Morphometry, Oil Yield and Fatty Acid Profile of Cannabis Achenes from the Chefchaouen Region

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Abstract: The achenes evaluated had average dimensions of 4.242 ± 0.329 mm long, 3.38 ± 0.294 mm wide, 2.75 ± 0.227 mm thick. They contained an average moisture of 7.614 ± 1.623 and an average mass of 0.01808 ± 0.0038 g. No significant differences in the yields of the oil obtained by hexane and press extraction (p-value > 0.05), they varied respectively from 31.36 % ± 0.05 to 36.86 % ± 1.79 and 21.47% ± 2.45 to 25.04 % ± 0.46. Among the fatty acids in the mechanically extracted vegetable oil from the five achenes varieties, the order of abundance of the identified components was the same. The linoleic acids they account for more than 86 % of the total α-predominant fatty acids are linoleic and fatty acids. PUFA/SFA ratio ranged from 5.5 ± 1.58 to 8.96 ± 0.23. The n-6/n-3 ratio varied from 1.53 ± 0.02 to 1.78 ± 0.0. The quotients recorded in our study are likely to be of nutritional interest since a ratio between 1/1 and 2/1 is considered ideal.

Keywords: Cannabis achene, morphometric characters, oil yields, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).

1. Introduction
In the Chefchaouen region of northern Morocco, interest in cannabis was mainly focused on the production of the resin (hashish); a product where the cannabinoids are concentrated making the psychoactive reputation of the plant (Merzouki 2001, Merzouki and Molero Mesa, 2002). The resin is the expected result of the entire cultivation cycle up to the production stage, it represents a highly sought-after commodity, endowed with great economic power both globally and nationally. Yet it remains informal in the country even with the arrival of the new law 13-21 regulating the legal use of cannabis in 2021 (Bulletin officiel, 2021).

According to the traditional method of cultivation and exploitation of cannabis in the Chefchaouen region, only pistillate plants are traditionally exploited for the production of resin; process during which a significant production of achenes takes place (Merzouki and Molero Mesa, 1999, Merzouki et al., 1994). The latter, either they are kept in part to guarantee the seed of the following year if the resin...
production of their progenitor generation has been judged quantitatively and qualitatively effective, or they are considered in the opposite case as being debris from the production to be eliminated by incineration or in a few special cases to feed poultry, their exploitation has never aroused interest in the society of farmers. Currently, it has become obvious that cannabis achenes have a wide range of industrial potential, given their physicochemical properties, particularly in the food industry and in medicine (Haddou et al., 2023a). According to the European Commission, cannabis achenes are generally recognized as a safe nutrient source (Kladar et al., 2021). Moreover, it is proven from a rich bibliography concerning the biochemical analysis of cannabis achenes, that they offer 26-38 % of oil compared to 15-24 % of cotton seeds, 17-21 % soy and 20-25 % in olive (Matthäus and Brühl, 2008, Abdollahi et al., 2020, Vosulipur et al., 2004, Xu et al., 2021, Crimaldi et al., 2017). The protein profile of achenes is comparable to that of soybeans, with an estimated content of 20 %–25 % and a ratio of amino acids necessary for human nutritional needs, the main amino acids identified are lysine, tryptophan and arginine (House et al., 2010, Liu et al., 2023, Neacsu et al., 2022, Chen et al., 2012). Cannabis proteins can serve as a rich thiol resource to formulate highly nutritious foods and as a nutritional tool to increase the antioxidant and anti-hypertensive capacity of the human body (Chen et al., 2012, Wang and Xiong, 2019, Shen et al., 2021, Aiello et al., 2020, Cerino et al., 2021). Carbohydrates, fibers and minerals are estimated at 20 %-30 % and 10 %-15 % respectively (Kostić et al., 2013, Anwar et al., 2006). Cannabis achenes also have high levels of vitamins A, C, E, k (Anwar et al., 2006, Turner et al., 1980, Orhan et al., 2000, Moczowska et al., 2020) and a rich range of minerals such as phosphorus, magnesium, calcium, potassium, iron and zinc (Moczowska et al., 2020, Latif and Anwar, 2009), some of which play important roles as cofactors for fatty acid metabolism in the human body (Deferne and Pate, 2009, Babiker et al., 2021).

