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Proceeding Paper

Evaluation of Technologies for the Co-Extraction of Phenolic Compounds and Proteinaceous Material from Olive-Derived Biomasses †

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Abstract: The current interest in using olive biophenols to promote functional ingredients and antioxidant additives is increasing. These compounds can be obtained from olive fruit and olive-derived biomasses using different technologies. However, other components can be co-extracted. Therefore, the main objective of this study was to evaluate the effect on protein solubilization of several extraction technologies, which were applied to obtain olive biophenols from olive-derived biomasses. For this purpose, conventional (Soxhlet and water bath) and non-conventional technologies (ultrasound and microwave) were evaluated. The total phenolic content was measured using the Folin and Ciocalteu method and the protein content was measured using the Dumas combustion method. The phenolic profile and the hydroxytyrosol content were also determined. Overall, the highest total phenolic content was obtained using the Soxhlet method, while the microwave-assisted extraction at 100 °C led to the highest protein solubilization (closer to 60%) using water.

Keywords: green extraction; microwave-assisted extraction; olive-derived biomass; protein solubilization

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1. Introduction

The current interest in using phenolic compounds to promote functional ingredients and antioxidant additives is increasing. This is also the case of olive biophenols obtained from olive fruits and by-products due to their high antioxidant activity and generally no adverse health effects have been shown. Besides the antioxidant activity in food systems, their biological properties mean they can potentially be used as multipurpose additives [1].

To recover olive biophenols, conventional technologies such as maceration and Soxhlet extraction have been applied, as well as new green trends include the use of ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction, and pressurized liquid extraction [2–6]. The latter technologies can shorten the extraction time, reduce solvent consumption, and present a lower energy cost; thus being more respectful to the environment.

In order to develop a bio-based economy for an efficient conversion of these biomasses, proteinaceous material can be another bioproduct obtained from olive byproducts [2] with potential to be applied in different sectors [7,8]. Some of the latter extraction technologies have also been applied to recover intact and partially hydrolyzed proteins from agri-food bioresources, generally using water, alkaline solutions, and buffers, obtaining different recoveries [7,9]. Therefore, the main objective of this study was to evaluate the effect of some extraction technologies to co-extract olive biophenols and protein.

2. Material and Methods

2.1. Samples and Reagents

Samples were obtained from different industries located in Jaén, Spain ('Spuny SA', 'SCA Unión Oleícola Cambil' and 'Peláez Renovables'). These biomasses were milled with an Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany).

The following reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): Folin and Ciocalteu′s phenol reagent, sodium carbonate, sodium hydroxide and gallic acid. Ethanol was procured from AppliChem (Barcelona, Spain). Hydroxytyrosol was obtained from Extrasynthese (Genay, France).

2.2. Extraction Technologies

Soxhlet extraction with a solid:solvent ratio of around 2.9:100 (*w/v*) was performed sequentially using water and ethanol for 24 h each step. The rest of methodologies were previously optimized in the laboratory to maximize the extraction of phenolic compounds from olive-derived biomasses. Aqueous water extraction using a water bath with agitation was performed at 85 °C for 90 min at 10% solid loading (*w/v*) [10]. UAE (probe-type) was performed using Branson Ultrasonics Corporation device (Danbury, CT, USA). The amplitude was 80%, the extraction time was 16 min, and the solid loading was 12% (*w/v*). Finally, MAE was performed at 100 °C for 16 min and the solid loading was 12% (*w/v*) in an Anton Paar microwave (Monowave 400, Graz, Austria). For both, UAE and MAE, water was used as solvent.

After extraction, the samples were vacuum filtered to separate the extract from the extracted solid fraction. The latter was dried and weighed to determine the solid recovery and the protein content.

2.3. Determination of the Total Phenolic Content and Phenolic Profile

The TPC was determined in the filtered extracts using the Folin and Ciocalteu method according to [10] using gallic acid as standard and a Bio-Rad iMarkTM microplate reader (Hercules, CA, USA) was applied.

The phenolic profile was determined by reversed phase (RP)-high-performance liquid chromatography (HPLC) with a diode array detector (Shimadzu Prominence UFLC system) (Kyoto, Japan) according to Contreras et al. [3]. The hydroxytyrosol content was determined at 280 nm using the external standard method.

2.4. Determination of the Protein Content

The protein content was determined through the nitrogen content, which was measured using an elemental analyzer TruSpec Micro (Leco, St. Joseph, MI, USA), and applying a conversion factor of 6.25.

3. Results and Discussion

3.1. Protein Content of Olive Biomasses

Figure 1 shows the protein content of raw olive-derived biomasses. The protein content in olive mill leaves and in the exhausted olive pomace (EOP) was higher than that of olive pulp and intermediate compared to that of other agri-food residues [7,9]. Thus, the former residues are interesting bioresources to obtain proteins; especially EOP.

