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Composition and Geographical Variations of Moroccan Date Seed Oils (*Phoenix dactylifera* L.) – A Targeted and Untargeted Metabolomic approach

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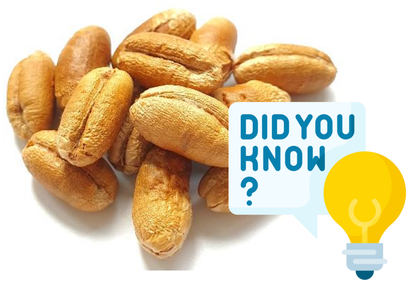
Introduction



In Morocco's arid landscapes, the date palm (*Phoenix dactylifera* L.) reigns supreme in arboriculture.
Astonishingly, over the past decade, Moroccan date palm production has surged from 101,351 tonnes in 2010 to 150,301 tonnes in 2021, leaving behind a surplus of 15,000 tonnes of seeds often dismissed as waste.
But here's our visionary proposal: We're about to reveal the extraordinary potential within these overlooked seeds. Join us as we unlock their hidden value through the extraction of valuable oil, turning waste into a precious resource.
Samples from three palm groves were investigated, analyzing their fatty acids, TAGs, tocochromanols, and phytosterols composition. Next, we probed the polar DSO fraction using advanced UHPLC-ESI-QTOF-MS to uncover geographic markers. Applying chemometrics, we classified date seed oil (DSO) samples by origin and, in time, identify the elusive key markers.

Material and Methods

Material

- Twenty-six date seed samples from three Moroccan regions: Allougoum (Ag), Alnif (Al), and Errachidia (Er) with coordinates Er (31°54'49"N 04°23'18"W), Al(31°07'07"N 05°09'52"W) and, Ag (30°16'31"N 06°49'34"W).

Methods for the chemical composition

- Fatty acid, triacyclglycerols and phytosterols were analyzed using gas chromatography (GC-FID) according to DGF C-VI 11d (19), DGF F-III 1 (98), and DGF C-VI 14(08), while tocochromanols were analyzed on a normal phase HPLC (DGF F-II 4a (00)).

Methods for the secondary metabolomic profiling

- 1 g date seed oil + 2 mL extraction solvent (80:20 methanol/H_2O, *v/v*) → Shake, Collect Supernatant → Evaporate, Reconstitute → Analyze using UHPLC-ESI-QTOF-MS (Negative bbCID, *m/z* 50–1,000).
- For statistical approaches MS-DIAL, Excel and Metaboanalyst were used. Normalization of untargated data with QCs carried along the analysis process.
- Compound identification using online metabolite databases with molecular formula confirmation (5 ppm mass accuracy and <50 mSigma isotope distribution)

Results and Discussion

1- Chemical composition: Targeted approach

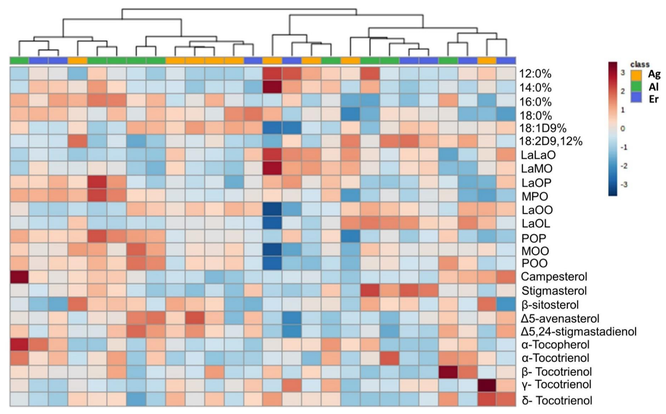


Fig. 1: Main fatty acids, TAGs, tocochromanols, and phytosterols in DSO

- Composition Heatmap: Shows DSO chemical variations by geographical origin.
- Dominant Fatty Acids: Oleic acid (40.8–50.2%), lauric acid (14.0–24.2%), myristic acid (9.0–12.6%), palmitic acid (9.0–11.6%), linoleic acid (7.1–10.7%), and stearic acid (2.4–4.8%).
- Tocochromanol Content: Significant levels (424–760 mg/kg) with *α*-tocotrienol prevalence.
- Main Phytosterols: *β*-sitosterol (2168–3079 mg/kg), Δ5-avenasterol (325–1101 mg/kg), campesterol (307–672 mg/kg); others < 200 mg/kg.