Due to its unique lipid composition, cannabis vegetable oil offers various health, pharmaceutical, nutritional and cosmetic benefits over any other known conventional oil. It is considered to be the most unsaturated plant-derived oil with a perfectly balanced 3:1 ratio of the two essential human polyunsaturated fatty acids; linoleic acid (ω6) and α-linolenic acid (ω3) (Da Porto et al., 2012b, Ciano et al. 2023, Dinicolantonio and Okeefe, 2019, Occhiuto et al., 2022). PUFAs represent 70% to 80% of the total fatty acids, which is considered an important criterion for assessing the nutritional value of cannabis vegetable oil (Da Porto et al., 2012a, Kriese et al., 2004, Matthäus et al., 2006, Xu et al., 2021). However, the rather high level of PUFAs attributes a less pronounced oxidative stability in cannabis vegetable oil compared to that presented in olive, rapeseed and sunflower oil (Porto et al., 2015, Li et al., 2022). Oxidative deterioration can affect the nutritional value of the oil, its color and taste, resulting in unpleasant flavors and organoleptic characteristics (Izzo et al., 2022). On the other hand, experimental, clinical and epidemiological data indicated that the potential health benefits of ω6 and ω3 are interesting given their neuroprotective, anti-inflammatory (Haddou et al., 2023b; Laws and Smid, 2022, Werz et al., 2014) antithrombotic, antiarrhythmic and hypolipidemic properties (Lowe et al., 2021, Callaway, 2004, Mikulec et al., 2019, Van Name et al., 2020), as well as their ability to play the roles of epigenetic factors in human cancer development (Cuvelier and Maillard, 2012). On the other hand, palmitic and stearic acid represent the most dominant saturated fatty acids in cannabis vegetable oil with a total content of about (9 g/ 100 g) (Matthäus et al., 2006). In addition to the aforementioned fatty acids, cannabis vegetable oil also contains Y-linolenic acid with minor levels (Matthäus et al., 2006, Anwar et al., 2006).

Y-linolenic and stearic acids are very important for human nutrition; they play the role of intermediates for the formation of polyunsaturated fatty acids and ecosanoids, which control vital processes ranging from inflammation and vascular tone to the initiation of contractions during childbirth.
(Ally and Horrobin, 1980, Alonso-Esteban et al., 2020). ɣ-linolenic acid is known both as a nutrient and as a therapeutic agent due to its wide potential contribution in improving various types of inflammation (Sergeant et al., 2016, Poorani et al., 2020, Rengachar et al., 2022, Kapoor and Huang 2006), in the correction of diabetic complications, cardiovascular and reproductive disorders (Kim et al., 2012) also in the amelioration of cancer (Andreassi et al., 2020, Miyake, 2009, Chas et al., 2019), as well as having antiviral implications (Horrobin, 1992).

Similarly, positive effects of ɣ-linolenic acid have been observed in patients with rheumatoid arthritis and atopic dermatitis (Horrobin, 1992, Zurier et al., 1996, Andreassi et al., 1997). In addition to the unsaturated fatty acids of the 18-carbon group identified in cannabis vegetable oil, there are other unusual molecular species that have been identified in trace amounts such as arachidic acid (20:0), eicosenoic acid (20:1, cis-11), behenic acid (22:0), and lignoceric acid (24:0) (Mölleken and Theimer, 1997). Also, in the unsaponifiable fraction of cannabis achenes oil, representing 1.5 % to 2 % (Blasi et al., 2022), an amalgam of minor antioxidant components accompanying the lipids have been identified and quantified (Montserrat-de la Paz et al., 2014, Liang et al., 2015, Russo and Marcu, 2017, Russo, 2018). This fraction is composed mainly of terpenes, phytosterols and some tocopherols (Haddou et al., 2023a; Faugno et al., 2019, Claro-Calà et al., 2022, Martinez et al., 2020, Nigro et al., 2020), offering therapeutic potential to reduce cholesterol and blood pressure, and even prevent cardiovascular disease and cancer (Liang et al., 2015, Alencar et al., 2018), they also act as anti-inflammatory and immunomodulators (Farinon et al., 2020, Rupasinghe et al., 2020). Moreover, the industrial and therapeutic importance of tocopherols comes from their ability to act both as natural antioxidants against oxidative deterioration of the oil (Spano et al., 2020) and as vitamin E in the human body with a remarkable antioxidant activity to scavenge free radicals (Izzo et al., 2020). Other minor bioactive components as new added value of cannabis vegetable oil is cannabidiolic acid (CBDA) and ligananamides. CBDA is able to differentiably modulate the release of pro-inflammatory cytokines and to act as mediators of chemokines (Nigro et al., 2022), also it exhibits antimicrobial activity against Gram (+) and Gram (-) bacteria (Martinenghi et al., 2020), while ligananamides offer strong anti-neuro-inflammatory potential (Zhou et al., 2018).

