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Extraction and Quantification of Bioactive Phenolic Compounds in Olive Oil by Acid Hydrolysis Method

Diya Alsafadi[1] · Jameel Aljariri Alhesan[2] · Aya Mansour[1] · Saba Oqdeh[1]

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Abstract

Phenolic compounds are responsible for healthy, nutritional, and sensory quality of olive oil. The present work deals with the optimization of a method for performing direct acid hydrolysis and extraction of the bound forms of hydroxytyrosol (Htyr) and tyrosol (Tyr) from olive oil. The proposed method was simpler and less time-consuming and required minimum sample pre-treatment steps, compared to liquid–liquid extraction and solid phase extraction (SPE) methods. The hydrolysis and extraction conditions were optimized in terms of extracting solvent, acid type and concentration, temperature, and mixing time. The maximum phenolic compound (Htyr and Tyr) contents were obtained when the olive oil sample was treated with 2.0 M H_2SO_4 and water as solvent for 4 h at 75 °C. The method showed satisfactory linearity ($R^2 > 0.99$), high precision (%RSD < 3.0%), and high recovery (> 94.0%) for Tyr and Htyr. The limits of quantification (LOQ) were 0.56 and 0.69 mg/L for Tyr and Htyr, respectively. Some commercial olive oil samples from Jordanian olive cultivars such as Nabali Baladi, Nabali Mohasen, K18, and Rumi were analyzed using the developed method. The results obtained varied depending on the type of cultivar, and highest value of total phenolic compounds (421.70 mg/kg) was reported for K18 variety that harvested at early time. Furthermore, phenolic compounds including phenols, secoiridoids, flavones, and lignans were determined using SPE method. The results presented statistically significant differences ($p < 0.05$) between SPE and acid hydrolysis methods.

Keywords Phenolic compounds · Hydroxytyrosol and tyrosol · Olive oil · Extraction method · Acid hydrolysis · Health claim

Introduction

Olive oil is widely used in the Mediterranean diet due to its beneficial effects on human health. The healthy effects of olive oil are attributed to the high ratio between monounsaturated/saturated fatty acids, a good amount of tocopherols content, and an appreciable presence of phenolic compounds in the form of polyphenols (Tripoli et al. 2007; Talhaoui et al. 2016). The olive oil polyphenols possess antioxidant and anti-inflammatory activities and reduce the risk of the development of chronic diseases, such as atherosclerosis, cancer, and cardiovascular diseases as proven by many in vivo and in vitro scientific studies (Cicerale et al. 2008; Servili et al. 2014).

The phenolic compounds in olive oil are classified into secoiridoid derivatives (aglycon derivatives of oleuropein and ligstroside), phenolic acids (benzoic and cinnamic acids derivatives), phenyl alcohols (hydroxytyrosol and tyrosol), flavones (luteolin and apigenin), and lignans (pinoresinol, 1-acetoxypinoresinol, and syringaresinol) (Brenes et al. 2000b; Kotsiou and Tasioula-Margari, 2016; Servili et al. 2004). However, the major polar phenolic compounds identified and quantified in olive oil are tyrosol (Tyr) and hydroxytyrosol (Htyr) (Di Maio et al. 2011; Ferro et al. 2019; Kotsiou and Tasioula-Margari, 2016; Servili et al. 2004). The formation of Tyr and Htyr compounds in olive oil results from the hydrolysis of secoiridoid aglycones, which are derived mainly from oleuropein degradation (Ferro et al. 2019).

The European Food Safety Authority (EFSA) recognized the health benefits of olive oil polyphenols; therefore, EFSA authorized the health claim "olive oil polyphenols

✉ Diya Alsafadi
diya.safadi@rss.jo

[1] Biocatalysis and Biosynthesis Research Unit, Advanced Research Center, Royal Scientific Society, Amman 11941, Jordan

[2] Faculty of Pharmacy, Zarqa University, Zarqa 13132, Jordan

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contribute to the protection of blood lipids from oxidative stress" (EFSA, 2011). The EFSA health claim is limited for olive oil samples containing at least 5 mg of hydroxytyrosol and/or its derivatives (e.g., oleuropein complex and tyrosol) per 20 g olive oil product (EFSA, 2011). The enacting of olive oil polyphenols regulation requires the development of simple, robust, and reliable analytical method for quantification of these compounds in olive oil to protect consumers and avoid unfair competition.

The extraction of phenolic compounds from the olive matrix before chromatographic separation step is the most time-consuming and error-prone part in the polyphenol determination protocols. Therefore, several extraction procedures such as liquid–liquid extraction (LLE) and solid phase extraction (SPE) have been used for the recovery of phenolic compounds from olive oil (Flores et al. 2012; Christophoridou et al. 2005; Pizarro et al. 2013; Mirón et al. 2020). The SPE method has been applied for the separation of different classes of phenolic compounds in olive oil using several types of sorbents phases such as C8 (Pirisi et al. 2000), C18 (Liberatore et al. 2001a, b; Pizarro et al. 2013), and diol–cartridges (Mateos et al. 2001). However, the low stability of the eluate during the separation process and the need of loading high sample content (e.g., 60 g of olive oil) and the use of considerable amount of solvent volumes are the main drawbacks of using this method (Alarcón Flores et al. 2012).

