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Antioxidative Activity of Olive Pomace Polyphenols Obtained by Ultrasound Assisted Extraction

Mohamed Khairy S. Morsi[1],Samy M. Galal[1,]*,Obaidh Alabdulla[2]
[1]*Food Science Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*
[2]*Food Science Department, Faculty of Agriculture, Damascus University, Syria*

Abstract: *This study was carried outto investigate the effect of ultrasound-assisted extraction (UAE) on polyphenols from olive pomace with methanol (80%) as an extracting medium and studythe effects of addingthe extract to refined sunflower oilduring accelerated oxidationconditions. Ahigh amount of polyphenols was extracted from the olive pomace with a solvent-to-solid ratio of 40 at 50% of the maximum output power for 30 min.Polyphenols of the extractswere identified and quantified by high-performance liquid chromatography.Sonicated extracts were characterizedby the presence of pyrogallol, 3-hydroxytyrosol andoleuropein.The most common flavonoids in the extracts were hesperidin, naringin, and hesperetin. The antioxidant activity of olive pomace extract was evaluated and compared to butylated hydroxytoluene (BHT) using the Rancimat assay. Olive pomace extract at 150 mg Gallic Acid Equivalents/kg oil was superior in protecting sunflower oil against oxidative deteriorative changes than BHT at 200 ppm.*

Keywords: *olive, oxidative stability, polyphenols,pomace,ultrasound*

I. Introduction

Antioxidants are substances, which are able to quench potentially damaging radicals [1] or reduce the rate of lipid oxidation, and stabilized free radicals [2]. The addition of antioxidants increases the shelf life of lipids. Rancidity results from oxidation, which takes place at the double bond sites in the triacylglycerol molecules [3]. Oxidation causes deterioration in flavour quality of oils and oil products and great economic losses to the food industry. Synthetic antioxidants have maximum permissible limits in foods. Synthetic antioxidants are controlled due to their carcinogenicity [4].Due to these safety concerns, many studies focus on the replacement of these synthetic antioxidants with natural ones [5].

Polyphenols constitute the active substances found in many plants [6]. They are one of the natural antioxidants present in a considerable quantity in olive fruits (*Oleaeuropaea* L.) [7]. During maturation of the olive fruit and processing of olives, chemical and enzymatic reactions take place which results in the formation of free phenols. The latter group is retained in minute amounts in the oil. The extraction system affects total phenol and o-diphenol content in the pomace. The oils produced by the continuous centrifugal system contain a lower polyphenol content than oils extracted with other systems. Diversities in the machinery of olive crushing, the contact time with the water and the total volume of water used affect significantly the total polyphenols content [8].

Olive pomace is an inexpensive heterogeneous solid waste from olive oil production that is available in large quantities in Mediterranean countries [9]. It could be considered a rich source of high added value compounds. The phenolic compounds of olive oil comprise about 1% of total fruit phenols, while the remaining was lost with wastewater and pomace [10]. Olive pomaceis considered a rich source of phenolic compounds with a wide array of biological activities. Ultrasound- assisted extraction uses acoustic cavitation to improve efficiency and decrease extraction time compared to conventional extraction techniques [11]. The quantity and type of phenolic compounds in olive pomace depend on the cultivar and maturity of the fruit, climatic conditions, storage time and processing technique [12]. The major phenolic compounds present in olive pomace are hydroxytyrosol, oleuropein and tyrosol [13], caffeic acid, p-coumaric acid, vanillic acid and rutin [10].

This study was carried out to evaluate the efficiency of phenolic compounds that were derived from olive pomace to protect sunflower oil (SFO) against oxidative deterioration.

II. Materials and Methods

2.1 Olive Pomace

Two-phase olive pomace sample (1 kg) was obtained from an olive oil factory (Mini Frantoio Oliomio-50-60 Centrifuge, Italy) located in the Agricultural Research Centre, Giza, Egypt. Pomace sample was dried in an oven at 70°C under vacuum before grinding using a laboratory mixer. The dried, powdered sample was extracted with petroleum ether (b.p. 40-60°C) to remove the residual oil using a Soxhlet apparatus.

2.2 Reagents and Standards

HPLC-grade solvents were purchased from Merck (Darmstadt, Germany). The Folin–Ciocalteau phenol reagent, BHT and polyphenol reference standards: syringic acid, gallic acid, pyrogallol, 4-aminobenzoic acid, 3-hydroxytyrosol, protocatechuic acid, catechin, chlorogenic acid, catechol, epicatechin, caffeine, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, ρ-coumaric acid, ferulic acid, oleuropein, ellagic acid, benzoic acid, coumarin, salicylic acid, naringin, hesperidin, rosmarinic acid, quercitrin, quercetin, naringenin, hesperetin, kaempferol, rhamnetin and apigenin were purchased from Sigma-Aldrich (St. Louis, MO).

