Analysis of Phenolic Compounds in Moroccan Olive Oils by HPLC

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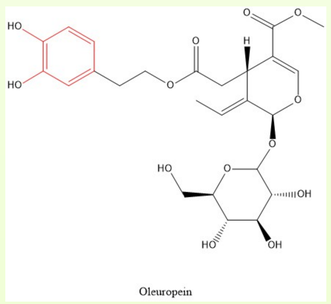
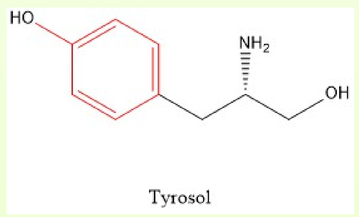
Abstract: Five extra virgin olive oils collected from farms in Morocco were analyzed for phenolic compounds. To complement these data, the chemical composition of the olive oils were analyzed by high-performance liquid chromatography. Several phenolic compounds were identified and quantified in the olive oils. Specifically, levels of oleuropein and luteolin were quantified in the low mg/kg range, and qualitative comparisons were made between the oil samples for tyrosol, vanillic acid, and apigenin. To compare extraction efficiency of different extraction solvents, methanol and DMF were used.

Introduction

- The purpose of investigating Moroccan olive oils is to correlate chemical composition with anti-cancer activity.
- In recent studies on colon cancer and breast cancer, a phenolic compound in extra virgin olive oil was found to induce cell death in human cancer cell lines(1,3).
- The main phenolic compound in green olives is oleuropein while the main one in black olives is tyrosol(1).
- In this study, HPLC was used to analyze various Moroccan olive oils for their phenolic compounds.
- This study will be used to develop a lab for undergraduate students in analytical chemistry.
- To compare the efficiency of different extraction solvents, DMF and methanol were used (2).

Figure 1. The chemical structures and formulas for the phenolic compounds oleuropein and tyrosol. The phenolic part of the molecule is highlighted in red.

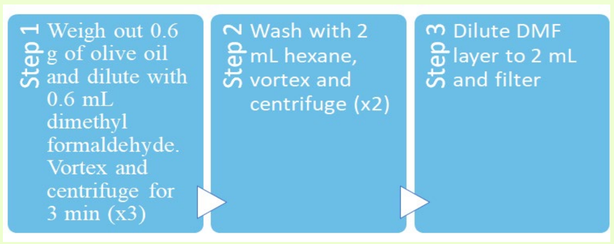




Oleuropein: $C_{25}H_{32}O_{13}$

Tyrosol: $C_8H_{10}O_2$

Figure 2. Extraction procedure



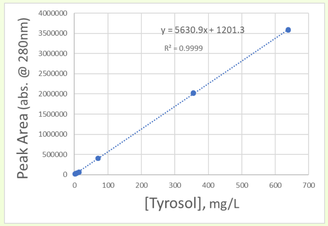
Experimental

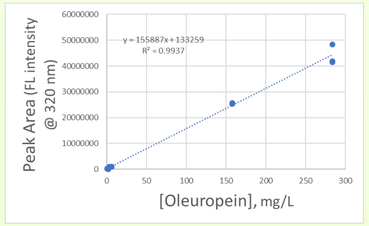
HPLC conditions

- Mobile phase A: 50 mM H3PO4
- Mobile phase B: acetonitrile
- Gradient: 10%B for 2 min, increase to 90% B to 18 min, hold 90% B for 2 min
- Column oven: 40 ºC
- Column: 150 x 4.6 mm, 5 µm particle, C18
- Detector: PDA at 220, 280, or 330 nm, and fluorescence (ex. 280 nm, em. 320 nm)

Olive oil samples were collected from 'Agropole Olivier' Meknes. The varieties of olive oil studied were 1: Moroccan Picholine; 2: Koroneiki; 3: Arbequine; 4: Picual; 5:Arbosana. All varieties are Extra Virgin on the Physicochemical and Sensory Plan. Extraction solvents were HPLC grade DMF and methanol.

Figure 3: Calibration curves representing the phenolic compounds tyrosol and oleuropein respectively.





Results and Discussion

Table 1. Quantitative analysis of four phenolic compounds identified in Moroccan olive oils(3). Average and ± standard deviation are given for three replicate samples.

	Luteolin	Oleuropein	Vanillic acid	Tyrosol
	average conc. (mg/kg oil)	average conc. (mg/kg oil)	average conc. (mg/kg oil)	average conc. (mg/kg oil)
Moroccan Picholine	2.06 ± 0.04	5.6 ± 0.4	1.9 ± 0.3	29.4 ± 0.6
Koroneiki	1.02 ± 0.02	5.8 ± 0.6	6 ± 2	14 ± 1
Arbequin	4.1 ± 0.1	3.9 ± 0.2	1.5 ± 0.3	22 ± 3
Picual	2 ± 1	1.5 ± 0.8	2 ± 1	12 ± 6
Arbosana	4.0 ± 0.3	3.0 ± 0.5	4.5 ± 0.1	13.8 ± 0.4

Table 2. Summary of quantitative analysis of four phenolic compound identified in the Moroccan olive oil, Arbequin(2). The extraction for each solvent was repeated twice.

	Oleuropein	Luteolin	Apigenin	Sinapic Acid
	Conc. (mg/kg oil)	Conc. (mg/kg oil)	Conc. (mg/kg oil)	Conc. (mg/kg oil)
DMF 1	5.546	0.701	0.985	0.995
DMF 2	5.832	0.699	1.043	1.171
MeOH 1	24.334	0.138	0.305	1.170
MeOH 2	25.188	0.100	0.385	0.912

Conclusions

- Six phenolic compounds in Moroccan olive oil were identified and quantified.
- The results in Table 1 indicate that tyrosol is the main contributor to the phenolic compounds in Moroccan olive oils, and that there are significant variations in the relative amounts of the other compounds that were included in the study.
- The results in Table 2 indicate methanol was a better solvent for oleuropein; however, DMF provided better extraction efficiency for luteolin and apigenin.
- The extraction efficiencies were different due to solubility variation of compounds.
- Both DMF and methanol provided extraction efficiency for sinapic acid.
- Future studies include methanol/ DMF comparison studies to establish the extraction efficiency, comparing all Moroccan oils, comparing other extra virgin olive oils to Moroccan, and correlating the data to cancer studies.

References

1. R. Concepión, M. Brenes, K. Yousfi, P. García, A. García, and A. Garrido. Effect of Cultivar and Processing Method on the Contents of Polyphenols in Table Olives. Journal of Agricultural and Food Chemistry, 2004, 52: 479-484.
2. Meyls H, Porter S. (2020). Analysis of Phenolic Compounds in Moroccan olive oils by HPLC. INCITE undergraduate journal of scholarship.
3. El Hilali H, El Hilali F, Porter S, Ghali SA, Meyls HM, Ouazzani N, Laziri F, Barber A. (2020) Moroccan olive oil varieties reduce reactive oxygen species and cell viability in human cervical cancer cells. Mediterranean Journal of Nutrition and Metabolism, In Press.