In the case of LLE, the polar fraction (PF) in olive oil matrix was extracted using pure polar organic solvents such as methanol (Owen et al. 2000), and N–N dimethylformamide (DMF) (Brenes et al. 2000a), or a mixture of solvents, such as methanol/water mixture with different methanol to water percent compositions (Aturki et al. 2008; Carrasco-Pancorbo et al. 2005; Suarez et al. 2008; Pizarro et al. 2013). In fact, the LLE approach requires large amounts of highly pure organic solvents, and complete extraction of polyphenol is considered time-consuming and tedious. Therefore, the LLE method was optimized by investigating the amount of olive oil sample size, solvent volume, and purification steps (Ricciutelli et al. 2017). In order to increase the recovery of the total amount of Tyr and Htyr, either free and/or linked to secoiridoidic molecules, the LLE procedure was followed by further step by application of an acidic hydrolysis for the polyphenol complex compounds extracted in the liquid solvent (Bellumori et al. 2019; Mulinacci et al. 2006). Romero and Brenes (2012) examined also the acid hydrolysis applied directly to oil (not the phenolic extract) to ease the determination of total Htyr and Tyr. So far, this promising approach has not been optimized.

Many analytical techniques have been implemented to evaluate the phenolic content after the extraction process including spectrophotometric (Folin-Ciocalteu) and chromatographic techniques (Olmo-García et al. 2019). High-performance liquid chromatography (HPLC) determination is characterized by its sensitivity, but the commercial availability of polyphenols standards restricted the HPLC application for a reliable quantification (Tuck and JHayball 2002; Cioffi et al. 2010; Capriotti et al. 2014; Ricciutelli et al. 2017; Siano et al. 2022; Romero and Brenes 2012). Paper spray tandem mass spectrometry (PS-MS/MS) and HPLC–MS/MS were used as more sophisticated chromatographic tools; however, these instruments are very expensive and not available in routine analysis laboratories (Bartella et al. 2018, 2020).

In this study, a simple method for direct acid hydrolysis of phenolic secoiridoids in olive oil has been developed to produce measurable amount of Htyr and Tyr compounds. The suggested method was optimized in terms of acid type/concentration, type of organic solvents, mixing time, and temperature. The method under the optimized conditions was validated using high-performance liquid chromatography-diode-array detection (HPLC–DAD). The validated method was implemented for the analysis of phenolic compounds in real Jordanian olive oil samples. Furthermore, quantitative determination of all phenolic compounds (simple phenols and phenolic acids, secoiridoids, flavones, and lignans) in the collected samples was carried out using SPE method. The SPE outcome results have been very thoroughly compared with the results obtained from acid hydrolysis method.

Materials and Method

Chemicals and Standards

All organic solvents used in this study were HPLC grade. Methanol (MeOH) and acetonitrile (ACN) were purchased from Honeywell Fluka (USA). Ethanol (EtOH), dimethylformamide (DMF), and phosphoric acid (85%) were obtained from Scharlau (Spain). Hydrochloric acid (HCl) (37%) and sulfuric acid (H_2SO_4) (95–97%) were supplied from Honeywell Fluka (USA). n-Hexane was sourced from Sigma-Aldrich. Tyrosol (≥99.5%), *p*-hydroxyphenylactic acid (98.0%), and *o*-coumaric acid (97.0%) were purchased from Sigma-Aldrich (Germany). Hydroxytyrosol (99.9%) was purchased from Apollo Scientific (UK). The solid phase extraction (SPE) cartridges, packed with diol-bonded phase (LC-Diol, 500 mg, 3 mL), were obtained from Supelclean (USA). Ultrapure water (EC 0.055 µS/cm) was obtained from Milli-Q® system apparatus (Millipore, Germany). Standard stock solutions (1000 mg/L) of both hydroxytyrosol (Htyr) and tyrosol (Tyr) were prepared using a mixture of methanol/water (1:1) as diluent. Five working standard solutions of Htyr and Tyr were prepared in the range of 1–25 mg/L. Internal standard (I.S) solutions, *p*-hydroxyphenylacetic and *o*-coumaric acid, were prepared in methanol with concentrations of 0.12 mg/L and 0.010 mg/L, respectively. All stock

solutions were kept in the dark at – 20 °C and warmed up to room temperature before use.

Olive Oil Samples

Virgin and extra virgin olive oil samples from different Jordanian varieties were collected as follows: Nabali Baladi from Azraq, Ma'an and Az Zarqa, Rumi from Irbid, Nabali Mohasen from Ajloun and K18 from Azraq. The samples were collected in the period from October to December, 2019. The samples were stored in dark glass bottles at 4 °C until performing the analyses. All of the samples fulfilled the limits of the category extra virgin and virgin olive oil in terms of peroxide value, free acidity, and UV absorption characteristics (K_{232} and K_{270}) values (IOC, 2019).