2-Metabolomic profiling: Untargeted approach

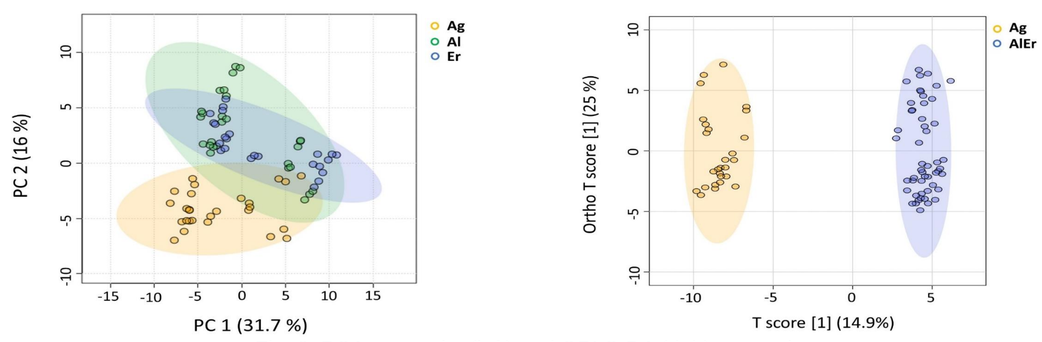


Fig. 2: PCA score plot (left) and OPLS-DA (righ) score plot

- PCA Score Plot (Fig.2, left): The climatic uniqueness of Ag, located further south, at a lower altitude, and closer to the Atlantic Ocean, distinctly separates its samples from Al and Er regions.
- OPLS-DA Comparison (Fig.2, right): We successfully validated our OPLS-DA model (Q2 and R2Y > 0.5) when comparing the Ag region against the composite group "AlEr" (Alnif and Errachidia samples).

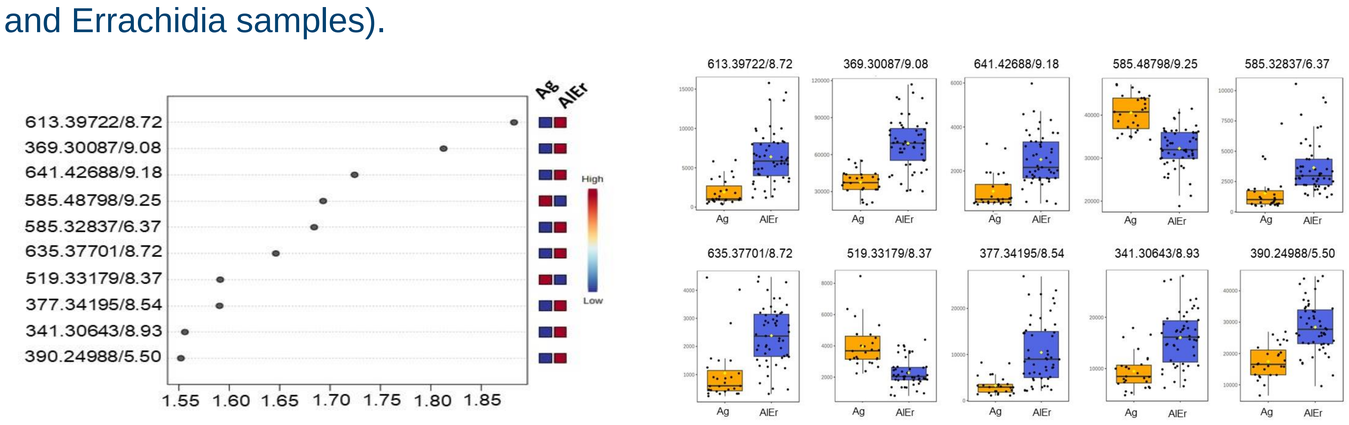


Fig. 3: VIP scores and Box plot variations for the top 10 features

- VIP scores (Fig.3): We ranked features (mz/Rt) based on their ability to discriminate geographical origins. Our work to identify these markers is still ongoing.

Conclusion

- DSO presents a rich source of essential fatty acids, including dominant oleic and lauric acids, coupled with substantial tocochromanol and phytosterol content.
- Our study revealed that DSO samples can be distinguished by their geographical origin, with the Ag region standing out due to its unique climatic conditions.
- The OPLS-DA model successfully validated these geographical differences (Q2 and R2Y > 0.5), offering a robust tool for classification.
- Ongoing Exploration: Our research sets the stage for ongoing efforts to identify specific geographic markers within DSO, further unlocking the potential of this valuable resource

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