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Evaluation of Olive Pomace as a Source of Phenolic Antioxidants for the Production of Functional Cosmetics

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Abstract
Olive pomace (OP), the solid by-product originating from the production of olive oil, was investigated as a potential source of phenolic compounds. Phenolic recovery was carried out using a multistage solvent extraction procedure. Different solvents, including water, acetone, ethyl acetate, ethanol and the 50:50 (v/v) ethanol–water mixture, were preliminarily screened for their extraction efficiency. Aqueous ethanol was the most effective solvent and was used toproduce phenolic extractsand evaluate the effects of OP stabilization by controlled drying. The optimal drying conditions were identified as: T = 120 °C and t = 1 h. Phenolic extracts produced from OP were incorporated in an amount of 0.5 to 3% by weight into a cosmetic base containing water, glycerin and liquid paraffin as main components. The antioxidant capacity of the resulting productsranged from 8.65 to 42.37 mg TE/mL, depending on the added amount of extract.
Overall, the results of this study indicate that OPis a valuable source of phenolic compounds and support the possibility of using phenolic-richextracts to produce functional cosmetics with high antioxidant capacity. The recovery ofvalue-added compounds from OP could notonly provide economic benefits to olive oil producers but also contribute to reduce theenvironmental impact of the olive oil industry.

Keywords: Olive Pomace, Phenolic Compounds, Solvent Extraction, Natural Antioxidants, Functional Cosmetics.

Introduction

According to estimates from FAO, about 3, 000, 000tons of olive oil are produced annually in the world [1]. More than 98% of thetotal production takes place in the Mediterranean region, with Spain, Italy, Greece and Portugal being the largest producers.
The production of olive oil by the traditional batch methodorby the continuous two- or three-phase systems generate large amounts of a solid by-product known as olive pomace (OP) [2]. This wasteconsists mainly of olive pulp, skin, stones and oil residues. OP is produced in a limited time period and is easily susceptible to microbial degradation due toits high moisture content, which can reach 60–70%. As a result, its disposal is a challenging task with important environmentalimplications.
OP is generally used for fuel or fertilizing purposes or, to a lesser extent, as a supplement for animal feed. At present, however, it remains largely underutilized. This has stimulated efforts to findnew strategies for adding value to it [3]. An examination of the composition of OP reveals that it contains valuable phenolic compounds, such ashydroxytyrosol, oleuropein, caffeic acid, verbascoside, catechol and rutin [4].In addition, the total amount of these compounds in OP is very high, since only a small fraction of themis transferred from the olive fruit to the oil during the extraction process [5].
Plant phenolic compounds, also known as polyphenols or biophenols, are secondary metabolites characterized by the presence of one or more hydroxyl groups attached to a benzene ring or other aromatic structures [6]. In the last decades, these substances have attracted increasing interest from nutritionists and food scientists due to their reported health benefits, including anti-oxidative, anti-inflammatory and anti-carcinogenic effects [7].Recently, the efficacy of formulations containing plant polyphenols to protect the skin from some diseases or premature ageing has been confirmed by a number of studies [8].
Despite the promising potential of OP as a source of phenolic compounds, only a few studies have examined the feasibility of their recovery from this waste [9–11]. In particular, systematic studies on the influence of solvent type and process conditions on theyield of polyphenolextraction from OP and on the properties of the resulting productsare currently lacking.
In this contribution we investigate the recovery of phenolic compounds from OP by a simple solvent extraction procedure. Different solvents were preliminarily screened for their ability to extract these compounds. The solvent with the most suitable properties was then used to analyze the effects of the number of extraction stages and of OP pretreatment conditions on the phenolic extraction yield. Finally, dry phenolic extracts were produced and incorporated into a cosmetic base to explore their suitability as bioactive ingredients for functional cosmetic products.

Experimental

Chemicals and olive waste

Acetone, ethanol, ethyl acetate, hydrochloric acid and sodium carbonate were purchased from Carlo Erba(Milano, Italy).Gallic acid (3, 4, 5-trihydroxybenzoic acid), DPPH (2, 2-Diphenyl-1-picrylhydrazyl), Trolox (6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid)and the Folin-Ciocalteu reagent were obtained from Sigma-Aldrich (Milano, Italy).All chemicals were reagent grade and used without furtherpurification. Aqueous solutions were prepared with

deionized water.