The yield efficiency and phytochemical profile of cannabis achenes oil, extracted by conventional mechanical and chemical methods (Matthäus and Brühl, 2008a, Anwar et al., 2012, Stambouli et al., 2006) as well as by alternative methods such as supercritical CO2 extraction (Aladić et al., 2015), microwave and ultrasonic extraction (Mookerjee et al., 2022), varied according to the variety of achenes (Xu et al., 2021, Izzo et al., 2020), the climatic conditions of the region and the cultivation techniques (Abdollahi et al., 2020, Faugno et al., 2019, Campiglia et al., 2017, Kalinowska et al., 2022, Irakli et al., 2019, Lan et al., 2019, Xu et al., 2021, Pacifico et al., 2015, Calzolari et al., 2021). Furthermore, the identification of cannabinoids (THC and CBD) in cannabis vegetable oil is another biochemical aspect that raises the problem of contamination. Generally, cannabis seeds do not contain cannabinoids (Russo, 2011, Russo and Marcu, 2017), yet studies have shown that even in the case of industrial cannabis achenes where the THC < 0.2 %, the oil obtained risks showing traces in cannabinoids including THC as results of contamination (Tura et al., 2023, Barthlott et al., 2021, Lachenmeier et al., 2020). The extent of the contamination is relative to the variety of achenes exploited, to the techniques of preparation, cleaning and storage of materials (Ross et al., 2000) and to the different extraction conditions (temperature and duration of the extraction) (Tura et al., 2023, Izzo et al., 2020). In the literature, a number of studies have shown that ingesting highly contaminated cannabis edibles can lead to elevated urinary levels of THC metabolites, positive immunoassay results, and even to exhibit the psychotropic effects specific to this molecule (Bosy and Cole, 2000, Grotenhermen and Elgoehly, 2001, Ouhtit et al., Mor. J. Chem., 2024, 12(1), pp. 43-60
In Canada, for safe extraction of vegetable oil from *cannabis*, where THC is < 10 ppm in the oil, the level of THC contamination of achenes must be < 2 ppm. A precautionary guideline value for THC estimated at 500 µg/kg has been established for edible oil and 10 mg·g⁻¹ in other products for various uses (Tura et al., 2023, AL Ubeed et al., 2022).

In order to overcome the problem of cannabinoid (especially THC) contamination in vegetable oil as well as in *cannabis* products, studies have already been presented on the creation of new varieties of non-psychoactive *cannabis* (Soler et al., 2017, Green, 2005, Piluzza et al., 2013, Salentijn et al., 2015, Xu et al., 2021), which are distinguished by their minimal content of cannabinoids including THC (or without THC), which excludes the possibility of their psychotic use and guarantees food products that comply with sanitary and legal standards.

Currently, in Europe, only certain varieties with a low Δ9-THC content (< 0.2 %) are allowed to be cultivated because they are certified as non-psychotic varieties, generally recognized as "industrial hemp" (Glivar et al., 2020, Montserrat-de la Paz et al., 2014, Hughes, 2017, Glivar et al., 2020, Montserrat-de la Paz et al., 2014, Moczkowska et al., 2020). In Morocco, according to the new law 13-21, the cultivation of *cannabis* varieties whose THC content exceeds the rate fixed by regulation, can only be granted for the medical and pharmaceutical industries (Bulletin officiel, 2021).

This work aims to characterize five *cannabis* cultivars (CB, CK, CM, CC, CA) from the Chefchaouen region according to the morphometric properties of achenes, mass, vegetable oil yield and fatty acids profile.

2. Methodology

2.1 Preparation of samples

The field trials were set up in El Kalâa, a small village in the region of Chefchaouen (35° 13’ 110" N, 5° 14’ 42" W), 873(m) of altitude. The village has a mountainous morphology with the famous Jbel el Kalâa summit (1721 m). The climate is typically mountainous, rainy and cold in winter and mild to hot in summer. Generally, the rainfall is between 800 and 1400 mm but sometimes it can exceed 2000 mm/year. During the summer (July, mid-August) a dry period occurs with scarce rainfall and high temperatures sometimes reaching or exceeding 40°C.

Five varieties of psychotic *cannabis* seeds corresponding to the CA, CB, CM, CK and CC cultivars were cultivated in Bour in the region of Chefchaouen under the same protocol of local traditional culture and monitoring, then subjected to the same processes of harvesting, drying and production of resin and achenes. Sowing was carried out between (March-April) and harvesting between (August, September). The quantification of the oil yields of achenes as well as the biochemical characterization of their lipid profile will be carried out on the harvest carried out in 2022.

2.2 Morphological characterization of achenes

Achenes dimensional characteristics were established on a small samples size (50 achenes of each cultivar of the five studied). Achenes were taken randomly after excluding unripe and empty achenes in the study samples. Measurements were taken for length (mm); width (mm); and thickness (mm), using a digital caliper with an accuracy of 0.01 mm. The length was taken from the hook of the achene to its end. The width was taken at the largest diameter. The mass (g) corresponding to each achene was measured through a digital precision balance. The count of achenes contained in one gram (1 g) for each achene variety was repeated 5 times for each cultivar.

The results were processed with SPSS version 23 software (IBM, Armonk, NY, USA) at a significance level of α=0.05. The variations in the dimensional properties of achene varieties were determined by

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independent t-test. The data distribution was checked for normality by the Shapiro-Wilk test, and the homogeneity of variance was determined by Levene’s test.

2.3 Procedure extraction

The moisture content of achenes was determined in duplicate according to AOAC Official Method 925.40 (2000), such that 5 grams of each achene’s variety were placed in a temperature-stabilized oven at (60 °C) until constant mass of the samples. Moisture content was calculated in percent (%) as the loss of recorded weight.

Oil content: oil yields were obtained by solvent extraction (hexane) following the method of (Kostić et al., 2013) with some modifications and by mechanical extraction using a mechanical press.

