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Chemical composition analysis of seed oil from the three wild Tunisian provenances of *Acer monspessulanum* L.

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Abstract - Maple genus is one of the highly distributed genera in the northern hemisphere. Maple species are known for their ecological, economical, and medicinal values. Some of them were mentioned for their seed oil yield and composition. One of the maple species native to the Mediterranean and West Asian regions is the Montpellier maple (*Acer monspessulanum* L.). This species is considered a rare one in Tunisia with only three localities mentioned in the Tunisian flora. As far as we know, the species seed oil wasn't investigated previously anywhere. In this study, Montpellier maple seed oil was extracted using a Soxhlet extractor. Seed oil composition was analysed using Gas Chromatography coupled with Mass spectrometry (GC/MS) and differences between the seed's provenances were investigated. Oil content ranged from 4.96 % for Bargou Mountain seeds to 10.56 for Serj Mountain seeds. A total of 7 fatty acids were detected in the seed oil, 5 of them are unsaturated fatty acids. The seed oil composition was predominated by Oleic acid 38.71±2.29 %, followed by Linoleic acid 28.88±2.09, Erucic acid 11.75±0.62, Palmitic acid 8.39±0.74, and cis-11-eicosenoic acid 7.43±0.28. Also, Stearic acid and gamma-Linolenic acid were present at a rate lower than 5 % of the total seed oil composition. This study shows great potential for the Montpellier maple seed oil and represents a significant contribution to the study of this rare species in Tunisia.

Key words: *Acer monspessulanum* L.; seed oil; fatty acids; GC/MS; Tunisia.

1. Introduction

Maple species are known for their use in traditional medicine. Many of them were used for relieving cough, pain, and eczema, as a laxative, for treating infections and some diseases, and for detoxification (Bi *et al*. 2016). Other species are known for their industrial value such as the maple syrup industry (especially red maple and sugar maple) and wood industry. Their seed oil is reported of high importance regarding its yield and composition (He at *al*. 2021, Qian *et al*. 2017, Qiao *et al*. 2017, Qiao *et al*. 2018, Su *et al*. 2021). For example, Hovanet *et al.* (2015) reported that *Acer platanoides* L. and *Acer pseudoplatanus* L. seed oil yield varies respectively between 3.3 to 4.89 % and 5.12 to 6.97 %. The composition of these oils is predominated by Linoleic acid (75.2 % and 47.1 % respectively). Another interesting fatty acid mentioned by the authors is the gamma-linolenic acid, found in lower rates (3.3 % and 2.5 % of the two seed oils respectively), and has several health benefits regarding cardiovascular and skin diseases. Another interesting fatty acid found in some maple species is nervonic acid which has an important role in brain health by helping regenerate nerve fibres and increasing the capacity of neurodevelopment (Su *et al*. 2021). He *et al*. (2021) reported that nervonic acid was found in 46 Chinese maple species seed oil varying from 2.84 % for *Acer cappadocicum* to 13.9 % for *Acer elegantulum*. The study conducted by He *et al.* (2021), using CO_2 critical extraction method on 46 maple species seeds germplasm oil revealed that the major fatty acids in these oils are Linoleic (From 15.1 to 44 %) and Oleic acid (From 5.6 to 43.5 %) with a yield varying between 1.8 and 44.8 % for *Acer sterculiaceum* subsp. *Franchetii* and *Acer coriaceifolium* respectively. High variability was observed between species regarding the yield and the oil composition. The same authors also reported that the *Acer tataricum* subsp. *semenovii* has the highest content of unsaturated fatty acids in its oil (94.2 %) while the *Acer amplum* has the lowest rate (73.3 %). SU *et al.* (2021) reported that *Acer triflorum* seed oil was composed of 16 fatty acids of which only six cumulate more than 90 %. These fatty acids are Linoleic acid

(44 %), Oleic acid (20.7 %), Docosadienoic acid (12.7 %), Peanutenedioic acid (6.8 %), Palmitic acid (5 %), and Nervonic acid (4.5 %).