OP was collected from a three-phase olive mill located in Central Italy (Villa Latina, FR). OPsamples were placed in plastic bags and stored at –20 °C. Before performing a set of experiments, appropriate amounts of the frozenmaterial were thawed in air at room temperature and characterized for moisture and phenolic content. When required, OP samples were dried in an electric oven at the appropriate conditions.

Analytical Methods

Moisture content was determined using an electronic moisture analyzer (model MAC 50/1, Radwag, Poland).

Total phenolics were determined by the Folin-Ciocalteu method with some modifications [12, 13]. The results were expressed as gallic acid equivalents (GAE) per unit weight of solid, or unit volume of liquid, using a calibration curve obtained with gallic acid standards.

Antioxidant activity was determined by the DPPH assay, following the procedure described in [13]. The results were expressed as Trolox equivalents (TE) per unit weight of solid, or unit volume of liquid, using a calibration curve obtained with Trolox standards.

Extraction Procedure

Solvent extraction experiments were carried out in batch mode, using the experimental setup described elsewhere [14].

Solvent screening was performed at 40 °C bya three-stage extraction procedure. In each stage, 0.2 g of OP were contacted with the appropriate amount of solvent (20, 10 and5 mL in the first, second and third stage) in magnetically stirred flasks placed in a thermostatic bath (±0.1 °C). After1-h stirring, a sample of theliquid was withdrawn, passed through a 45-μm nylon filter and assayed for phenolic content. The total amount of extracted polyphenols was calculated as the sum of the values obtained at each stage.

Production and characterization of phenolic extracts were carried out using the solvent with the highest extraction efficiency. Experiments were conducted at 60 °C on 1 g of OP. The plant material was extracted four times using an extraction time of 1 h and a liquid-to-solid ratio of 20, 10, 5 and 2.5 mL/g in the first to fourth stage. The total amount of extracted polyphenolswas determinedas reported previously.

Dry phenolic extracts were obtained in a rotary evaporator (Rotavapor R-215, BÜCHI Labortechnik AG, Switzerland) operated at 40 °C and reduced pressure(15–20 mbar). The extracts were incorporated by hand mixing at room temperature into a cosmetic base containing water, glycerin and liquid paraffin as main ingredients.

Results and Discussion

In the first part of the study, the influence of solvent type on the recovery of phenolic compounds from OP was investigated. Extraction was carried in three stages and the following solventswere considered: water, acetone, ethyl acetate, ethanol and the 50: 50 (v/v) ethanol–water mixture.The results of solvent screening are shown in Fig. 1.The percentage amounts of polyphenols recovered in the first, second and third stage were in the following ranges: 56.2–75.3%, 17.8–34% and 5.3–9.8%. This suggests that at least two stages are necessary to achieve a satisfactory recovery (>90%).From the values of the total amount of extracted phenolicsreported in Table 1, the followingorder of solvent effectiveness can be established: 50: 50 ethanol–water mixture >acetone > water > ethanol >ethyl acetate. Interestingly, quite similar results were obtained for the extraction of phenolic compounds from artichoke waste, a lignocellulosic material with some similarities to OP [15].

Solvent effects are thought to arise fromtwo main factors: (a) affinity of the phenolic compounds for the solvent and (b) solvent-induced changes in the plant material. Regarding the first point, it should be considered that the presence of one or more hydroxyl groups in the molecule imparts a polar character to polyphenols. Accordingly, the greater the solvent polarity, the higherthe phenolic extraction yield. However, as can be seen in Table 1, this is not strictly true. In fact, if we take the dipole moment or the Hansen polar solubility parameter as a measure of polarity, we see that the extraction yield does not increase regularly withthe solvent polarity. One reason may be that phenolic compoundsare often bound to cell components such as polysaccharides and proteins [16]. Furthermore, solvents can induce structural changes in the plant material. In particular, they can swell the plant tissue, favoring the penetration of solvent molecules and the diffusion of the solute out of the solid matrix [17, 18]. The overall effect of the solvent will thus result from both chemical affinity and matrix changes.

Aqueous ethanol, the most effective solvent, was used to produce phenolic extracts from OP. The total amount of polyphenols obtained using four extraction stages was 11.9 ± 0.5 mg GAE/g and over 90% of them were recovered in the first two stages (Fig. 2).