2.3.1 Chemical extraction

Determination of the oil yield for each achene variety was carried out in duplicate using a Soxhlet apparatus with six devices (Figure 1). Achene samples were natural dried in a cool, windy and dry location. The extraction process was carried out for 6 h at 70 °C, using 10 g of ground achene samples and 100 mL of n-hexane according to the solvent: seed ratio of 10:1 mL/g. The solvent was removed at (50°C) under reduced pressure using a Rotavapor until mass stabilization. The oil obtained had a clear color ranging from bright green to yellow.

Figure 1. Experimental device for chemical extraction

Extraction 1

10 grams of achenes of each variety (CA, CB, CM, CK and CC) were manually crushed in a ceramic mortar. The sample was labeled and then placed in a filter paper cartridge (rod shape) and placed in the glass body, with 100 ml of hexane in the ratio 10: 1 ml/g, and then connected to a condenser. The flask containing the hexane is placed in a water bath thermostated at a predetermined temperature (70°C), accompanied by a built-in magnetic stirrer. The 6 vials were catalogued with the same labels as the corresponding samples.

- The extraction process lasted 6 hours; the time necessary for the total exhaustion of the sample.
After the start of the extraction, the devices 1 and 4 corresponding to the cultivars (CB, CC), stopped evacuating the solvent because of mechanical problems. It took us 23 minutes to restart them, after adding 50 ml of solvent in the two bottles concerned. Devices 1 and 4 were left on for another 23 minutes after 6 hours for all vials.

- The hexane was removed at (50°C) under reduced pressure. The mass of the vial was noted after every 5 minutes of evaporation to constant mass.
- The yield was expressed in grams of oil per 100 grams of achenes according to the formula: 
  crude oil (%) = \((A2 - A1) / A\) * 100, such that A1= the mass of the empty flask; A2= the mass of the flask after extraction and evaporation; A= the mass of the sample.

- The oil obtained was immediately stored in hermetically sealed glass bottles and then wrapped with aluminum foil and stored in a refrigerator at 4°C for future characterization of various phytoconstituents.

**Extraction 2**

We used a sample of 10 grams of ground achenes corresponding to the five varieties CA, CB, CM, CK and CC, with the same sample-solvent ratio of 10:1 (ml/g), then followed the same protocol mentioned above in test 1.

2.3.2 Mechanical Extraction

Oil extraction was carried out in duplicate using a mechanical press P500R, frequency 50HZ, voltage 400V and a power of about 1.5 KW. The achenes of our samples were air-dried beforehand and had an average moisture content of 7.614 ± 1.623% (oven-dried according to the AOAC Official Method 925.40 (2000). The first and second extractions were performed on a 150 g sample of achenes for each variety for 6 min. The yield of oil extracted by mechanical press was expressed as grams of oil per gram of total dry matter. Percentages were calculated according to the following equation: Oil yield (%) = \((A2 - A1) / A\) * 100, such as A1= the mass of the empty vial; A2= the mass of the vial after extraction (vial + extracted oil); A= the mass of the seed sample (150 g). The oil obtained was immediately filtered through the filter paper, then stored in hermetically sealed glass bottles, wrapped with aluminum foil and kept in a refrigerator at 4°C for the future characterization of various phytoconstituents. The Figure 2 shows oil samples from the five varieties of achenes. The oil obtained had a green to brown color with an almond taste.

**Figure 2.** Oil samples from five varieties of achenes by mechanical extraction
2.4 Determination of fatty acids with gas chromatography (GCFID)

FAMEs analyses were performed using an Agilent gas chromatograph 8860 (GC) equipped with a flame ionization detector (FID). Prior to injection, the extracted oils were converted to methyl esters, 10 microliters of oil were accurately weighed and dissolved in 490 microliters of hexane, 200 microliters of sodium methoxide 20 % methanol were added, and the closed tube vortex for 2 min, then placed in oven 55°C for 30 min. This step is meant to transform carboxylic into methyl ester groups. Samples were heated to improve the reaction rate, RCOOH+NaOCH \rightarrow RCOOCH3+NaOH. 400 microliters of 1 N HCl were added, the solution was shortly vortex for 2 min, few mgs of MgSO4 were added. This step was done to basify the NaOH that is formed. One microliter was injected into split/splitless mode, the gas chromatographic column was a DB5-MS (30 m X 0.25um) (Agilent J&W) with a (5%-phenyl)-methylpolysiloxane stationary phase. Column flow was 1 ml/min. The oven temperature program was as follows: from 50°C for 1 min to 175°C at 10°C/min and stays 10 min to 230 5°C/min for 9.5 min -to 300 °C 10°C/min (run time 61 min); Injector 250°C, Detector 260°C. The results were expressed in percentage, after the identification of fatty acids by comparison with those of standards mixture FAME 37 components (Sigma Aldrich).

