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Valorization of olive pomace: bioactive compounds and antioxidant properties

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ABSTRACT

Olive pomace (OP), the major by-product of the olive oil processing, contains high levels of bioactive compounds. This feature anticipates new opportunities to recover functional ingredients for further applications, namely for cosmetics and food industry.

OP from a PDO olive oil region (Trás-Os-Montes, Portugal) was obtained to perform this study. A fraction of the raw OP was freeze-dried and milled. A solid-liquid extraction was carried out to obtain the lipidic fraction. Vitamin E and fatty acids profile were analysed by HPLC/DAD/FLD and GC-FID, respectively. Total phenolics (TP) and antioxidant activity (DPPH• inhibition and FRAP) were assessed for both OP fractions: raw, and freeze-dried and milled.

Regarding total vitamin E content, levels of 0.15 mg/g of oil were found. Oleic acid was the predominant fatty acid, followed by palmitic, linoleic, and stearic acids. Richer antioxidant extracts were obtained from the freeze-dried and milled OP samples. Nevertheless, both OP fractions revealed to be natural rich sources of bioactive compounds.

1. INTRODUCTION

Global olive oil production has doubled in the last 20 years with a positive socio-economic impact in Europe, particularly in the Mediterranean countries. As the production of olive oil is increasing, olive oil agro-industry generates a large amount of by-products such as OP [1].

Consumers are demanding for innovative and healthier new ingredients. In food industry, natural chemical compounds, as OP antioxidants, can have several applications, for instance, to improve the nutritional profile or stability of foodstuffs. Indeed, OP phenolic extracts have been studied to enrich OP oil, maize, soy, sunflower, olive, and rapeseed oils, as well as virgin olive oil [2].

Usually, OP is used to the production of edible OP oil. There is a well-established technology and food legislation that allows the recovery and processing of the remaining OP oil in refineries. In this process, OP is storage and dried. Then, it is extracted using chemical solvents. Defatted OP is the last remaining product of this process [3].

This work aimed to test the best processing method to accomplish the challenging goal of finding eco-friendly procedures to recover phenolic compounds from the defatted OP.

2. MATERIAL AND METHODS

2.1. Samples collection and preparation

OP was obtained from an olive oil production unit in the PDO olive oil region Trás-Os-Montes (Portugal). The OP was divided into two fractions: one analysed directly **(A)** and the other was freeze-dried and milled into powder before analysis **(B)**. Both fractions were defatted by solid-liquid extraction (Soxhlet; 4 h). Afterwards, aqueous extracts were obtained from the defatted OP (Figure 1) (100% deionised water; 60 min; 40 °C; 600 rpm).

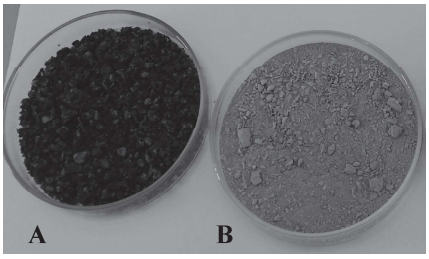

Figure 1. OP raw (A) and OP freeze-dried, milled (B).

2.2. Lipidic fraction analysis

The vitamin E profile was determined by normal phase HPLC/DAD/FLD (Jasco, Tokyo, Japan). The quantification was performed based on the internal standard method. The compounds were identified accordingly on their UV/vis spectra and by the comparison of their retention time with those of the authentic standards [4]. The results were expressed in mg/g of OP oil.

For determination of fatty acid (FA) composition, methyl esters were prepared [5]. A GC-FID Shimadzu GC-2010 *Plus* (Shimadzu, Tokyo, Japan) coupled with a split/splitless auto-injector, was used. FA methyl esters were identified by comparison with a standard mixture. The results were expressed in relative percentage of each FA.

2.4. Defatted fraction analysis

2.4.1 Total phenolics content

The total phenolics content was determined spectrophotometrically [6]. Briefly, 500 μL of each extract were mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of a $Na_2CO_3 \cdot 10H_2O$ solution. The mixture was left to react, protected from light, for15 min at 45 °C. After 30 min at room temperature, the absorbance was read at 765 nm. Total phenolics content was expressed as μg of gallic acid equivalents (GAE)/g of OP (dw).

2.4.2 Antioxidant activity

Antioxidant activity was assessed using the Ferric Reducing Antioxidant Power (FRAP) and the $DPPH^{\bullet}$ inhibition assays.

The FRAP assay was carried out by mixing an aliquot of 90 µL of extract with 270 µL of distilled water and 2.7 mL of the FRAP reagent. The mixture was kept protected from light (30 min; 37 °C). Absorbance was measured at 595 nm [7]. The Ferric Reducing Antioxidant Power was expressed as µmol of ferrous sulfate equivalents (FSE)/g of OP (dw).
For the DPPH$^{\bullet}$ inhibition assay, diluted sample extracts (20 µL) were added to 180 µL of a DPPH$^{\bullet}$ ethanolic solution ($6x10^{-5}$ mol/L). The absorbance decrease was monitored at 525 min. The results were expressed as percentage of radical scavenging activity (%) [8].