In order to evaluate the possibility of stabilizing OP by reducing its moisture content prior to storage, OP samples were dried at 120 or 150 °C for 1 or 6 h. Dried samples were solvent extracted with aqueous ethanol at 60 °C and the resulting extracts characterized for phenolic content and antioxidant activity. The results are presented in Table 2, where the values relative to a mild drying treatment of OP (12 h at 40 °C) are also reported.

High-temperature drying caused a significant reduction in moisture content (from 2–3 to <0.5%), independently of the severity of treatment.Furthermore, compared to samples treated at 40 °C for 12 h, 1-h drying at 120 °C resulted in an increase in both the phenolic content of the extract (from 412 to 437 mg GAE/L) and its antioxidant capacity (from 231 to 253 mg TE/L). By contrast, prolonging the drying time to 6 h or increasing the temperature to 150 °C gave rise to a progressive decrease in the phenolic content and its antioxidant activity. Therefore, based on these results, the optimal drying conditions can be identified as: T = 120 °C and t = 1 h. Because of the very low residual moisture content, OP dried under these conditions is expected to withstand long storage without deterioration.

The apparently anomalous increase in phenolic content after 1-h drying at 120 °C is consistent with the results of Ahmad-Qasem and co-workers [19], who found higher amounts of polyphenols in OP dried attemperatures exceeding 70 °C. A possible explanation is that high temperatures induce non-enzymatic formation of new phenolic compounds from

precursors present in the plant material [20]. Of course, above a certain temperature or for prolonged times, thermal degradation reactions will prevail over the formation of new polyphenols, leading to a decrease in the net phenolic content.
Finally, dry phenolic extracts were produced by solvent evaporation and used to prepare a cosmetic formulation. Dry extracts had a moisture content close to 3% (w/w). The yield of the extract, calculated with respect to the initial weight of OP, was 9.8% (w/w) and its phenolic content was 75.31±0.22 mg GAE/g. Appropriate amounts of this extract (0.5 to 3% by weight) were incorporated into the cosmetic base. Fig. 3 shows the appearance ofthe functionalcosmetic productcontaining 2% phenolic extract. Depending on the added amount of extract, the antioxidant activity of the cosmetic formulation, expressed as Trolox equivalents, ranged from 8.65 to 42.37 mg TE/mL.
Topical formulations containing phenolic compounds are considered very effective for the prevention ofphotoageing and the therapy of some skin diseases [21]. Photoageing is predominatelya result of exposure to solar radiation. In particular, short-wavelength UV radiation is known to produce detrimentaleffects in the connective tissue due to the formation of lipid peroxidesand reactive oxygen species (ROS). Overproduction of ROS can lead to severe damage to tissues and cellular components, a situation referred to as oxidativestress. Phenolic compounds are strong antioxidant agents whichact as free radical terminators, thus providing protection to the skin against oxidative damage [22]. In addition, plant phenolic extracts have beenreported to possess anti-collagenase, anti-elastase and anti-hyaluronidaseactivities, i.e., to inhibit the enzymesresponsible for the hydrolysis of collagen, elastin and hyaluronicacid in the dermis [23]. Therefore, the development of functional cosmetics containing olive phenolic compounds can be an attractive and viable strategy to exploit the potential of these natural antioxidants. Since the early 2000s, olive polyphenols are extracted and commercialized from oil mill wastewater (and later on, also from olive leaves) [24]. The results presented here clearly demonstrate that OP, a by-product produced in large amounts by the olive oil industry, could be an alternative promising source of bioactive phenolic compounds.

Table 1. Extraction yields of phenolic compounds (q) in different solvents together with the solvent dipole moment (D) and Hansen polar solubility parameter (δ_P).

Solvent	μ (D)	δ_P ($MPa^{½}$)	q (mg GAE/g)
Water	1.85	16.0	7.18 ± 0.25
Acetone	2.88	10.4	9.43 ± 0.36
Ethyl acetate	1.78	5.3	3.69 ± 0.32
Ethanol	1.69	8.8	6.22 ± 0.39
Ethanol–water (50:50 v/v)	1.77*	12.4*	10.48 ± 0.42

* Estimated as a weighted averageof the values for the pure solvents, using volume fractions as coefficients

Table 2. Effect of OP drying conditions on the phenolic content (c) and Trolox equivalent antioxidant capacity (TEAC) of the extracts.

