

Origin and identification of valuable components in the extra virgin olive oil proteome.

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INTRODUCTION AND MOTIVATION

Early analyses have detected proteins as minor components of extra virgin olive oil (EVOO); however, precise identification of the olive oil proteome has not been yet accomplished.

An insufficient quantity and/or quality of the starting material used and the lack of transcriptomic and genomic records for this species until 2015 might explain this fact.

In the present work, all these restrictions have been overcome, with the objective of identifying the main protein constituents of EVOO.

MATERIALS AND METHODS

• Materials

Freshly bottled EVOO (cv. Picual) of the Protected Designation of Origin "Montes de Granada" was used.

• *In situ* digestion of extra virgin olive oil proteins

Extra virgin olive oil proteins were extracted, electrophoresed on 1-D polyacrylamide gels and stained as described in [1]. The gel lane containing the EVOO proteins was systematically cut (Fig 1A). *In situ* digestion was performed using the MassPREP Station. Gel slices were washed with 25 mM NH₄HCO₃: acetonitrile (ACN) (1:1, v/v). The Cys residues were reduced by dithiothreitol (DTT) and alkylated by iodoacetamide. Proteins were digested overnight at room temperature with modified porcine trypsin. Finally, a double extraction was performed with ACN in formic acid, and then 100% ACN.

• Database search and protein identification

Protein identification was performed using the Mascot Server v2.2.07 against an ad hoc-generated database composed of protein entries retrieved from the olive genome and transcriptome records. Peptide identifications extracted from Mascot result files were validated at a final peptide FDR of 1%. Peptide matches were also manually validated if their score was close to the Mascot homology threshold for a given Mascot p value.

RESULTS AND DISCUSSION

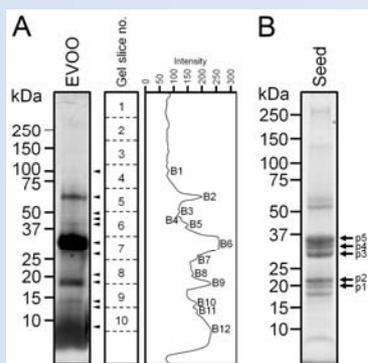


Figure 1. (A) Flamingo-stained polyacrylamide gel showing protein 1-D profile of extra virgin olive oil (EVOO). The densitometric profile of EVOO protein extracts is also plotted. This gel was systematically cut into slices of ~1 cm width each from the top (slice S1) to the bottom (slice S10) for further mass spectrometry analysis. (B) Flamingo-stained polyacrylamide gel showing protein 1-D profile of olive seeds. Protein markers are displayed on the left of each figure.

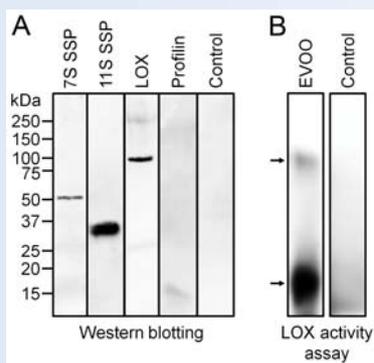


Figure 2. (A) Immunodetection of 7S and 11S globulin, lipoxigenase (LOX) and profilin (Ole e 2) proteins in EVOO total protein extracts by Western blotting. A negative control was performed by omitting the primary Ab. Protein markers are displayed on the left. (B) *In-gel* assay of lipoxigenase activity (arrows) from EVOO total protein extracts. A negative control was performed by excluding the substrate from the reaction mixture.

CONCLUSIONS

- The extra virgin olive oil (EVOO) proteome comprises at least 40 unique proteins. This proteomic dataset provides for the first time a comprehensive list of olive fruit and fungal proteins present in this oil.
- Seed storage proteins of globulin-type are the most abundant proteins in EVOO
- EVOO also contains an active 13S-lipoxygenase and several potential allergenic proteins
- The olive seed is the main source of proteins in EVOO

- Starting from these proteomic data, the impact of different protein components present in EVOO on its stability and quality could be further studied.
- The effect of the olive oil proteins on human health and their potential use as functional food components could be also evaluated.
- This dataset provides a resource for use in further functional comparisons across other vegetable oils.
- Expanding the proteomic resources to non-model but agronomically relevant species will allow further comparative inter-species studies.

REFERENCES

[1] Castro AJ, Lima-Cabello E, Alché JD. Identification of seed storage proteins as the major protein constituents of the extra virgin olive oil proteome. *Food Chemistry: X* Volume 7, 30 September 2020, 100099.

[2] Castro AJ, Lima-Cabello E, Alché JD. A comprehensive dataset of the extra virgin olive oil (EVOO) proteome. *Data in Brief* (2021) Volume 35, April 2021, 106822.

[1] [2]



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