2.3 Extraction of Polyphenols from Olive Pomace

In brief, one hundred grams of the dried defatted olive pomace (ddp) were extracted with aqueous methanol (80%) using solvent/pomace ratios (v/w) of 20:1 for 7 min (Technique 1) and 40:1 for 30 min (Technique 2), at room temperature. UAE was conducted using Fisher-Sonic, Dismembrator Model 300, N.Y., USA at 50% of the maximum output power (300 W). The resulting slurry was centrifuged at 3000g. After filtration, the solventwas removed under vacuum at 40°C using a rotary evaporator (Eyela, Japan). Subsequently, the concentrate was kept at −18°C for further analysis.

2.4 Total Polyphenols Content

The total polyphenols content was determined using the Folin-Ciocalteu reagent and Unico visible UV-2000 Spectrophotometer, USA at 750 nm according to the method described by [14]. A standard calibration curve was prepared using gallic acid (50–800 µg/mL). The total polyphenol concentration was calculated from the calibration curve (r^2= 0.9951). Spectrophotometric analysis was carried out in triplicate. Results were expressed as mg GAE/g ddp ± SD.

2.5 HPLC Analyses of Pomace Polyphenols Extract

Chromatographic analyses were performed on an Agilent Technologies 1200 HPLC series, USA, equipped with Agilent 1200 Series quaternary pump, vacuum degasser, a Zorbax ODS HPLC column (25 cm x 4.6 mm, i.d.) at room temperature. The mobile phase was acetic acid 8% (A) and acetonitrile (B), flowing at 1 ml/min and Agilent UV-VIS detector. The separation was obtained with the following gradients: at 0 min, 5 % A and 95% B; at 5 min, 25% A and 75% B; at 10 min, 45% A and 55% B; at 15 min, 65A and 35% B; at 20 min, 85% A and 15% B; and from 25 to 30 min, 99% A and 1% B. The wavelength of the detector was set at 330 nm for polyphenols and 280 nm for flavonoids. The results were expressed as mg /g ddp. Quantification of the identified compounds was carried out using calibration curves of the reference standards.

2.6 Quality Indices of Oil

Free acidity (oleic acid%) and peroxide value (PV) expressed as milliequivalents of active oxygen per kilogram of oil (meq. O_2/kg) were determined according to the analytical methods described in [15]. Parameters were determined in triplicate.

2.7 Evaluation of the Antioxidant Activity by the Rancimat Method

The antioxidant potential of the olive pomace extract (rich in polyphenols) was evaluated on freshly refined SFO (free of synthetic antioxidants). Oxidative stability was expressed as the oxidation induction time (h), measured with the Rancimat 679 apparatus (Metrohm Co., Basel, Switzerland), using an oil sample of 3 g heated to 110°C under a dry air flow of 20 l/h. The volatile products stripped from the oil during oxidation were collected into a cell containing double distilled water, whose conductivity increased progressively. The time taken to reach the conductivity inflection point was recorded as the induction period (IP) and it was expressed in hours. According to the polyphenols content of the olive pomace extract, a known weight of the concentrated extract corresponding to the required amount of polyphenols was transferred to 10 ml volumetric flask before addition of the oil to the mark. The extract concentration was experimentally prepared at the levels of 150 and 300 mg GAE/kg oil. To compare the antioxidant activity of the investigated extract, BHT was used at 200 ppm (maximum allowed concentration). The protection factor was calculated as the ratio of the induction period of the SFO sample with BHT or olive pomace extract to the induction period of the control.

2.8 Statistical Analyses

The results of quality indices of SFO and total polyphenols content were obtained from three independent experiments and averaged. Data were expressed as mean ± SD.

III. Results and Discussion

UAE using methanol: pomace ratio of 40:1 for 30 min (technique 2) yielded extract with total polyphenols of 86.13 ± 0.80 mg GAE/g ddp. On the other hand, the total polyphenols content of the extract obtained at a lower solvent ratio (20:1) for a short time (7 min) (technique 1) did not exceed 23.15± 0.14 mg GAE/g ddp. These results indicate that solvent to solid ratio and extraction time have positive effects in extraction by increasing the extracted total polyphenols. These results are in agreement with those found by [16,17].

HPLC chromatograms for olive pomace extracts are displayed in Figures 1 and 2. Phenolic compounds identified and quantified in the methanol extracts of pomaceare presented in Table 1.

