MATERIALS AND METHODS

• The olive seed is the main source of proteins in EVOO.

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 diithiothreitol (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.

REFERENCES


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