Optimizing Hydrolysis and Extraction of Phenolic Compounds

Extra virgin olive oil sample (Nabali Baladi) was used as a reference sample to optimize the experimental conditions of hydrolysis and extraction of the phenolic compounds. A portion of 2.5 g oil was extracted using 50 mL of different solvent mixtures; (MeOH:H_2O 20:80, MeOH:H_2O 80:20, EtOH:H_2O 20:80, EtOH:H_2O 80:20, DMF:H_2O 20:80, DMF:H_2O 80:20, ACN:H_2O 20:80, ACN:H_2O 80:20 and 100% H_2O) containing 2.0 M HCl. The sample was incubated in an orbital shaking incubator at 250 rpm for 5 h at 25 °C. Then, 1.0 mL of the aqueous phase solution was taken and filtered through 0.22 µm cellulose acetate filter and directly analyzed by HPLC–DAD (see "Chromatographic Conditions" section). In order to investigate the effect of the acid type/concentration and temperature on the extraction and hydrolysis of phenolic compounds, eight olive oil samples were prepared as described previously, and then hydrolyzed with 50 mL of H_2SO_4 and HCl, with different concentrations (1.0 M and 2.0 M). The samples were agitated using shaking incubator at 250 rpm for 5 h at 25 °C and 75 °C. The extraction time was finally optimized by mixing 2.5 g of olive oil samples with 50 mL of 1.0 M and 2.0 M H_2SO_4 at different periods of time. The sample mixtures were placed in 250 mL glass bottles and then closed with a polypropylene cap. The samples were agitated at 250 rpm for 2, 4, 5, and 6 h at 75 °C. In the all studied samples, the hydrolyzed extracts were analyzed immediately (in the same day) using HPLC–DAD.

Chromatographic Conditions

Quantification of Tyr and Htyr compounds in olive oil samples was performed using high-performance liquid chromatography (HPLC; Agilent 1100) equipped with diode array detector. The column was Synergi 4u Fusion-RP C18 (4.0 mm i.d. × 250 mm, particle size 4 µm), (Phenomenex, Germany) maintained at 30 °C. The injection volume was 20 µL and the detection wavelength was set at 280 nm. The elution was performed at a flow rate of 1.0 mL/min, using water/phosphoric acid (95:5, v/v) (solvent A) and methanol (solvent B). The solvent gradient changed according to the following conditions: first, 90% of solvent A and 10% of solvent B were set as initial eluent composition for 10 min. The concentration of B solvent was increased to 30% over 8 min. Finally, the methanol percentage (solvent B) was increased to 100% over 7 min and maintained there for 10 min. The total time of analysis was 35 min.

Method Validation

Validation parameters, precision, accuracy, selectivity, sensitivity, limit of detection (LOD), limit of quantification (LOQ), linearity, and recovery, were used to assess the developed method. Linear calibration curves based on low concentration levels of Tyr and Htyr standard solutions (0.5, 1.0, 2.0, 3.0 and 5.0 mg/L) were established in order to determine the values of LOD and LOQ (supplementary information). The LOD and LOQ values were calculated based on the standard deviation of the response and the slope of Tyr and Htyr calibration curves, as reported in the ICH harmonized (ICH, 2021). The method selectivity was verified by the chromatographic resolution between Tyr and Htyr standard solutions (Fig. 3). Method accuracy was evaluated in terms of trueness and it was measured from the percent recovery when a known concentration of the target material was spiked in the olive oil sample (Sazali et al. 2019). In more details, the olive oil samples were spiked with 10 mg Htyr and Tyr per 20 g olive oil. The phenolic compounds in the spiked samples were extracted at the optimized extraction conditions and then analyzed using HPLC–DAD. The precision of the method was evaluated using intra- and inter-day (five consecutive days) repeatable measurements of Tyr and Htyr standard solutions. The precision was assessed using five replicates of spiked samples ($n = 5$) and the results were expressed in terms of % relative standard deviation (%RSD).

Identification and Quantification of Phenolic Compounds by SPE Method and HPLC–DAD Analysis

Identification and quantification of phenolic compounds were performed using SPE method developed by Mateos et al. (2001) with some modifications. Briefly, diol cartridge was placed in a vacuum elution apparatus (Thermo Scientific manifold) and pre-conditioned by passing 6.0 mL methanol and subsequently 6 mL n-hexane. The internal standard solutions (500 µL, *p*-hydroxyphenylacetic and *o*-coumaric acid dissolved in methanol) were added to 2.50 g of olive oil

sample (accurately weighed). The mixture was mixed thoroughly and the methanol was evaporated by gentle nitrogen flow at 25 °C. The sample was dissolved in 6.0 mL n-hexane and the resulting solution was loaded on the pre-conditioned SPE column, leaving the sample mixture on the solid phase under vacuum. The column was washed with 6.0 mL n-hexane and, subsequently, 4.0 mL of n-hexane:ethyl acetate (85:15, % v/v) solvent mixture until all nonpolar fractions were washed out. After that, 10.0 mL of methanol was passed through the column to elute the phenolic compounds. Then, the methanol was evaporated until dryness under a stream of nitrogen at 25 °C. The dried residue was then redissolved in 500 µL of methanol:water (1:1, %v/v) mixture and the solution was shaken for 1 min using vortex. The solution was then filtered through cellulose acetate syringe filter (0.45 µm) in to a HPLC vail. The vail was placed in the dark for at least 4 h at 25 °C prior chromatographic analysis.