A total of 21 polyphenols and 11 flavonoids were detected in the pomace extracts. Results indicate that the yield of the individual polyphenols is varied, though the polyphenolic profile is not remarkably affected by the extraction technique. The most abundant polyphenols in the sonicated extracts were oleuropein, hydroxytyrosol, pyrogallol, catechol, ellagic acid and benzoic acid.

The higher yield of identified polyphenols for the extract obtained by technique 2, is mainly associated with the higher contents of oleuropein, pyrogallol,catechol, 3-hydroxytyrosol, and 4-hydroxybenzoic acid (Table 1). However, the extract obtained by technique 1 is characterized by higher levels ofcaffeic acid and coumarin. The most common flavonoids in the investigated extracts were hesperidin, naringin, and hesperetin. Rutin, quercetin, rosmarinic acid, naringenin, and quercitrinwere also found in considerable quantities.

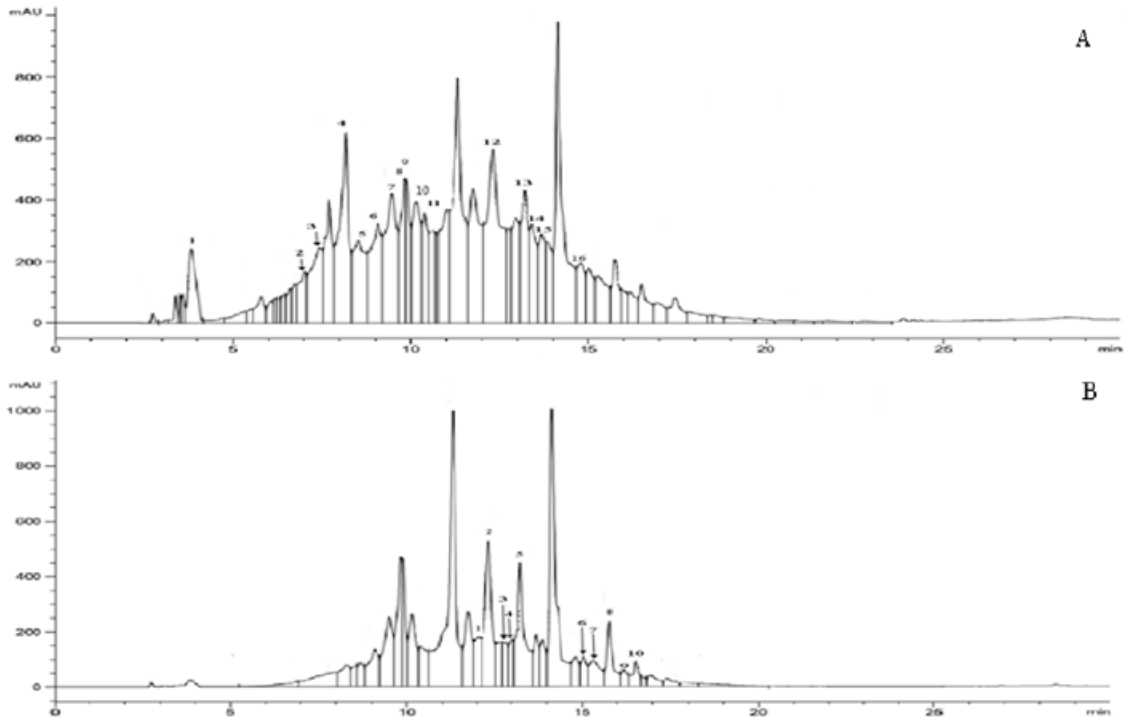

Fig.1 HPLC chromatograms of olive pomace extract obtained by ultrasonic technique 1 using methanol: pomace ratio of 20:1 for 7 minutes. (A) Polyhenols, Peaks: (1) syringic acid, (2) gallic acid, (3) pyrogallol, (4) 3-Hydroxytyrosol, (5) protocatechuic acid , (6) catechin, (7) chlorogenic acid, (8) catechol, (9) caffeine, (10) 4-hydroxybenzoic acid, (11) vanillic acid, (12) ferulic acid, (13) oleuropein, (14) ellagic acid, (15) benzoic acid, (16) coumarin. (B) Flavonoids, Peaks: (1) naringin, (2) rutin, (3) hesperidin, (4) rosmarinic acid, (5) quercetrin, (6) quercetin, (7) naringenin, (8) hesperetin, (9) kaempherol, (10) rhamnetin, (11) apigenin.