Acer monspessulanum L. (Montpellier maple) is a Mediterranean and west Asian deciduous tree from the *Sapindaceae* family. The species is known for its three-lobed green leaves that turn red-greenish than yellow-brownish in autumn. Tree bark is dark brown and fissured for adult trees. Fruits are di-samaras with a capacity for parthenocarpy development (Van Gelderen *et al*. 1995). It is used traditionally for carpentry and gardening. It was reported to relieve foot pain and cough and is also used as a laxative in traditional medicine (Bi *et al*. 2016). In Tunisia, it is classified as a rare species with only three stands located in the Dorsal Mountain chain. It has high ecological importance as one of the few deciduous tree species encountered in this region.

The aim of this work was to compare three provenances of Montpellier maple seeds in Tunisia regarding their oil content and to report, for the first time, the composition of the species seed's oil content and composition. This study will add more value to the Montpellier maple as a Mediterranean species and help us preserve it more in Tunisia.

2. Materials and methods

2.1. Plant material

Seeds were collected randomly from different trees in three provenances (Serj, Bargou, and Zaghouan) located in the Dorsal Mountain chain in Tunisia (Figure 1) in October 2020.

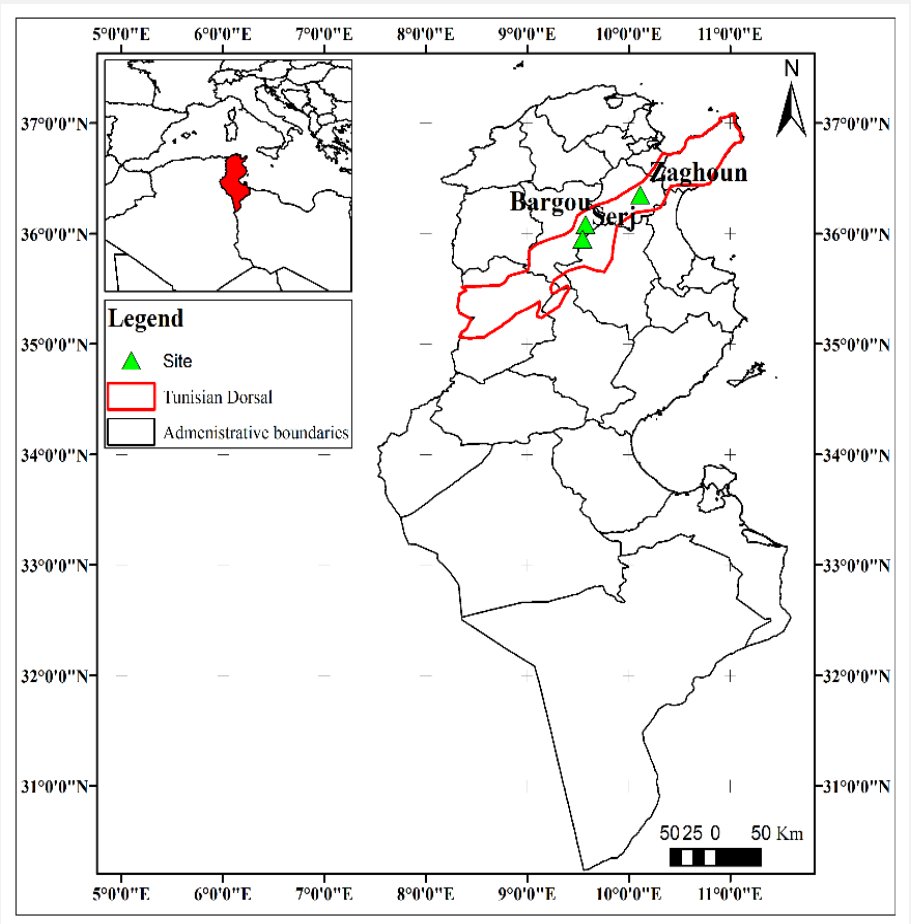

Figure 1. Location of the Montpellier maple in Tunisia.