Chromatographic analysis was performed according to the method reported by Mateos et al. (2001) with minor modifications. The extracted samples were analyzed using HPLC equipped with diode array detector. The phenol separation was achieved using Lichrospher 100RP18 column (4.0 mm i.d × 250 mm, particle size 5 µm) (Merck, USA). The separation was carried out using gradient elution with 0.5% phosphoric acid as eluent A and a mixture of methanol and acetonitrile (1:1, v/v) as eluent B. The eluent gradients were changed as the following: 95% of eluent A and 5% of eluent B as initial conditions for 15 min, 70% of eluent A and 30% of eluent B for 15 min, 62% of eluent A and 38% of eluent B for 5 min, 55% of eluent A and 45% of eluent B for 5 min, 47.5% of eluent A and 52.5% of eluent B for 5 min and 100% of eluent B for 5 min. Finally, 95% of eluent A and 5% of eluent B were passed through the column for 10 min. The flow rate was adjusted at 1.0 mL/min and the total analysis time was 60 min. The sample injection volume was 20 µL. The phenolic compounds were detected at 280, 235, and 335 nm. Quantitative analysis of Htyr, Tyr, dialdehydic form decarboxymethyl oleuropein aglycon (DDOA), dialdehydic form decarboxymethyl ligstroside aglycone (DDLA), aldehydic form oleuropein aglycon (AOA), aldehydic form ligstroside aglycone (AOL), vanilic acid, vanilin, *p*-coumaric acid, tyrosyl acetate, pinoresinol, and 1-acetoxypinoresinol was carried out at 280 nm using *p*-hydroxyphenylacetic acid as internal standard. The ferulic acid, luteolin, and apigenin compounds were quantified at 335 nm using *o*-coumaric acid as internal standard.

The total phenolic compounds were calculated as the sum of individual phenols.

Statistical Analysis of the Experimental Data

All experiments were carried out in duplicate and the results were expressed as mean ± standard deviation. The data were analyzed using the analysis of variance (ANOVA) procedure. Duncan's multiple range test range test ($p < 0.05$) was applied to determine the significant difference between the means.

Results and Discussion

Optimizing Phenolic Compound Extraction

In this study we present the effect of various parameters that are involved in the direct acid hydrolysis and extraction of the free and bounded forms of tyrosol (Tyr) and hydroxytyrosol (Htyr) in the olive oil.

Effect of Solvents on Phenolic Compound Extraction

The acid hydrolysis treatment of olive oil was conducted using different solvents (MeOH, EtOH, ACN, DMF, and H_2O) mixed with 2.0 M HCl. The extraction efficiency for these solvents was investigated at fixed shaking time and temperature (Fig. 1). Compared to other solvent mixtures, MeOH:H_2O and ACN:H_2O (80:20, v/v) gave slightly higher extraction phenolic yield and showed similar phenolic compound recovery (155.50 ± 2.00 mg/kg). In previous studies, the liquid–liquid extraction method was adopted for the recovery of phenolic compounds from olive oil samples using water and organic solvent mixtures. The results showed that a maximum yield of phenolic compounds was achieved when organic solvent–water mixture, containing 80%, v/v MeOH (Bartella et al. 2020) or 80%, v/v DMF (Brenes et al. 2000a), were used. However, in the proposed acid hydrolysis method, the yield of phenolic compounds was maximized at water as solvent (160.00 ± 2.00 mg/kg) (Fig. 1). This was in agreement with another study which reported the extraction of phenolic compounds such as oleuropein, phenolic acids, phenolic alcohols, and flavonoids from olive leaves using water (Irakli et al. 2018). The increase of organic solvent concentration (more than 20%) reduces the recovery of phenolic compounds (data not shown). Based on this result, water was used as extraction solvent in the acid hydrolysis treatment. The high content of phenolic compounds extracted by water could be related to their higher polarity and better solubility of phenolic components present in olive oil.

Effect of Acid Type/Concentration and Temperature on Phenolic Compound Extraction

In the subsequent step, hydrolytic procedures with different conditions such as temperature and type/concentration of acid were performed to identify the optimal hydrolysis and separation of phenols. Table 1 shows that 152.52 ± 3.95 mg/

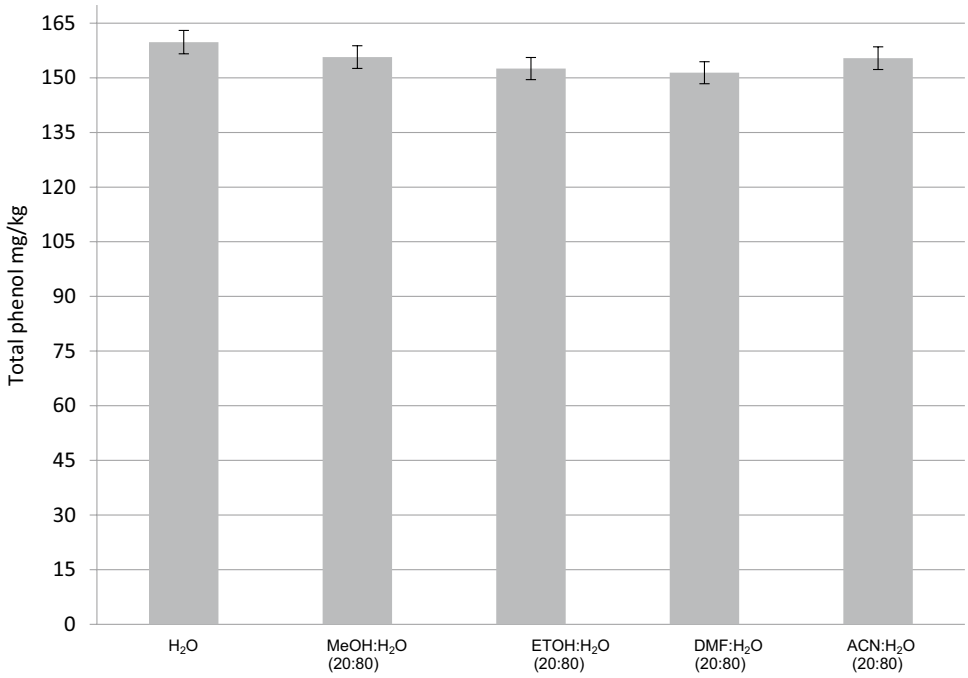

Fig. 1 Total phenolic compounds extracted using different solvent ratios and 2.00 M HCl. The samples were incubated in shaker incubator for 5 h at 25 °C. The analyses were performed in two replicates. Different letters are significantly different for each extract separately ($p < 0.05$)