DryingConditions			Extractproperties	
Temperature (°C)	Time (h)	Residualmoistureconte nt (%w/w)	c (mgGAE/L)	TEAC (mgTE/L)
40	12	2–3	412 ± 10	231 ± 11
120	1	<0.5	437 ± 13	253 ± 16
120	6	<0.5	409 ± 18	225 ± 12
150	1	<0.5	387 ± 22	215 ± 10
150	6	<0.5	375 ± 23	198 ± 15

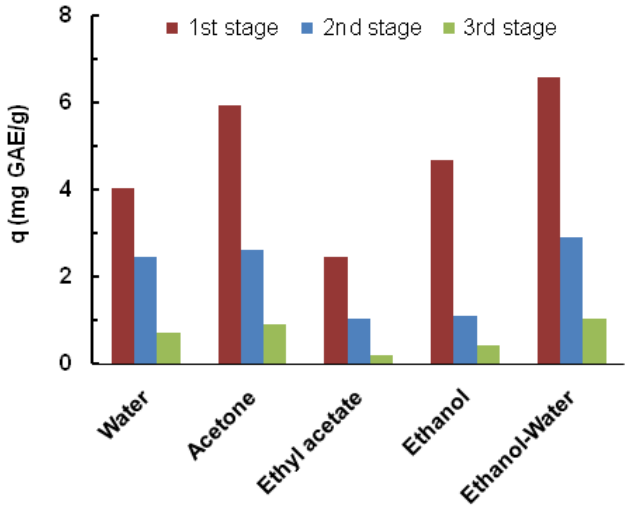

Fig. 1. Effect of solvent type on the recovery of phenolic compounds from OP. q is the extraction yield, expressed as GAE per dry weight of plant material.

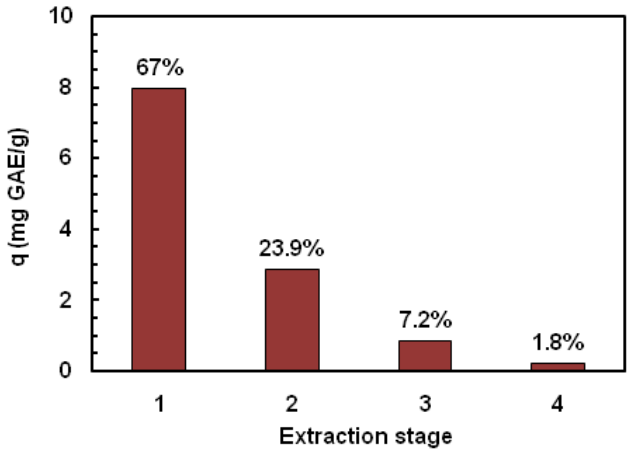

Fig. 2. Extraction yield of phenolic compounds (q) and recovery percentages in the four extraction stages.

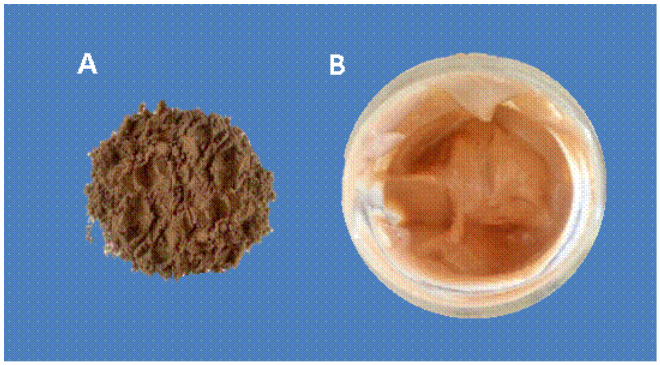

Fig. 3. Dry phenolic extract (A) and cosmetic composition (B) containing 2% by weight of the phenolic extract.

Conclusion

The results of this study indicate that OPis a rich source of phenolic antioxidants and that they can be easily recovered from this waste using aqueous ethanol as solvent and appropriate extraction conditions. Water and ethanol are generally recognized as safe (GRAS) and environmentally benign compounds and the resulting extracts can thus be used in food, nutraceutical and cosmetic applications. Regarding the latter, we have shown that phenolic extracts obtained from OP can be used as bioactive ingredients to produce new functional cosmetic products with high antioxidant activity.The exploitation of OP for such purposes could notonly provide economic benefits to olive oil producers but also contribute to reduce theenvironmental impact of the olive oil industry.

Future research should be directed atinvestigating the extraction process on a larger scale andperforming an accurate cost-benefit analysis. Assessment of the functional properties of the fortifiedcosmetic products is another important issue to be addressed in the future.

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