For each provenance, random trees were selected to collect seeds. Tree's locations, altitude, slope, and aspects are described in Table 1. For each provenance, seeds were cleaned and mixed to get a representative

sample from the site (Figure 2). Seeds were air-dried at room temperature and then grinded at a 0.5 mm diameter.

Figure 2. di-samara fruits and seeds of the Montpellier maple in Tunisia observed in the autumn of 2020.

Table 1. Location, altitude, slope and aspect of the collected seeds from three Tunisian provenances.

Provenance	Latitude (DD)	Longitude (DD)	Altitude (m)	Slope (degree)	Aspect
Zaghouan	10°120278	36°361611	1105	35-40	North
Zaghouan	10°111392	36°352550	1262	25-30	North
Zaghouan	10°110806	36°353047	1194	35-40	North
Zaghouan	10°109556	36°354583	1128	30-35	North
Zaghouan	10°111639	36°355972	931	25-30	North
Bargou	09°621194	36°070556	1095	25-30	North
Serj	09°556472	35°939889	1258	30-35	North
Serj	09°555669	35°939528	1283	30-35	North
Serj	09°554083	35°941694	1109	30-35	North
Serj	09°559353	35°940628	1225	15-20	North
Serj	09°553500	35°939472	1267	30-35	East
Serj	09°537272	35°929192	1307	15-20	North
Serj	09°537156	35°929036	1307	15-20	North
Serj	09°537763	35°928789	1310	10-15	North
Serj	09°538750	35°930689	1287	20-25	North

2.2. Oil content and Fatty acid composition analysis

Seed oil extraction was held by a Soxhlet apparatus using 20g of seeds from each provenance. Petroleum ether 60% was used as solvent for seed oil. The Soxhlet apparatus was held for 3 hours and performed 15 cycles (Hu *et al*. 2017). The obtained extract was then dried under 40°C temperature to eliminate the residual Petroleum Ether. To keep the quality and avoid the possibility of oxidation, seed oil was conserved in a low-temperature chamber (4°C) in the dark for further analysis (Liang *et al*. 2019). Seed oil yield was calculated for the three provenances by dividing the weight of the oil by the wheight of the used seeds and multiplied by 100 to get a percentage. A precision balance was used to get the exact wheight.

Based on the method ISO 5509:2000, Seed oil was methylated twice, the first step was the pre-esterification to reduce the acid value to less than 1 mg KOH/g using H_2SO_4-CH_3OH, and the second step was trans-esterification using KOH-CH_3OH. Fatty acid methyl ester composition of the obtained seed oils was performed using Gas Chromatography coupled with Mass spectrometry (GC/MS). The used chromatograph is of type Agilent 7890 equipped with a capillary polar column of type HP-5MS (30m x 0.25mm; 0.25µ), a dividing injector programmed to 250°C, and a selective mass detector of type Agilent 5975C MSD. The oven temperature is programmed from 150 to 250°C with a reason of 4°C/min before it is held for 10 min at 250°C. Helium was used as a vector gas with a flow of 0.8ml/min. The samples were injected in split mode with a reason of 1µl each. The identification of different peaks was performed using Mass Spectrometry. The NIST W8N08 mass spectral library was used to identify the different components of the oil. For every analysis, three repetitions were performed.

2.3. Statistical analysis

Statistical analysis including ANOVA test and Tukey Post-Hoc test between fatty acids composition within the three provenances was performed using IBM SPSS 26 software to verify the importance of each fatty acid in the composition (p-value <0.05). ArcMap software was used for map presentation. R software ggplot2 package was used for the graphical representation of the results.

3. Results and discussion

The average seed oil content for the Tunisian Montpellier maple seeds was 7.89 % with a significant difference between provenances. The highest yield of 10.56 % was observed for Serj provenance followed by Zaghouan and Bargou seeds with a yield of 8.17 % and 4.97 % respectively (Table 2). These yield values are higher than other maple species. For example, seed oil yields range between 3.30 and 4.89 % for *Acer platanoides* and between 5.12 and 6.97% for *Acer pseudoplatanus* (Hovanet *et al*. 2015). A lower value of 4.97 % was observed for the Bargou mountain seeds. This low value could be explained by the importance of parthenocarpic seeds in the Bargou samples since maple oil is contained mainly in the germplasm but not in the parthenocarp (He *et al.* 2021).