Table 1 Total phenolic compounds extracted from olive oil sample at different hydrolysis parameters: (i) the acid used for hydrolysis (HCl = 1.00 M and 2.00 M; H_2SO_4 = 1.00 M and 2.00 M); (ii) the hydrolysis temperature 25 °C and 75 °C; (iii) the hydrolysis time was kept constant at 5 h

Acid type	Acid concentration (M)	Temperature °C	Htyr mg/kg	Tyr mg/kg	Total phenol mg/kg ± SD
HCl	1.00	25	70.41 ± 3.64^a	65.62 ± 4.71^b	136.03 ± 1.07^c
HCl	1.00	75	79.97 ± 2.93^a	72.55 ± 1.98^b	152.52 ± 3.95^c
HCl	2.00	25	80.44 ± 3.41^a	71.55 ± 0.32^b	151.99 ± 3.09^c
HCl	2.00	75	89.23 ± 3.87^a	76.64 ± 4.21^b	165.86 ± 0.34^c
H_2SO_4	1.00	25	67.96 ± 2.06^a	63.61 ± 3.91^b	131.56 ± 4.97^c
H_2SO_4	1.00	75	89.62 ± 1.05^a	76.44 ± 4.00^b	166.06 ± 2.95^c
H_2SO_4	2.00	25	74.20 ± 3.11^a	67.76 ± 2.31^b	141.95 ± 4.79^c
H_2SO_4	2.00	75	100.58 ± 1.54^a	83.00 ± 0.22^b	183.58 ± 1.32^c

Values are means ± standard deviation ($n = 3$). Different letters within a row are significantly different ($p < 0.05$)

kg of Tyr and Htyr were recovered from the treatment of the olive oil sample with 1.00 M HCl at 75 °C. This value was reduced to 136.03 ± 1.07 mg/kg when the same process was carried out at 25 °C. The effect of temperature on the phenol yield was more significant when H_2SO_4 was used as hydrolytic acid, resulting in 23% higher yield when the sample was extracted at 75 °C compared to 25 °C. The rising of temperature caused a significant improvement of the wetting ability of the olive oil sample and resulting in an enhancement of water penetration power within the olive oil matrix and so higher extraction yield of the target compounds was obtained. Increasing the temperatures to higher than 75 °C was avoided since the phenolic compounds could be oxidized or degraded at high temperatures (Czemplik et al. 2017; Taamalli et al. 2012). Table 1 also shows that the treatment of the olive oil samples with 2.0 M acids gave a higher recovery of phenolic compounds compared to 1.0 M acid concentrations. For example, 151.99 ± 3.09 mg/kg of total phenolic content was obtained when the olive oil samples were treated with 2.0 M HCl, while only 136.03 ± 1.07 mg/kg was obtained after hydrolysis the samples using 1.0 M HCl at the same temperature (25 °C). The high recovery of phenolic compounds obtained with 2.0 M of the acid concentration could be attributed to the releasing of non-extractable phenolic acids (insoluble phenolic acids) such as caffeic acid, chlorogenic acid, *p*-coumaric acid, ferulic acid, *p*-hydroxybenzoic acid, protocatechuic acid, salicylic acid, sinapic acid, syringic acid, and vanillic acid (Pérez-Jiménez and Torres, 2011). These phenolic acids have been identified practically in cereal, black olives, and black currant pomace samples after the hydrolysis process using acid concentration (2.0 M-6.0 M HCl or H_2SO_4). Here, the acid hydrolysis process at high acid concentration (more than 2.0 M) resulted in a reduction in the phenol recovery (data

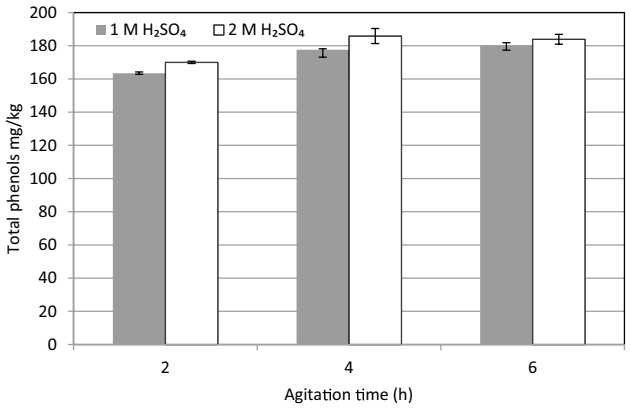

Fig. 2 Effect of agitation time on total phenols extracted from olive oil sample using H_2SO_4 (1.00 M and 2.00 M) at 75 °C The analyses were performed in two replicates. Different letters are significantly different for each extract separately ($p < 0.05$)