On the other hand, the sonicated extract prepared by technique1 was characterized by higher yields of quercitrin, naringin and rhamnetin versus the other investigated extract (technique2). Furthermore, there was no remarkable difference in rosmarinic acid content between both extracts. These results are consistent with those reported by [18].The quality characteristics of the SFO were evaluated by free acidity and peroxide value. Acid value and peroxide value were 0.217 mg KOH/g oil and 0.753 meq. O_2 /Kg oil, respectively. The determined parameters were within the limits of the standards [19] for refined SFO. The Rancimat method used to measure the oxidative stability of oil is based on the determination of IP as a result of the accumulation of secondary oxidation products.The oxidative stability time obtained during the analysis allows for estimating the oil resistance to oxidation under accelerated conditions.

Analysis displayed that the addition of olive pomace extract at concentrations of 150 and 300 mg GAE/kg oil increased SFO oxidative stability by 36% and 96% (IP = 11.8 h and 17 h, respectively) in comparison with the control sample (IP = 8.65 h) (Fig. 2). The addition of BHT at 200 ppm made it possible to extend IP of SFO by 26% (IP = 10.9 h). A positive correlation between the total phenolic content of olive cake extracts and antioxidant activity using β-carotene/linoleic acid assay was observed [20]. These results for SFO are comparable with those reported in the literature [21]. Addition of BHT at 200 ppm prolonged induction period of SFO by 25% [22].

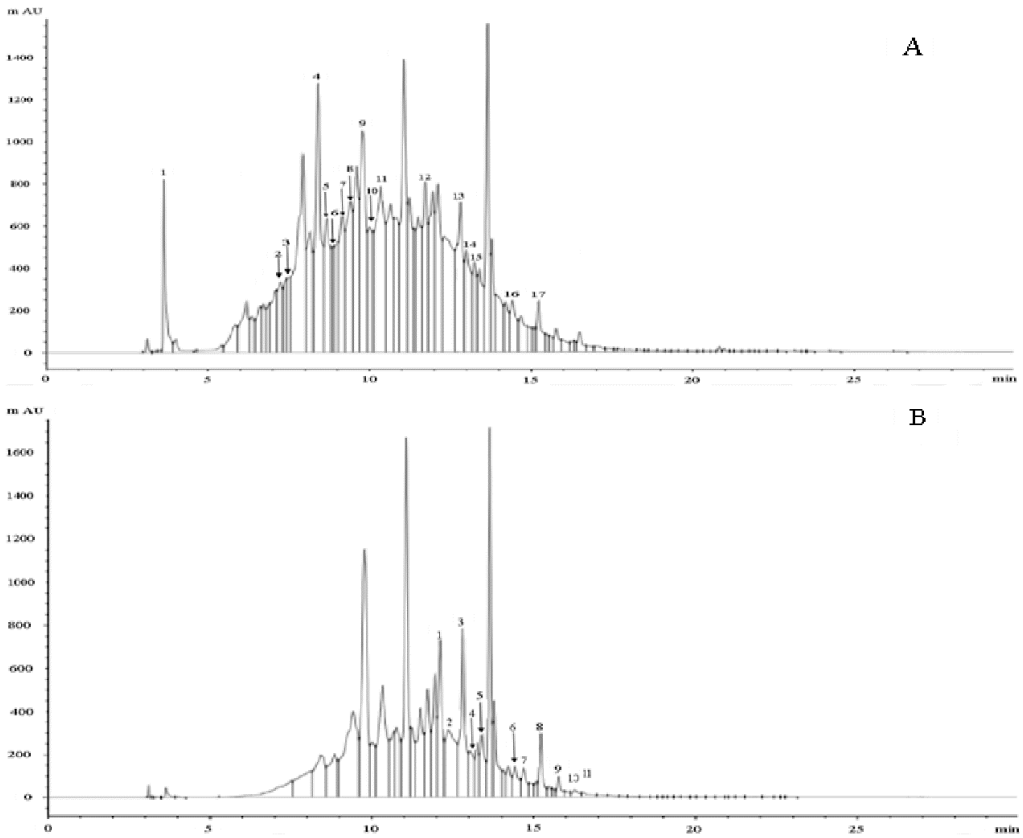

Fig.2 HPLC chromatograms of olive pomace extract obtained by ultrasonic technique 2 using methanol: pomace ratio of 40:1 for 30 minutes. (A) Polyhenols, Peaks: (1) syringic acid, (2) gallic acid, (3) pyrogallol, (4) 3-Hydroxytyrosol, (5) protocatechuic acid , (6) catechin, (7) chlorogenic acid, (8) catechol, (9) caffeine, (10) 4-hydroxybenzoic acid, (11) vanillic acid, (12) ferulic acid, (13) oleuropein, (14) ellagic acid, (15) benzoic acid, (16) coumarin. (B) Flavonoids, Peaks: (1) naringin, (2) rutin, (3) hesperidin, (4) rosmarinic acid, (5) quercetrin, (6) quercetin, (7) naringenin, (8) hesperetin, (9) kaempherol, (10) rhamnetin, (11) apigenin.

