IECM 2025 Conference
The 4th International Electronic Conference on Metabolomics
13-15 October 2025 | Online
MDPI

Metabolomic Characterization of Portuguese *Ceratonia siliqua* Varieties

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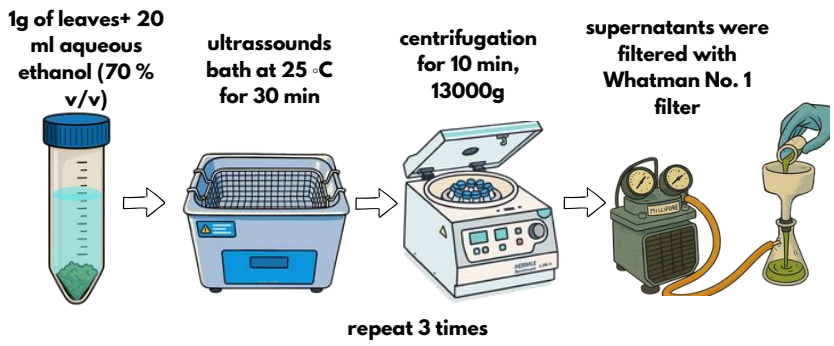
INTRODUCTION & OBJECTIVE

The carob tree (*Ceratonia siliqua* L.) is a Mediterranean species highly tolerant to salinity and drought stress, making it well-suited to regions with degraded or overexploited soils, and supporting ecosystem recovery. It is also known for its resistance to pests and diseases due to the synthesis and accumulation of protective compounds. Data from leaf extracts reveal the accumulation of metabolites with antioxidant and antimicrobial properties, highlighting the potential of these extracts. This study focused on the detailed metabolomic profiling of ten Portuguese varieties.

METHODOLOGY

Plant Material: Mature leaves from 10 portuguese carob varieties were collected from field-grown plants located at the national carob field collection in Tavira (Portugal). Samples were randomly collected from three trees per variety.

Metabolome extraction: performed through an exhaustive extraction protocol.



UHPLC-DAD: Untargeted analysis was performed using chromatographic separation with a high-performance liquid chromatograph (HPLC) equipped with a diode-array detector (DAD). MassLynx software was used for data processing (see example in Fig.1).

A preliminary assay was conducted to assess variability among biological replicates within each variety. As no significant differences were observed, a single sample per variety was considered.

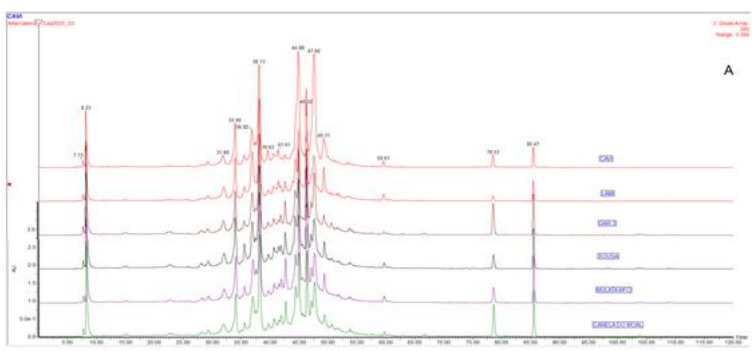


Fig. 1: Representative chromatographic profile of 6 samples at 280 nm

RESULTS AND DISCUSSION

- **No significant differences** were observed **between biological replicates** within each variety.
- The metabolome profile revealed **differences across varieties** (Table 1 shows the most different varieties).

Table 1: Retention time (Rt) and area (A) of the main peaks detected at 280 nm of the three portuguese varieties showing the representative profiles identified.

SOUSA		CANELA DO MOAL		GALHOSA		COMPOUND
Rt	A	Rt	A	Rt	A	
ND	ND	ND	ND	8,43	239408	1
22,71	63795	22,78	56434	ND	ND	2
28,12	67362	28,17	55738	ND	ND	3
29,29	82080	29,32	65688	29,75	49545	4
32,03	266396	31,95	252891	33,02	72312	5
34,01	609278	34,04	497789	34,29	342641	6
35,56	174366	35,59	71974	ND	ND	7
36,97	534300	36,99	512109	ND	ND	8
38,18	1441616	38,21	1218171	38,38	1147754	9
39,68	68079	39,71	50228	ND	ND	10
40,65	166461	40,69	70281	40,85	79603	11
41,45	106300	41,5	31832	ND	ND	12
41,88	102885	41,91	79602	ND	ND	13
42,64	199320	42,68	159225	42,78	229865	14
44,35	438269	44,39	418680	ND	ND	15
44,95	655354	44,98	582187	45,08	488900	16
46,33	641220	46,37	506776	ND	ND	17
47,1	141293	47,13	146911	47,23	113112	18
47,64	428128	47,67	460829	ND	ND	19
49,34	120952	49,37	87198	49,43	72085	20
78,58	134879	78,63	315147	78,63	354872	21
85,5	293205	85,53	338442	85,53	404843	22

ND: Not detected

- The results suggest the presence of **over 20 compounds**, primarily belonging to the phenolic class, particularly **flavonoids and hydrolyzable tannins**, as well as **fatty acid derivatives** (Table 1).
- By comparison with Serio et al. (2024), several metabolites were tentatively identified, including Siliquapyranone acid form (n° 11), Siliquapyranone (n° 14), Myricetin 3-O-hexoside (n° 18), Myricetin 3-O-pentoside (n° 20), 1,2,3,6-Tetragalloyl glucose (n° 22), Quercetin O-deoxyhexoside (n° 28), Kaempferol O-deoxyhexoside (n° 30), Trihydroxy-octadecadienoic acid (n° 38) and Trihydroxy-octadecenoic acid (n° 39).
- The variety '**Sousa**' appears with the **most complex metabolome.**

CONCLUSION

The metabolomic profile revealed differences among varieties, which may indicate variations in plant responses to biotic and abiotic stresses. Further research will focus on the role of the identified compounds in enhancing plant defense mechanisms.

Bibliography: Serio, S., Santoro, V., Celano, R., Fiore, D., Proto, M. C., Corbo, F., Clodoveo, M. L., Tardugno, R., Piccinelli, A. L., & Rastrelli, L. (2024). Carob (Ceratonia siliqua) leaves: A comprehensive analysis of bioactive profile and health-promoting potential of an untapped resource. Food Chemistry, 468, 142392. https://doi.org/10.1016/j.foodchem.2024.142392

ACKNOWLEDGMENTS

This work is funded by national funds through FCT (Foundation for Science and Technology) under the Project UIDB/05183, and through the project ALCAROB (ref. PD24-00024), funded through "la Caixa" Foundation's Promove Program, in partnership with FCT (Projetos I&D Mobilizadores 2024). The authors would like to thank MED (https://doi.org/10.54499/UIDB/05183/2020; https://doi.org/10.54499/UIDP/05183/2020), and CHANGE (https://doi.org/10.54499/LA/P/0121/2020).

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