not shown), probably due to the effect of high concentration in the degradation of these compounds. It should be noted that the H_2SO_4 was more efficient to hydrolyze phenolic secoiridoids in olive oil than the HCl. Table 1 shows that higher content of phenol (183.58 ± 1.32 mg/kg) was achieved when 2.0 M H_2SO_4 was used at 75 °C compared to 165.86 ± 0.34 mg/kg when 2.0 M HCl was used at the same temperature. The using of H_2SO_4 as hydrolytic acid is also in agreement with previous studies, where the H_2SO_4 has been applied successfully to hydrolyze phenolic compounds in many food matrices such as cereal (Arranz et al. 2010; Balli et al. 2020a, b), fruits (Arranz et al. 2009), vegetables (Saura-Calixto et al. 2007), and wheat bran (Arranz and Saura Calixto, 2010). The stability of phenolic compounds during acid hydrolysis extraction process could be investigated in the future, especially the stability of easily degradable phenolic compounds such as Htyr.

Effect of Time

The extraction (mixing) time was optimized to achieve a maximum recovery of phenolic compounds within short period of extraction time. Olive oil samples were incubated in 50 mL H_2SO_4 solutions (1.00 M and 2.00 M) at 75 °C, and the extraction time was changed in the range of 2–6 h. Figure 2 shows that the extraction efficiency of phenolic compounds was quite low when the mixing process was maintained for 2 h and it increased over time for both acid concentrations (1.00 M and 2.00). An increase in the yield of phenolic compounds from 163.50 mg/kg to 177.60 mg/kg at 1.0 M H_2SO_4 was observed when the extraction time maintained at 4 h. Increasing the hydrolysis time to 6 h caused a reduction in the extractable phenolic compounds from 186.90 mg/kg to 183.80 mg/kg when the sample was treated with 2.0 M H_2SO_4 at 75 °C. Accordingly, 4 h was enough to obtain complete hydrolysis of secoiridoid aglycons and so the diffusion of Tyr and Htyr from the olive oil matrix to the solution. In summary, high recovery of total phenolic compounds was obtained when the olive oil sample was hydrolyzed with 2.00 M H_2SO_4 for 4 h at 75 °C. In comparison with previous studies, the extraction of phenolic compounds from olive oil samples was carried out using 2.00 M HCl and the mixture was mixed for 4–6 h at 25 °C (Romero and Brenes 2012). The results indicated that the acid hydrolysis of secoiridoid aglycone was completely accomplished under the optimized conditions by liberation of the maximum amount of phenolic compounds.

Method Validation

The validation of the proposed analytical method for the determination of Hytr and Tyr in olive oil samples followed the International Council for Harmonisation (ICH) guidelines (ICH, 2021). The linearity, precision, recovery, limit of detection (LOD), and limit of quantitation (LOQ), selectivity and sensitivity were investigated. The validation parameters tested are summarized in Table 2. The linearity of the method was examined in the range 1–25 mg/L for both Hytr and Tyr compounds. The results showed a good linear response for the two compounds as the correlation coefficient values (R^2) of the calibration curves were found to be ≥ 0.999 (supplementary information). The LOD refers to the minimum concentration of both Tyr and Hytr that can be detected but not quantified while the LOQ associated with the minimum concentration of both compounds that can be detected and accurately quantified (ICH 2021). The results indicated that Hytr had LOD of 0.22 mg/L and LOQ of 0.69 mg/L while the LOD and LOQ of Tyr were 0.10 mg/L and 0.56 mg/L, respectively. Method precision was studied in terms of intra- and inter-day assay using Hytr and Tyr standard solutions. The intra-day precision of the two compounds was evaluated by calculating the percent relative standard deviation (%RSD) for replicates ($n = 5$). The inter-day precision was determined by analyzing the both standard solutions within five consecutive days. The % RSD values for the intra-day measurements were 1.3% for Hytr and 2.3% for Tyr while the % RSD values for the inter-day measurements were 0.7% and 1.7% for Hytr and Tyr, respectively. The results indicated a high repeatability of the method since the results were lower than those set by the Association of Official Analytical Chemists (AOAC) (%RSD limit: 15% for 0.1 mg/kg; 11% for 1 mg/kg; 7.3% for 10 mg/kg and 5.3% for 100 mg/kg) (AOAC 2016). Previous studies had indicated that the %RSD for intra-day values were 3.0% and 0.6% for Htyr and Tyr, respectively, while the %RSD for inter-day values were 3.7% for Htyr and 2.3% for Tyr. These results were obtained when a single solution containing 5 µg/

Table 2 Validation parameters for acid hydrolysis method

Phenolic compounds	Rt (min)	Range of linear calibration (mg/L)	Slope	Intercept	Linearity R^2	LOD (mg/)	LOQ (mg/)	Recovery (%)	Inter-day precision (%RSD, $n = 5$)	Intra-day precision (%RSD, $n = 5$)
Hydroxytyrosol	7.8	1.0–25.0	14.82	8.199	0.999	0.22	0.69	109.9	0.7	1.3
Tyrosol	11.7	1.0–25.0	11.79	1.7183	0.999	0.10	0.56	94.8	1.7	2.3