3. RESULTS AND DISCUSSION

OP consists of the hulls, residues from pulp and fragments of stones. The main components of olive stone are hemicellulose, cellulose and lignin [9]. However, valuable components as phenolic compounds have been a point of interest. Few studies have focused on the phenolic composition of the olive stones. Moreover, during the extraction of olive oil, only part of the seed oil is included in olive oil. A significant amount remains, therefore, in the OP [10].

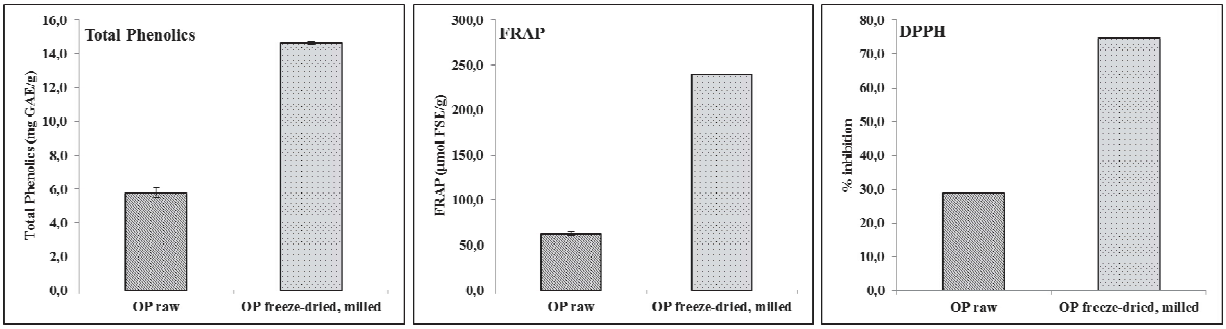

Figure 2. TP compounds and antioxidant activity (FRAP and DPPH$^{\bullet}$ inhibition) of defatted OP. Results are reported as mean ± SD.

In this study, data showed a higher TP content in freeze-dried and milled OP comparatively to the raw OP (Figure 2). That shows clearly that OP milling allow a greater recovery of bioactive compounds since it increases the surface area in contact with the solvent. Furthermore, the stone chemical compounds are also recovered. The antioxidant activity was also greater in sample B than in A. The raw OP presented 62.7 µmol FSE/g and the freeze-dried and milled OP presented 239.3 µmol FSE/g. Sample A showed lower radical scavenging activity (28.8%) than the freeze-dried and milled OP (74.6%) (Figure 2).
Phenolic compounds such as nuezhenide and salidroside are detected only in the olive seed [10]. Further research is needed to evaluate the content of these compounds in milled OP. It has been shown that olive seed oil is richer than olive oil in polyunsaturated FA, especially linoleic acid [11]. OP contained 10% of fat (dw), a good source of oleic and linoleic acids (Table 1). Most of the FA are unsaturated. Oleic acid is the predominant FA followed by palmitic acid. The other FA were found in small quantities. OP has a content in vitamin E of 0.15 mg/g of fat being α-tocopherol the major vitamer followed by γ-tocotrienol. Due to the antioxidant potential of Vitamin E, which also prevents the degradation of the unsaturated

FA, this oil is an interesting product to be used in food industry, for instance, for direct consumption or as a natural preserving ingredient.

Table 1. Fatty acids composition (%) of OP oil.

	Fatty acids	Raw OP	Freeze-dried, milled OP
SFA	Palmitic (C16:0)	10.72 ± 0.01	11.11 ± 0.06
	Heptadecanoic (C17:0)	0.14 ± 0.00	0.13 ± 0.01
	Stearic (C18:0)	2.73 ± 0.00	2.58 ± 0.02
	Arachidic (C20:0)	0.33 ± 0.00	0.41 ± 0.00
UFA	Palmitoleic (C16:1)	0.63 ± 0.00	0.57 ± 0.01
	Heptadecenoic (C17:1)	0.26 ± 0.00	0.22 ± 0.00
	Oleic (C18:1)	79.06 ± 0.01	76.57 ± 0.26
	Eicosenoic (C20:1)	0.24 ± 0.01	0.35 ± 0.09
	Linoleic (C18:2)	5.23 ± 0.00	7.12 ± 0.09
	Linolenic (C18:3n3)	0.66 ± 0.01	0.95 ± 0.02

SFA - Saturated fatty acid; UFA - Unsaturated fatty acid. Results are reported as mean ± SD.

4. CONCLUSIONS

Due to OP composition in bioactive compounds, its valorisation should be promoted. Freeze-dried and milled OP extracts presented higher TP content as well as antioxidant activity. Additionally, the OP lipidic fraction can be a good source of vitamin E and oleic acid for food or, even, cosmetic industries.

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