The colour of the obtained Montpellier maple seed oil is green yellowish, it is the same colour range observed for *Acer platanoides* L. and *Acer pseudoplatanus* L. seed oil (Hovanet *et al*. 2015). It has a high viscosity and crystallises in temperatures lower than 5°C. The oil is composed of five unsaturated fatty acids [Oleic acid (C18:1), Linoleic acid (C18:2), Erucic acid (C22:1), cis-11-eicosenoic acid (C20:1), and gamma-Linolenic acid (C18:3)) and two saturated fatty acids (Palmitic acid (C16:0) and Stearic acid (C18:0)] (Table 2).

Table 2. Yield and composition of the seed oil of the Tunisian Montpellier for the three provenances including the ANOVA results for a p-value under 0.05, Oleic acid by Linoleic acid index, the C20-24 by C16-18 content, and the saturated and unsaturated fatty acids content in the oil.

Provenance	Fatty acid %							Oil content %	O/L	C20-24 / C16-18	Sum of Saturated fats %	Sum of unsaturated fats %
	C16:0	C18:3	C18:2	C18:1	C18:0	C20:1	C22:1					
Zaghouan	9.16 ±0.05	1.67 ±0.06	31.22 ±0.13	36.45 ±0.15	3.05 ±0.01	7.31 ±0.23	11.14 ±0.05	8.17	1.17	0.23	12.21 ±0.20	87.79 ±0.62
Bargou	7.80 ±0.05	1.49 ±0.05	27.01 ±0.12	40.96 ±0.19	3.03 ±0.01	7.39 ±0.50	12.32 ±0.02	4.96	1.52	0.25	10.83 ±0.06	89.17 ±0.98
Serj	8.02 ±0.06	1.70 ±0.05	28.95 ±0.18	39.43 ±0.24	3.39 ±0.01	6.95 ±0.22	11.57 ±0.22	10.56	1.36	0.23	11.41 ±0.28	88.59 ±0.25
Total mean	8.32 ±0.59	1.62 ±0.11	29.06 ±1.73	38.95 ±1.88	3.16 ±0.16	7.21 ±0.31	11.68 ±0.50	7.89	1.34	0.24	11.48 ±0.90	88.52 ±0.88
ANOVA	***	*	***	***	***	ns	***	***	*NC*	*NC*	*NC*	*NC*

C16:0: Palmitic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, C18:3: gamma-Linolenic, C20:1: cis-11-eicosenoic acid, C22:1: Erucic acid *NC*: Not calculated. O/L: Oleic-Linoleic ratio.; ***: Very highly significant, *: Significant and ns: non-Significant, p-value < 0.05%.

Tunisian Montpellier maple seed oil fatty acids composition showed a predominance of Oleic acid (C18:1) followed by Linoleic acid (C18:2) with a rate of 38.71±2.29 % and 28.88±2.09 % respectively. At a lesser level, Erucic acid (C22:1), Palmitic acid (C16:0), and cis-11-eicosenoic acid (C20:1) were found with rates

of 11.75±0.62, 8.39±0.74, and 7.43±0.28. Stearic acid (C18:0) and gamma-Linolenic acid (C18:3) were also identified with a rate lower than 5 % of the total seed oil composition (Figures 3 and 4).