Rt, Retention time; R^2, squared regression coefficient; *RSD*, relative standard deviation

mL of each compound was used in precision study (Godoy-Caballero et al. 2013). The effectiveness of the hydrolytic procedure in the extraction of the phenolic compounds was investigated by evaluating the recovery of Htyr and Tyr, previously spiked into the olive oil samples (see "Materials and Method" part). The phenolic compounds were extracted at the optimized conditions described previously, and the analysis was performed using HPLC–DAD. The results showed that 94.8% recovery of the Tyr was obtained while higher recovery (109.9%) was observed for Htyr (Table 2). The difference in the percent recovery between Tyr and Htyr refers to their polarities. Tyr showed lower recovery due to its lower polarity compared to Htyr. Generally, the obtained recovery values for both Tyr and Htyr were acceptable and met the normal range of recovery (80–110%), proposed by the Association of Official Analytical Chemists (AOAC 2016). In a previous study carried out by Tsimidou et al. (2019), the percent recovery of Htyr and Tyr was 105.0% and 87.6% respectively (Tsimidou et al. 2019), suggesting a similar trend as observed in this study. The selectivity measurement investigates the ability of the method to distinguish the peaks of the analytes from the peaks of the interferences (ICH 2021). The HPLC chromatogram (Fig. 3) shows good separation and resolution between Tyr and Htyr peaks, indicating a high selectivity of the method to determine both Htyr and Tyr. In addition, the proposed analytical method was sensitive as it gave a measurable peaks for both Htyr and Tyr when low concentrations of both compounds were analyzed using the HPLC–DAD (Fig. 3).

Analysis of Real Olive Oil Samples Using the Validated Method

Recently, the interest in Jordanian olive oil has increased due to their specific organoleptic qualities and high production quantity (34,500 tons of olive oil produced in 2019) (Mukundi, 2021). So far, there is no sufficient data about the content of phenolic compounds in Jordanian olive oil varieties and all the previous published studies were limited to investigate the phenolic compounds in Jordanian olive oil mill wastewater (Deeb et al. 2012) and olive cake (Alhamad et al. 2017). The validated method in this study was used to quantify the phenolic compound content existing in virgin and extra virgin olive oil samples collected from different locations in Jordan at different harvesting times. The collected samples were classified as the following: from 1/10 to 30/10 and from 1/11 to 30/12 for the season 2019. The major varieties tested were Nabali Baladi, Nabali Mohasen, K18, and Rumi (Table 3). The analysis of the phenolic compounds in different Jordanian olive oil varieties showed significant differences, which mainly depended on the harvesting time. The results in Table 3 show the samples being harvested in early time (1/10–30/10) containing higher content of phenolic