IV. Conclusion

Olive pomacecould be utilized as a rich source of natural antioxidants.The enrichment of refined SFO with oleuropein and hydroxytyrosol rich extract from olive pomaceinhibits the deterioration of oil and improve its oxidative stability.

Table 1. Identifiedphenolic compounds in olive pomacemethanolic extracts

Compounds	Yield (mg/g ddp*)	
	Extraction Technique 1**	Extraction Technique 2**
Polyphenols		
Syringic acid	1.243	0.574
Gallic acid	0.016	0.196
Pyrogallol	4.590	12.834
4-Aminobenzoic acid	0.033	0.484
3-Hydroxytyrosol	10.70	13.028
Protocatechuic acid	0.600	1.213
Catechin	0.333	1.347
Chlorogenic acid	0.247	0.909
Catechol	0.387	4.976

Epicatechin	0.815	1.675
Caffeine	0.498	0.254
4-Hydroxybenzoic acid	1.107	3.991
Caffeic acid	0.343	0.179
Vanillic acid	0.842	1.862
ρ-coumaric acid	0.104	0.833
Ferulic acid	0.126	1.181
Oleuropein	2.383	13.112
Ellagic acid	1.400	2.374
Benzoic Acid	2.030	2.992
Coumarin	0.232	0.169
Salicylic acid	0.310	0.630
Flavonoids		
Naringin	0.723	2.342
Rutin	0.937	0.570
Hesperidin	2.709	4.413
Rosmarinic acid	0.181	0.178
Quercitrin	0.281	0.031
Quercetin	0.161	0.230
Naringenin	0.035	0.274
Hesperetin	0.241	1.671
Kaempferol	0.360	0.064
Rhamnetin	1.830	0.065
Apigenin	0.005	0.031

*****ddp: dried defatted pomace.******Ultrasonic assisted extraction conducted at solvent to solid ratio of 20:1 for 7 min (Technique 1),at solvent to solid ratio of 40:1 for30 min (Technique 2).

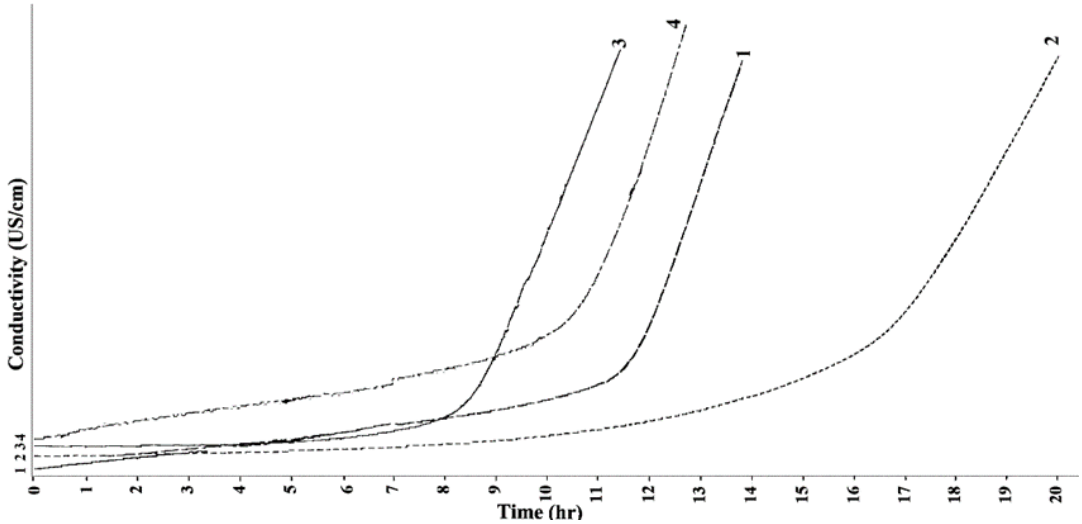

Fig.3 Oxidative stability of sunflower oil before (3) and after enrichment with the olivepomace extract (corresponded to the addition of 150 mg Gallic acid equivalent/kg oil (1) or 300 mg Gallic acid equivalent/kg oil (2)) or BHT at 200 ppm (4).

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