2.5 Statistical analysis

The data represent the means ± standard deviations, they are expressed in mg/ g of the oil. Normality tests were performed according to the Kolmogorov-Smirnov and Shapiro-wilk models. Differences between the five oil varieties were assessed using a non-parametric test (one-way ANOVA), followed by Tukey & Duncan's correction for multiple comparison, the differences were considered significant at the 5% probability level. A principal component analysis (PCA) was performed on the basis of the principal components to visualize the possible correlations between the variables and the oils varieties. Statistical analyses were performed using SPSS version 23 software (IBM, Armonk, NY, USA).

3. Results and Discussion

3.1 Morphological characterization of achenes

No significant differences in the dimensional parameters of the five achene varieties according to the results of the Kolmogorov-Smirnov and Shapiro-Wilk normality tests, the p-value is above the 0.05 threshold for all variables (Table 1).

Table 1. The data represent the means ± SED of different dimensional parameters (L, La, DG) expressed in (mm), mass (g) and moisture content (%) measured on achenes of the five cultivars. Normality tests reveal a p-value > 0.05 for all measurements. (n = 50) represents the number of achenes measured from each variety.

<table>
<thead>
<tr>
<th>Achenes varieties</th>
<th>Length (mm)</th>
<th>Width (mm)</th>
<th>Thickness (mm)</th>
<th>Mass (g)</th>
<th>Moisture content (%)</th>
<th>Achenes number in (1g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB (n=50)</td>
<td>3.83 ± 0.29</td>
<td>2.98 ± 0.25</td>
<td>2.43 ± 0.20</td>
<td>0.01 ± 0.00</td>
<td>6.24 ± 1.04</td>
<td>117 ± 8.40</td>
</tr>
<tr>
<td>CK (n=50)</td>
<td>3.99 ± 0.24</td>
<td>3.09 ± 0.19</td>
<td>2.63 ± 0.16</td>
<td>0.01 ± 0.00</td>
<td>7.48 ± 1.16</td>
<td>89 ± 10.68</td>
</tr>
<tr>
<td>CM (n=50)</td>
<td>4.28 ± 0.23</td>
<td>3.45 ± 0.20</td>
<td>2.83 ± 0.16</td>
<td>0.02 ± 0.00</td>
<td>10.37 ± 2.07</td>
<td>84 ± 6.60</td>
</tr>
<tr>
<td>CC (n=50)</td>
<td>4.52 ± 0.23</td>
<td>3.53 ± 0.24</td>
<td>2.90 ± 0.16</td>
<td>0.02 ± 0.00</td>
<td>7.33 ± 1.30</td>
<td>78 ± 10.95</td>
</tr>
<tr>
<td>CA (n=50)</td>
<td>4.59 ± 0.26</td>
<td>3.74 ± 0.21</td>
<td>3.00 ± 0.17</td>
<td>0.02 ± 0.00</td>
<td>6.64 ± 0.96</td>
<td>73 ± 10.07</td>
</tr>
</tbody>
</table>

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The different dimensional parameters (L, La, G) as well as mass (M) represented estimated means of 4.24 mm ± 0.38 mm; 3.38 mm ± 0.34 mm; 2.76 mm ± 2.67 mm; 0.02 g ± 0.00 g, respectively, for all five achene varieties. CA, CC, CM achenes had more pronounced dimensional and mass values compared to CK and CB achenes. Since Figure 3, the five varieties of achenes had dimensional parameters ranging from 3.83 ± 0.29 mm to 4.59 ± 0.26 mm for length; from 2.98 ± 0.25 mm to 3.74 ± 0.21 mm for the width; and from 2.43 ± 0.20 mm to 3.00 ± 0.17 mm for the thickness. As these parameters increase, the equivalent mass of the achene also increases. On the other hand, the average moisture content for all five achene varieties was 7.61 % ± 1.62 %. The CM achenes had the highest moisture content (10.37 % ± 2.07) compared to the CB achenes which marked the lowest content (6.24 % ± 1.04).

**Figure 3.** Comparison of dimensional parameters and achene mass for the five varieties. The results of the normality tests express a p-value > 0.05.

Furthermore, the count of achenes contained in one gram showed significant differences between the achenes of the five cultivars studied (Table 1). The number of achenes varied from 73 ± 10.07 seed/1 g of dried seeds for CA to 117 ± 8.40 seed/1 g of dried seeds for CB, these differences can be explained by the morphological and physiological properties mentioned above. Generally, the average number of achenes in 1 gram in all five achene varieties was 88.20 ± 17.19 seed/1 g of dried seeds.

### 3.2 Oil yield by hexane and mechanical extraction

Table 2 summarize the means and standard deviations of the yields obtained from two soxhlet and two mechanical extractions expressed in %. The yield of oil expressed as an average in all five varieties of achenes represented 34.86 % ± 1.14 % by extraction with hexane while it represented 23.93 % ± 1.43 % by mechanical extraction, the latter allowed us to recover 71.80 % of the oil compared to the extraction with soxhlet, this reduced yield can be explained by the fact that the cake resulting from the pressing still contains a significant fraction of oil that is not recoverable. On the other hand, hexane allowed a complete extraction by total exhaustion of lipids in the sample.