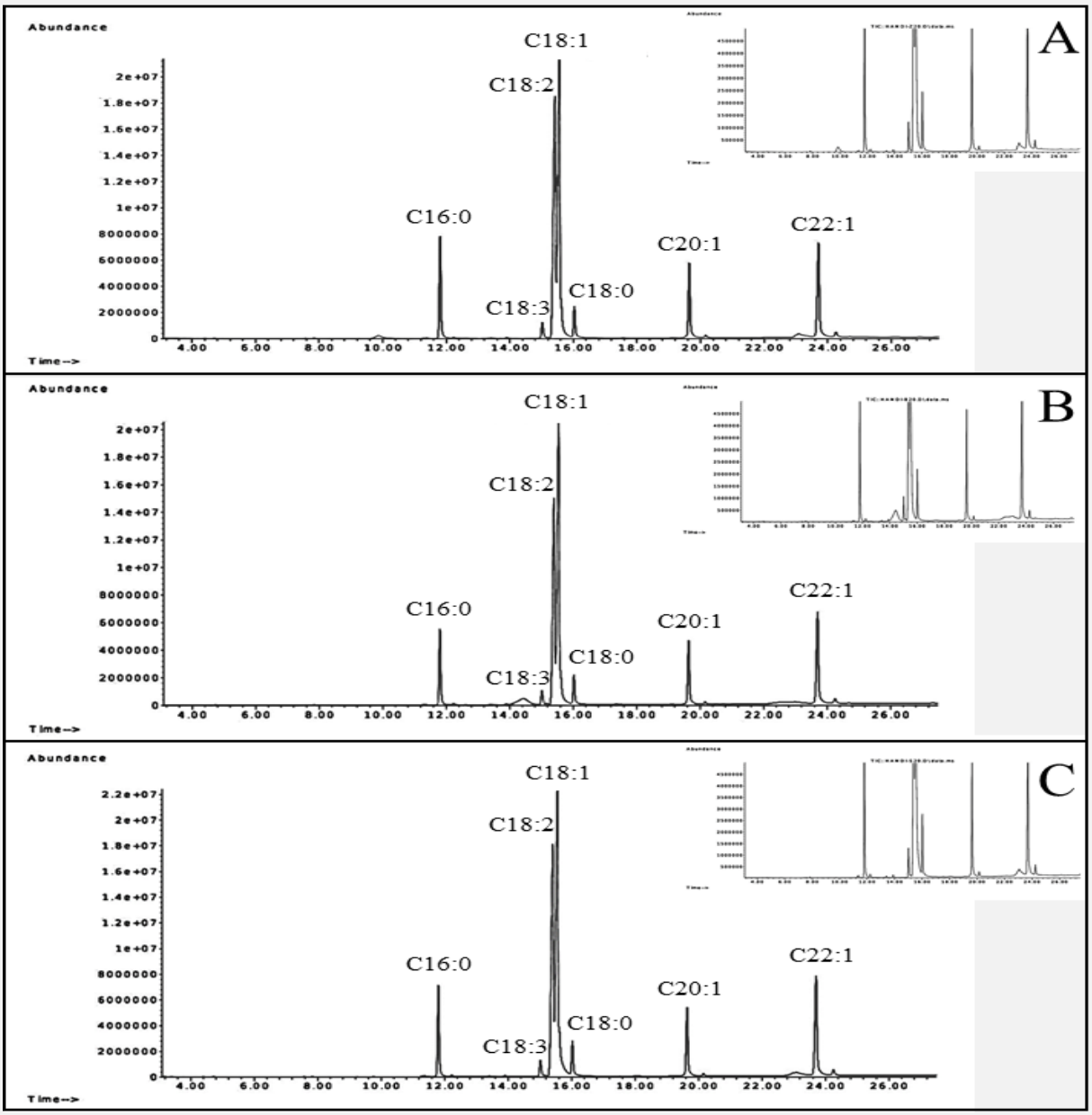

Figure 3. Chromatographs of the time of the appearance in minutes (X-axis) and the importance (Y-axis) of the different fatty acid peaks using the GC/MS for the seed oil samples of the Montpellier maple collected from A (Zaghouan), B (Bargou), and C (Serj) mountains in the Tunisian Dorsal. C16:0: Palmitic acid, C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, C18:3: gamma-Linolenic, C20:1: cis-11-eicosenoic acid, C22:1: Erucic acid. 11.81, 15.02, 15.41, 15.55, 16.03, 19.65, and 23.71 minutes were the retention times for each fatty acid respectively.