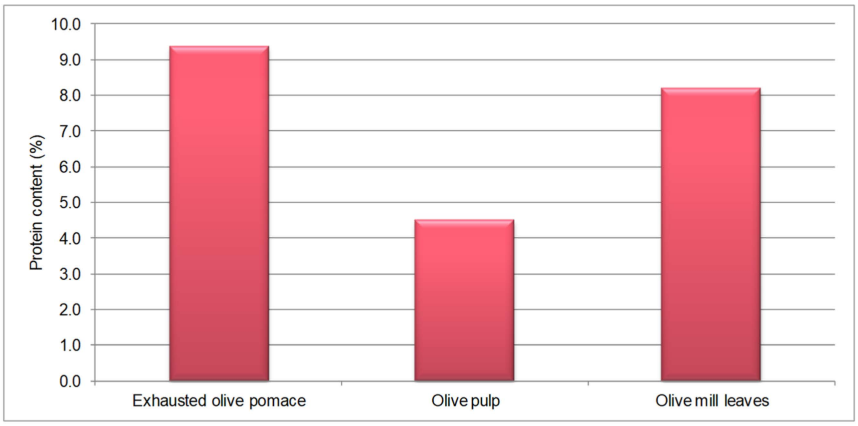

Figure 1. Crude protein content in olive-derived biomasses, adapted from Contreras et al. [3].

3.2. Total Phenolic Content of the Extracts

Exhausted olive pomace (EOP) presented the highest TPC value using conventional technologies; particularly using Soxhlet extraction when the aqueous extract was determined (4.5 g/100 g biomass). However, UAE and MAE reached up to 85% recovery values compared to Soxhlet extraction in a short time.

The phenolic profiles were qualitatively similar and the chromatographic peak corresponding to hydroxytyrosol was the major one. Its content was of 0.6 g/100 biomass in all the extracts. As an example, Figure 2 shows the phenolic profile of the extract obtained by MAE.

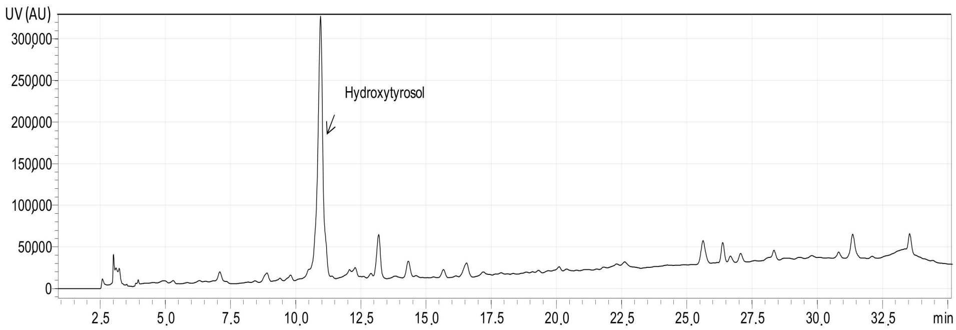

Figure 2. Chromatogram (280 nm) of the aqueous extract of exhausted olive pomace obtained using microwave.

3.3. Protein Solubilization

EOP was selected for further study. Figure 3 depicts the protein solubilization, taking into account the initial protein in the raw biomasses and the solid recovery after extraction. All the extraction methodologies seem to provoke the solubilization of a part of the protein content of EOP in the aqueous extracts. The highest protein solubilization was reached using MAE.

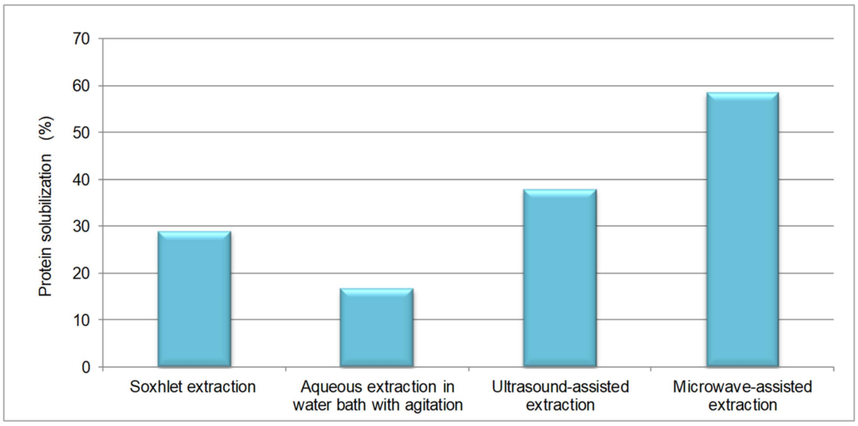

Figure 3. Protein solubilization from exhausted olive pomace after applying different extraction technologies.

4. Conclusions

The present results showed that the technologies applied to extract phenolic compounds can provoke the co-extraction of proteins, as is the case with EOP. MAE is a green method, considering that water was used as extractive agent and a shorter time was applied, which can be applied to co-extract both phenolic compounds, including hydroxytyrosol, and protein from EOP.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/Foods2021-10968/s1, Poster: Evaluation of Technologies for the Co-Extraction of Phenolic Compounds and Proteinaceous Material from Olive-Derived Biomasses.

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