**Table 1.** Oil yields by chemical and mechanical extraction. Data represent the mean ± SED expressed as (%).

<table>
<thead>
<tr>
<th>Achenes variety</th>
<th>Soxhlet extraction</th>
<th>Oil yield (%)</th>
<th>Mechanical extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB</td>
<td>31.37 ± 0.05</td>
<td></td>
<td>24.32 ± 0.06</td>
</tr>
<tr>
<td>CK</td>
<td>36.43 ± 1.17</td>
<td></td>
<td>25.04 ± 0.46</td>
</tr>
<tr>
<td>CM</td>
<td>34.76 ± 2.88</td>
<td></td>
<td>24.02 ± 5.12</td>
</tr>
<tr>
<td>CC</td>
<td>34.87 ± 0.37</td>
<td></td>
<td>21.47 ± 2.45</td>
</tr>
<tr>
<td>CA</td>
<td>36.87 ± 1.79</td>
<td></td>
<td>24.81 ± 1.75</td>
</tr>
</tbody>
</table>
The results obtained in this study are comparable to those identified in several publications on the quantification of oil in different varieties of *cannabis* achenes and according to different extraction methods. In Morocco, pilot studies carried out on the characterization of the lipid profile of *cannabis* achenes grown in the northern region of the country (Merzouki and Molero Mesa, 1997, Stambouli et al., 2006, Taaifi et al., 2021), revealed an average content of oil extracted by hexane with soxhlet of 30, 96 % ± 1.29 % to 34.60 % ± 1.28 %, these results are comparable to those identified in northern Italy, where the quantification of achenes oil corresponding to 4 industrial cultivars by hexane in soxhlet revealed a content of 19.50 % ± 0.24 % to 30.70 % ± 0.32 % (Porto et al., 2015).

Furthermore, the oil content obtained by cold pressing of 3 varieties of *cannabis* oilseeds grown in Pakistan ranged from 26.90 % to 31.50 % (Anwar et al., 2006). In another study (Latif and Anwar 2009), enzyme-assisted cold pressing oil extraction revealed an oil content of 28 % to 32.80 %, while oil extraction from 51 genotypes of *cannabis* achenes by supercritical CO₂ revealed a yield of 26.30 g/100g to 37.50 g/100g (Deferne, 1996). Also, contents estimated at 21.50 % and 22 % have been determined respectively in other studies relating to the supercritical CO₂ extraction of oil from *cannabis* achenes of different regions (Da Porto et al., 2012b). Since Figure 4 there are no significant differences in the yields of soxhlet extracted oil between the five achen variety. The results of normality tests (Shapiro-Wilk) reveal a p-value > 0.05. The CB achenes scored the minimum yield of 31.36 % ± 0.05 %, while the CA achenes offered the maximum estimated yield of 36.86 % ± 1.79 %.

![Figure 4](image-url)

**Figure 4.** Comparison of oil yields for the five-achene varieties. Bars represent means ± standard deviations of two independent extractions. The p-value is above the 0.05 significance level.

From Figure 5, no significant differences in yields of mechanically extracted oil from the five-achene varieties, results of normality tests (Shapiro-Wilk) reveal a p-value > 0.05. Yields ranged from 21.47 % ± 2.45 % identified in CC achenes to 25.04 % ± 0.46 % in CK achenes.

### 3.3 Fatty acids composition of extracts by GCFID

In addition to the yield efficiency of vegetable oil in the *cannabis* achenes corresponding to the five cultivars studied, the fatty acid profile is another very important criterion for characterizing the suitability of the oil of different cultivars for certain uses. The chemical composition of fatty acids in the oils obtained by mechanical press is presented as means ± SED and expressed in (g/100 g) in Table 3. The results of normality test according to Kolmogorov-Smirnov and Shapiro-Wilk reveal a p-value < 0.05 for some fatty acids, whereas it is higher than 0.05 for other species. Nonparametric analysis was performed according to ANOVA One-Way.
Figure 5. Comparison of oil yields for the five-achene varieties. Bars represent means ± standard deviations of two independent extractions. The p-value is greater than the 0.05 significance level.

Among the fatty acids in the vegetable oil of the five cultivars, the order of abundance of the identified components was the same: C18:2 (n-6) > C18: 3 (n-3) > C16: 0 > C18: 0 > C20: 1 (n-9) > C18: 3 (n-6) > C20: 3 (n-6) > C22: 1 (n-9) > C16: 1 > C17: 0 > C17: 1. The Figure 6 below shows an example of a fingerprint obtained from three replicates from the injection of 10µl of the oil (methodology) corresponding to the CB achenes into the chromatographic system. A first visual analysis shows that the general profile of the chromatogram is different. The most intense peak is attributable to linoleic acid C18: 2 (n-6) representing a percentage of 52 %, followed by α-linoleic acid C18: 3 (n-3) (34 %), palmitic acid C16: 0 (8 %) and stearic acid C18: 0 (3 %). The acids C20: 1 (n-9) > C18: 3 (n-6) > C20: 3 (n-6) > C22: 1 (n-9) > C16: 1 > C17: 0 > C17: 1 are distinguished by percentages below 1 %.