Considering the results shown in Table 2, and figure 4, a high significant difference between the three Montpellier maple provenances for the majority of fatty acids. Anova test coupled with Tukey Post-Hoc test showed an important variance between provenances and between fatty acids composition with only cis-11-eicosenoic acid (C20:1) content showing a non significant difference. The largest observed difference is remarcable between Zaghouan and Bargou seeds while Serj seed oil composition has an intermediate position. For the two major fatty acids, Bargou seeds have the highest Oleic acid (C18:1) content while Zaghouan seeds have the highest Linoleic acid (C18:2)).

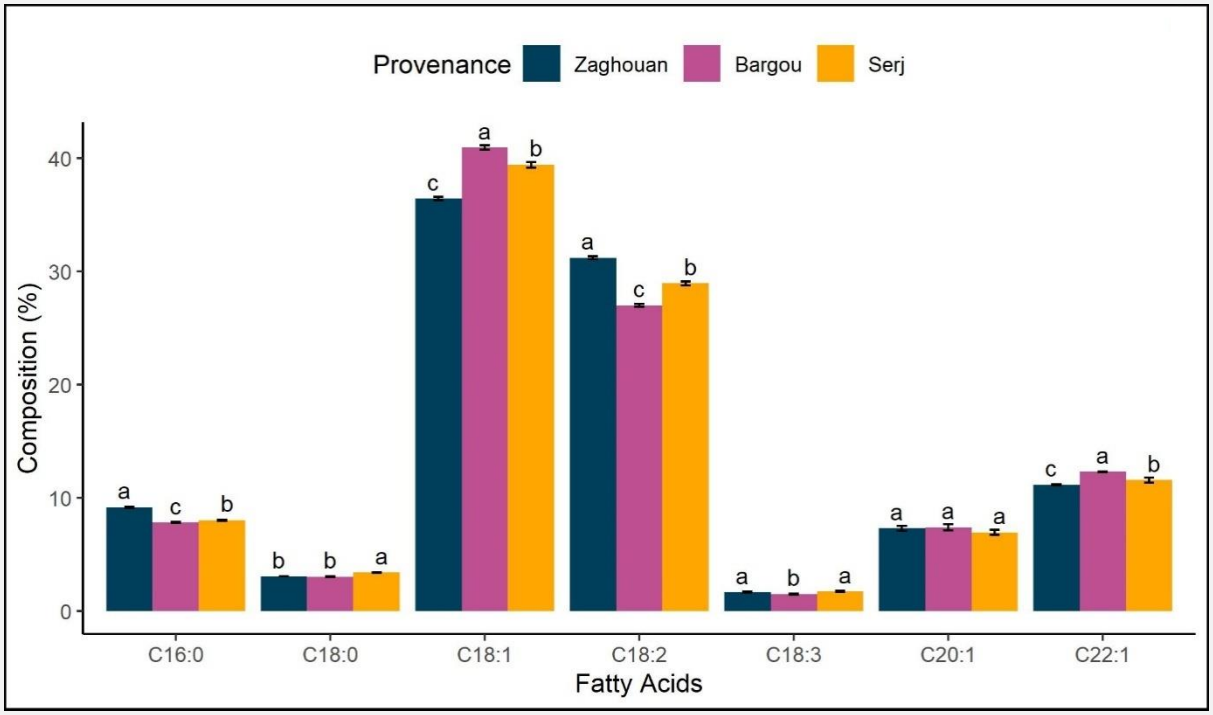

Figure 4. Composition of the Seed oil of Montpellier maple in Tunisia: Percentage of different fatty acids (C16:0: Palmitic acid,C18:0: Stearic acid, C18:1: Oleic acid, C18:2: Linoleic acid, C18:3: gamma-Linolenic, C20:1: cis-11-eicosenoic acid, C22:1: Erucic acid) abundance in the seed oil for every provenance.

