td>
</tr>
</tbody>
</table>

Averages in one row followed by the same superscript do not differ significantly according to the SNK post hoc test. Superscripts a → b: indicate increasing values.

acid composition has a high percentage of unsaturated fatty acids.

The low temperature properties of *P. atlantica* methyl esters were determined by CP and CFPP, which indicate whether the biodiesel can be used in low-temperature regions. The three standards do not specify a low-temperature parameter in their lists of specifications. Each country using these standards can specify certain temperature limits for different times of the year, depending on the local climate. Low CP and CFPP imply better cold flow properties. The CP and CFPP of the methyl esters produced in this study (Table 2) were considerably good because the unsaturated fatty acid content is high compared to the saturated fatty acids (Table 3). A too high cold filter plugging point in biodiesel leads to wax formation and engine starving due to a reduced fuel flow [16].

Heat of combustion or heating value is another important in-use property, though there is no specification for it in the three biodiesel standards (Table 3). However, a European standard for using biodiesel as heating oil, EN 14214-08, specifies a minimum heating value of 35 MJ kg⁻¹. The HHVs for *P. atlantica* fruit methyl esters (Table 2) were above, with values ranging from 39.5 to 40.6 MJ kg⁻¹. As a result, the methyl esters prepared from *P. atlantica* oil exhibited a high energy content.

Other fuel properties of *P. atlantica* fruits at two different ripening stages such as KV and IV, satisfied the three biodiesel standards (Tables 2 and 3).

According to the European standard for biodiesel (EN 14214-08), the concentration of linolic acid (C18:3) in fatty acid methyl esters should not exceed the limit of 12%. *P. atlantica* fruit oil only contained linolic acid in amounts of 0.63 to 0.85% (Table 2).

In order to verify the biodiesel quality of the *P. atlantica* oil methyl esters from the two development stages, some physico-chemical properties, such as density, kinematic viscosity, cloud point, IV and SN were determined experimentally and comparison was made with limit values specified in the standards (Table 3). The experimental values for KV, ρ, CP, IV and SN agree with those given by standards (Table 3), which confirms that the biodiesels of immature and mature *P. atlantica* oil methyl esters satisfied the limits imposed by European (EN 14214-08), Chinese (GB/T 20828-07) and American (ASTM D6751-2010) standards.

In Moreover, the fatty acid profiles produced by *P. atlantica* fruits were suitable for biodiesel production. These findings support previous reports that short chain fatty acids, which contain high amounts of SFAs (palmitic acid) and MUFA's (oleic acid), are suitable for biodiesel production because they lead to substantially less fuel polymerization during combustion than fuel derived from polysaturated fatty acids [16].

Biodiesels emit more nitrogen oxides (NOx) than conventional diesels. Mirhashemi and Sadmira [21] reported that the NOx emissions are correlated with increasing CNs, saturation and length of the fatty ester chain, and with decreasing IV values. In this sense, biodiesels of both regions obtained at the immature stage (June) with high CN, low IV and high percentages of saturated fatty acids (Table 3) are the best environmentally-friendly candidates with low NOx emissions.
Currently, the raw materials for over 95% of commercially produced biodiesel come from edible oils, such as rapeseed in Canada, soybean in US, sunflower in Europe and palm in Southeast Asia, thus leading to a competition of their usage as food versus fuel. Non-edible oils plant are studied to solve the problem of competition with food production [16]. However, the problem of water requirement, water availability, and mainly, the quantity of greenhouse gases generated by the large amount of exploitable land can not be solved using this raw material. For these reasons, *P. atlantica* fruit oil can be proposed as a very important candidate source of fuels and chemical feedstock, because this species is well adapted to arid or semi-arid conditions and requires low fertility and moisture to grow. Further, *P. atlantica* may use land that is largely unproductive [11]. Moreover, *P. atlantica* is often used as a rootstock for *Pistacia vera*, *terebinth* (*P. terebinthus* L.), and the mastic tree (*P. lentiscus* L.), where these latter have edible fruits and a considerably higher commercial importance than *P. atlantica* [11]. In Mediterranean countries, the use of *P. atlantica* is usually focused on its resin or gum, while the fruits hardly are used. The resin of *P. atlantica* has a variety of industrial and traditional uses, including food and medicine. The fruits are used as a rootstock fruit source and sometimes for fruit consumption or soap production [11]. The availability of *P. atlantica* feedstock in Mediterranean countries represents one of the most significant factors for biodiesel production.

For all these reasons and according to the above results the use of *P. atlantica* fruit oil as raw material is a promising way for biodiesel production.

### IV. CONCLUSION

The tested quality parameters of biodiesel, derived from oil extracted at two stages of fruit maturity were found to comply with the industrial standards. It is quite common to harvest fruits, when they become morphologically mature because of the high oil content resulting in diesel with conformity to ASTM D6751-10, EN 14214-08 and GB/T 20828-07 organization standards. The results of the study could help to use targeted fruit collection during ripening, and guide to use the most suitable fruit for industrial products preparation, both for food and biodiesel properties.

### REFERENCES


<table>
<thead>
<tr>
<th>Parameters</th>
<th>Standards</th>
<th>Experimental</th>
<th>Laghouat</th>
<th>Ain oussera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASTM D6751-10</td>
<td>EN 14214-08</td>
<td>GB/T 20828-07</td>
<td></td>
</tr>
<tr>
<td>SN (mg KOH g⁻¹ oil)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>207.44</td>
</tr>
<tr>
<td>IV (g l⁻¹ 100 g⁻¹ oil)</td>
<td>–a</td>
<td>120 max</td>
<td>–a</td>
<td>93.31</td>
</tr>
<tr>
<td>CN</td>
<td>47 min</td>
<td>51 min</td>
<td>49 min</td>
<td>–</td>
</tr>
<tr>
<td>LCF</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CFPP (°C)</td>
<td>–a</td>
<td>–b</td>
<td>–c</td>
<td>–</td>
</tr>
<tr>
<td>CP (°C)</td>
<td>–a</td>
<td>–b</td>
<td>–c</td>
<td>–3.56</td>
</tr>
<tr>
<td>DU</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>OS (h, 110°C)</td>
<td>3.0 min</td>
<td>6.0 min</td>
<td>6.0 min</td>
<td>–</td>
</tr>
<tr>
<td>KV (mm² s⁻¹; 19-60°C)</td>
<td>1.9-6.0</td>
<td>3.5-5.0</td>
<td>1.9-6.0</td>
<td>3.53</td>
</tr>
<tr>
<td>KV (mm² s⁻¹; 40-60°C)</td>
<td>–a</td>
<td>860-900</td>
<td>820-900</td>
<td>840.67</td>
</tr>
<tr>
<td>HHV (MJ kg⁻¹)</td>
<td>–</td>
<td>35 min</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Note: Not Reported

- a: No specified limit
- b: Not specified. Variable by location and time of year
- c: Low temperature properties are not specified, but should be agreed upon by the fuel supplier or purchaser

IM: immature biodiesel samples
MA: mature biodiesel samples
ED%: percent error deviation between estimated and experimental values