Figure 6. Type of chromatogram of fatty acids in oil obtained from CB achenes (repeat 1).

The predominant fatty acids in the oil of our achene samples are C18: 2 (n-6) linoleic acid and C18: 3 (n-3) α-linoleic acid (Figure 7). Together, they account for over 86 % of the total fatty acids for each variety of the oil. Linoleic acid C18: 2 (n-6) was found in amounts ranging from 51.89 ± 0.22 g/100 g in the corresponding CB oil to 55.65 ± 0.44 g/100 g in the CM oil. On the other hand, α-linoleic acid C18: 3 (n-3) varied from 31.81 ± 0.03 g/100 g in CM oil to 34.53 ± 0.40 g/100 g in CB oil.
Figure 7. Linoleic acid and α-linoleic acid structures.

These values identified in our work are comparable to those published in studies on the characterization of lipids in the oil of cannabis achenes from the Chefchaouen region (Stambouli et al., 2006, Merzouki and Molero Mesa, 1997, Taaifi et al., 2021). Similarly, the levels of linoleic acid C18: 2 (n-6) and α-linoleic acid C18: 3 (n-3) identified in the oils from our sample of achenes are consistent with published results for cannabis from other regions (Deferne and Pate, 1996, Babiker et al., 2021, Van Name et al., 2020, Lan et al., 2019). Another property of achene oil from the Chefchaouen region is the low content of saturated fatty acids, which consisted mainly of palmitic acid (C16: 1) > stearic acid (C18: 0) > heptadecanoic acid (C17: 0). Together, these three fatty acids represented from 9.94 ± 0.21 g/100 g in CC oil to 10.91 ± 0.37 g/100 g in CK. It can be deduced that the SFA content seems to be a very stable characteristic independently of the achene’s variety. The most interesting feature of the fatty acid composition in the oil of the five varieties of cannabis achenes studied in comparison with the published results on cannabis from the Chefchaouen region (Merzouki and Molero Mesa, 1997, Stambouli et al., 2006) is the content of γ-Linoleic acid (C18: 3 (n-6)), the level of which ranged from 0.47 ± 0.05 g/100 g in CB oil to 0.58 ± 0.06 g/100 g in the CM. Our results are in agreement with those identified in the study (Deferne and Pate, 1996). Done on the characterization of the achenes oil from 51 cannabis genotypes and where the levels of γ-Linoleic acid (C18: 3 (n-6)) varied from 0.70 g/100 g to 4.10 g/100 g in the oil of cannabis achenes harvested in 2000 and from 0.00 g/100 g to 3.50 g/100 g in 2001. In the present work, the vegetable oil obtained from the five cultivars is characterized by a dominance of PUFA over SFA (Table 4). PUFA represented 87.97 ± 0.43 g/100 g to 89.73 ± 0.27 g/100 g respectively in the oil of CK and CA achenes. The PUFA content was a fairly stable property among the five cultivars, accounting for more than 87% of total fatty acids. The PUFA/SFA ratio is very high ranged from 5.50 ± 1.58 in CB oil to 8.96 ± 0.23 in CC, this property is a highly appreciated value from a nutritional point of view. On the other hand, the n-6/n-3 ratio varied from 1.53 ± 0.02 in the CB oil to 1.78 ± 0.01 in the CM oil, these quotients recorded in our study are likely to be of nutritional interest since a ratio between 1/1 and 2/1 is considered ideal (Granados et al., 2006, Gómez Candela et al., 2011).

The results obtained in this study suggest that the cannabis achenes cultivated in the region of Chefchaouen is a good source of vegetable oil based on the high yields obtained by chemical and mechanical extraction and the richness of PUFA fatty acids (87 %), including linoleic acid C18: 2 (n-6) and α-linoleic acid C18: 3 (n-3), as well as the content of γ-linoleic acid C18: 3 (n-6).

3.4 Principal component analysis (PCA)

The data analysis was performed by principal component analysis (PCA), on a data matrix of 5 observations (5 cultivars) and 11 variables (fatty acids). The XLSTAT software was used for the different data processing. From the graph resulting of the variables and the cultivars projection on the
two first axes of the PCA (Figure 8), CC and CM have positive factorial coordinates on the first principal component of the PCA (F1) which explains 53.57% of the total inertia, these cultivars are associated with the variables C22: 1 (n-9), C18: 2 (n-6) and C16: 1.