By comparing the composition of seed oil of the Tunisian Montpellier maple with other maple species, many differences were revealed. He *et al*. (2021), Su *et al*. (2021) and Hovanet *et al*. (2015) reported in their work that focused on comparing the seed oil composition of several maple species that the major fatty acid observed in the oil was linoleic acid followed by oleic acid which is the major fatty acid observed for our case. Gamma-linolenic acid which ranges for the Montpellier maple seed oil between 1.57 % (Bargou) and 1.73 % (Serj) was found also in low rates in *Acer pseudoplatanus* and *Acer platanoides* and reported to be beneficial for protecting the body from cardiovascular diseases (Hovanet *et al*. 2015). Nervonic acid, found in 46 maple species seed oils wasn't identified in the Montpellier maple seed oil.

4. Conclusion

Montpellier maple seed oil from Tunisia presents a satisfying source of mono and polyunsaturated fatty acids which is an important finding regarding the potential use of this oil. With a yield that could reach more than 10 % of the seed mass and composition that encloses around 1.6 % of gamma-linoleic acid. Seed oil composition varies significantly between provenances of the seeds. This study shows a promising potential use of the Montpellier maple seeds in the future. Further studies are to be done to investigate the effect of environmental conditions on seed oil.

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5. References

Bi W, Gao Y, Shen J, He C, Liu H, Peng Y, Zhang C, Xiao P (2016) Traditional uses, phytochemistry, and pharmacology of the genus *Acer* (maple): A review. Journal of Ethnopharmacology189 : 31–60. https://doi.org/10.1016/j.jep.2016.04.021

He X, Li D-Z, Tian B (2021) Diversity in seed oil content and fatty acid composition in *Acer* species with potential as sources of nervonic acid. Plant Diversity 43(1): 86–92.https://doi.org/10.1016/j.pld.2020.10.003

Hovanet M-V, Dociu N, Dinu M, Ancuceanu R, Morosan E, Oprea E (2015) A Comparative Physico-chemical Analysis of *Acer platanoides* and *Acer pseudoplatanus* Seed Oils. Revista de Chimie - Bucharest 66(7): 987–991.

Hu P, Xu XB, Yu LL (2017) Physicochemical properties of *Acer truncatum*Seed Oil extracted using supercritical carbon dioxide. Journal of the American Oil Chemists' Society 94: 779-786. http://doi.org/10.1007/s11746-017-2983-1

Liang Q, Wang W W, Yuan F L, Liu X, Li D L, Yang K Q (2019) Characterization of yuanbaofeng (*Acer truncatum*Bunge) samaras: Oil, fatty acid, and phytosterol content. Industrial Crops and Products 135: 344-351. http://dx.doi.org/10.1016/j.indcrop.2019.04.032.

Qian Q, Mei-Jing Y, Fen-Fen S, Hong-Jian R, Kai A, Zhen F, Lin Z, Zhong-Kui S (2017) Variability of seed oil content and fatty acid composition in Shantung maple (*Acer truncatum*Bunge) germplasm for optimal biodiesel production. African Journal of Biotechnology 16(48): 2232–2241. https://doi.org/10.5897/AJB2017.16250

Qiao Q, Xue W, Feng Z (2018) Variability of seed oil content, fatty acid composition, and nervonic acid content in *Acer truncatum*, native to 14 regions of China. Grasas y Aceites 69(4): 274. https://doi.org/10.3989/gya.0465181

Qiao Q, Wang X, Ren H, An K, Feng Z, Cheng T, Sun Z (2019) Oil Content and Nervonic Acid Content of *Acer truncatum* Seeds from 14 Regions in China. Horticultural Plant Journal 5(1): 24–30. https://doi.org/10.1016/j.hpj.2018.11.001

Su Y, Xue W, Jia B, Chu J, Wang L, Feng Z, YuX (2021) *Acer triflorum*: A maple species that serves as a resource for nervonic acid and that has an ultralow erucic acid content. Food Science and Technology 41(suppl 2): 633–636. https://doi.org/10.1590/fst.30520

Van GelderenD M, OterdoomH J, De Jong P C (1995) Maples of the world. Timber Press, USA.