Table 2. Data represent means ± SED for 11 fatty acids and are expressed in g/100g. Values in the same row with different superscript letters are significantly different (p < 0.05) according to nonparametric one-factor ANOVA measures with Tukey and Duncan tests.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>CB (%)</th>
<th>CM (%)</th>
<th>CA (%)</th>
<th>CK (%)</th>
<th>CC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:1 Palmitoleic acid</td>
<td>10.63 ± 1.79</td>
<td>10.60 ± 0.31</td>
<td>10.28 ± 0.27</td>
<td>10.91 ± 0.37</td>
<td>9.94 ± 0.21</td>
</tr>
<tr>
<td>C16:0 Palmitic acid</td>
<td>1.16 ± 0.11</td>
<td>1.05 ± 0.07</td>
<td>1.05 ± 0.04</td>
<td>1.12 ± 0.16</td>
<td>1.11 ± 0.17</td>
</tr>
<tr>
<td>C17:1 cis-Heptadecenoic acid</td>
<td>88.46 ± 0.42</td>
<td>89.4 ± 0.31</td>
<td>89.73 ± 0.27</td>
<td>87.97 ± 0.43</td>
<td>88.95 ± 0.37</td>
</tr>
<tr>
<td>C18:0 Stearic acid</td>
<td>5.50 ± 1.58</td>
<td>8.44 ± 0.28</td>
<td>8.74 ± 0.25</td>
<td>8.07 ± 0.31</td>
<td>8.96 ± 0.23</td>
</tr>
<tr>
<td>C20:3n6 cis-8,11,14-Eicosatrienoic acid</td>
<td>1.53 ± 0.02</td>
<td>1.78 ± 0.01</td>
<td>1.70 ± 0.18</td>
<td>1.69 ± 0.01</td>
<td>1.72 ± 0.03</td>
</tr>
</tbody>
</table>

In contrast, CA and CK were plotted on the negative side of this principal component, these cultivars are more associated with C18: 3 (n-6), C17: 1, C17: 0 and C18: 0. On the other hand, the second component of the PCA (F2) which explains 29.11% of the total variability discriminates CB from all four cultivars, CB interacts with higher records of C18: 3 (n-9), C16: 0, C20: 1 (n-9) and C20: 3 (n-6). The 2 principal components are genetic in nature since they allowed discrimination of the five cultivars into three different groups according to their fatty acid profiles. Taking into account only variables representing more than 1%, CC and CM represent the source of the richest vegetable oil in C18: 2 (n-6) (Linoleic acid). CA and CK present an oil richer in C18: 0 (Stearic acid), while CB offers a vegetable oil rich in C18: 3 (n-3) (α-linoleic acid) and C16: 0 (Palmitic acid).
Figure 8. Representation of the correlations between the variables (fatty acids) and the cultivars on the plane of the F1, F2 axes of the PCA. The eigenvalues are symbolized by red segments representing the parameters that most affect each principal component.

Conclusion

The five varieties of *cannabis* achenes studied did not show significant differences in their dimensional parameters and masses. Similarly, we did not find significant differences in the yield of vegetable oil between the achenes corresponding to the five cultivars, either by mechanical or chemical extraction. Vegetable oil yields averaged 34.86 % ± 1.14 % and 23.93 % ± 1.43 % by chemical and mechanical extraction respectively. On the other hand, the PUFA analysis in the oil of the five achene varieties was above 87 %. Linoleic acid (C18: 2 n-6) was the most dominant acid, followed by α-linoleic acid (C18: 3 n-3). These two polyunsaturated fatty acids accounted for more than 87 % of the total fatty acids with n6/n3 ratios ranging from 1.53: 1 to 1.78: 1. The PUFA/SFA ratio were in the range of 5.5: 1 to 8.96: 1 in the oil of the five achene varieties.

The most interesting feature of the fatty acid composition of the oil of the five varieties of *cannabis* achenes studied in comparison with the published results on *cannabis* from the Chefchaouen region is the content of γ-linoleic acid (C18:3 (n-6)), the level of which ranged from 0.47 ± 0.05 g/100 g in the CB oil to 0.58 ± 0.06 g/100 g in the CM.

From the results of the principal component analysis (PCA), CC and CM achenes represent the source of the richest vegetable oil in C18: 2 (n-6) (Linoleic acid). CA and CK present an oil richer in C18: 0 (Stearic acid), while CB offers a vegetable oil rich in C18: 3 (n-3) (α-Linoleic acid) and C16: 0 (Palmitic acid). The whole of the characteristics identified in this study places the vegetable oil of *cannabis* among other agro alimentary products advised for their nutritional and physiological effects, favorable to the regulation of physiological disorders such as the blood cholesterol level and to the prevention of cancer and cardiovascular diseases.
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Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

References


Calzolari D., Rocchetti G., Lucini L., Amaducci S. (2021) The variety, terroir, and harvest types affect the yield and the phenolic and sterolic profiles of hemp seed oil, Food Res. Int., 142, 110212.


Miyake J.A. (2009) Gamma-linolenic acid inhibits both tumour cell cycle progression and angiogenesis in the orthotopic C6 glioma model through changes in VEGF, Flt1, ERK1/2, MMP2, cyclin D1, pRb, p53 and p27 protein expression. _Lipids Health Dis._, 8:8.


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