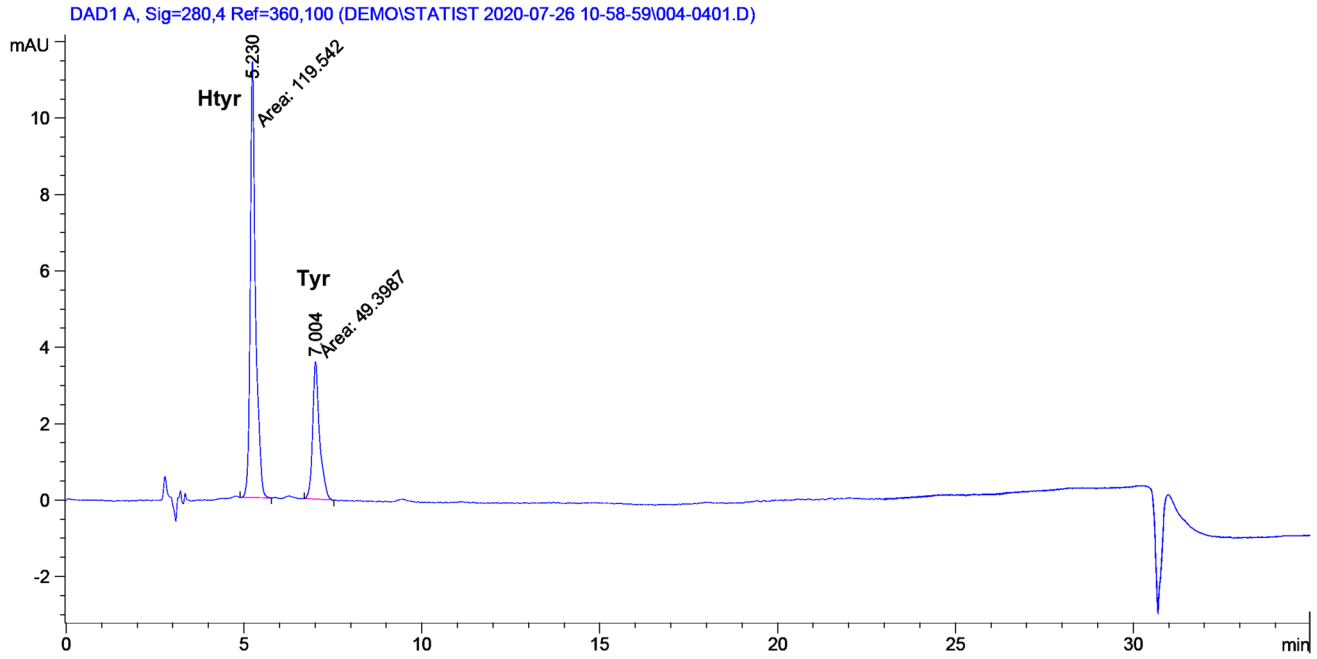

Fig. 3 HPLC–DAD profiles of the Tyr and Htyr standards at 280 nm

compounds than samples harvested at the end of the season (1/11–30/12), with one exception for Nebali Baladi/Ma'an sample. The previous trend is not unusual and has been previously reported by other studies (Dag et al. 2011; De Lorgeril et al. 1999; López-Yerena et al. 2019) which reported reduction in phenol levels at later harvest time of the olive. The delay in harvesting time might have the effect of reducing the activity of endogenous enzymes (polyphenol oxidase, peroxidase, and β-glucosidase), which are involved in the biosynthesis of phenolic compounds in olives during fruit ripening stage (Cirilli et al. 2017; Hachicha Hbaieb et al. 2015). The analyzed oil varieties in Table 3 show a large range of variability in the total phenolic compound amounts; the K18 variety harvested in early time showed the highest phenolic compound content (421.70 ± 4.90 mg/kg), followed by Rumi and Nabali Baladi varieties with 318.20 ± 4.30 mg/kg and 254.50 ± 3.50 mg/kg of phenols, respectively. Interestingly, the phenols recovered from Nabali Mohasen depended on the climate and the location. This variety showed very a low phenolic compound level in Azraq area (33.20 ± 1.20 mg/kg) while the content of phenolic compounds was increased to (244.80 ± 1.50 mg/kg) when this variety sourced from Ajloun (Table 3). The content of each individual phenolic compound Tyr and Htyr obtained for all the analyzed oils is reported in Table 3. The results obtained from low phenolic compound samples (less than 200 mg/kg) highlighted a predominance of Tyr compound in the acid extract such as Nabali Baladi/Azraq sample containing total phenol of 138.29 ± 3.00 mg/kg and Rumi/Irbid (2) samples (total phenol 74.34 ± 3.90 mg/kg). On the contrary, the Htyr was largely predominant in the high phenol samples (more than 200 mg/kg). For example, the extracted contents of Htyr and Tyr were 202.46 ± 1.73 mg/kg and 115.73 ± 1.30 mg/kg, respectively, in Rumi/Irbid (1) sample (total phenol 318.19 ± 4.30 mg/kg). This trend was also observed in K18/Azraq sample containing total phenol of 421.70 ± 4.90 mg/kg.

The results in Table 3 also show that a significant number of Jordanian olive oil samples (5 out of 16 samples) reached the minimum amount of phenolic compounds (5 mg/20g_{oil}), requested by the EFSA for the application of the polyphenol health claim (EFSA, 2011). The K18 variety which originated from Azraq has the highest value (8.43 mg/20g_{oil}), followed by Nabali Baladi/Ma'an (6.17 mg/20g_{oil}) and Rumi/Irbid (1) (6.36 mg/20g_{oil}). These findings provide the Jordanian olive oil advantages over some of extra virgin olive oil from the Italian retail market which found only 3 samples out of 32 samples passed the EFSA claim limit (Caporaso et al.2015a, b).

Identification and Quantification of Phenolic Compounds in Olive Oil Samples by SPE Method

The SPE technique has been implemented ("Materials and Method" part) to determine the individual phenolic compounds extracted from the collected olive oil samples. Figure 4 illustrates representative HPLC chromatograms of the phenolic compounds obtained from olive oil extract at wavelengths of 280 nm and 340 nm.

Table 3 The main phenolic compounds (mg/kg) present in the olive oil samples extracted by acid hydrolysis

Variety/location	Harvesting time					
	1/10–30/10			1/11–30/12		
	Htyr mg/kg	Tyr mg/kg	Total phenol mg/kg ± SD	Htyr mg/kg	Tyr mg/kg	Total phenol mg/kg ± SD
K18/Azraq	224.87 ± 1.50	196.83 ± 1.21	421.70 ± 4.90[a]	57.61 ± 1.05	80.40 ± 0.69	138.01 ± 1.50 [b]
Nabali Mahasen/Azraq	11.74 ± 0.85	21.47 ± 0.36	33.21 ± 1.20[a]	5.82 ± 0.37	16.84 ± 0.12	22.66 ± 1.00 [b]
Nabali Baladi/Azraq	136.07 ± 0.80	118.38 ± 1.50	254.45 ± 3.50[a]	50.05 ± 1.25	88.24 ± 1.45	138.29 ± 3.00 [b]
Nabali Baladi/Az Zarqa	84.47 ± 0.85	114.66 ± 0.77	199.10 ± 1.10[a]	31.17 ± 1.10	33.83 ± 1.20	65.00 ± 2.10[b]
Nabali Mohasen/Ajloun	143.55 ± 1.70	101.26 ± 1.10	244.81 ± 1.50[a]	90.38 ± 1.45	68.07 ± 1.70	158.45 ± 1.80[b]
Nabali Baladi/ Ma'an	68.53 ± 0.51	59.94 ± 2.10	128.47 ± 3.00[a]	218.41 ± 6.10	89.90 ± 4.50	308.31 ± 8.30[b]
Rumi/Irbid (1)	202.46 ± 1.73	115.73 ± 1.30	318.19 ± 4.30[a]	93.93 ± 2.53	76.98 ± 3.75	170.91 ± 5.30[b]
Rumi/Irbid (2)	63.02 ± 2.25	74.81 ± 1.80	137.83 ± 4.40[a]	24.29 ± 2.58	50.05 ± 3.80	74.34 ± 3.90[b]

Values are means ± standard deviation ($n = 2$). Different letters within a row correspond to statistically different means ($p < 0.05$)

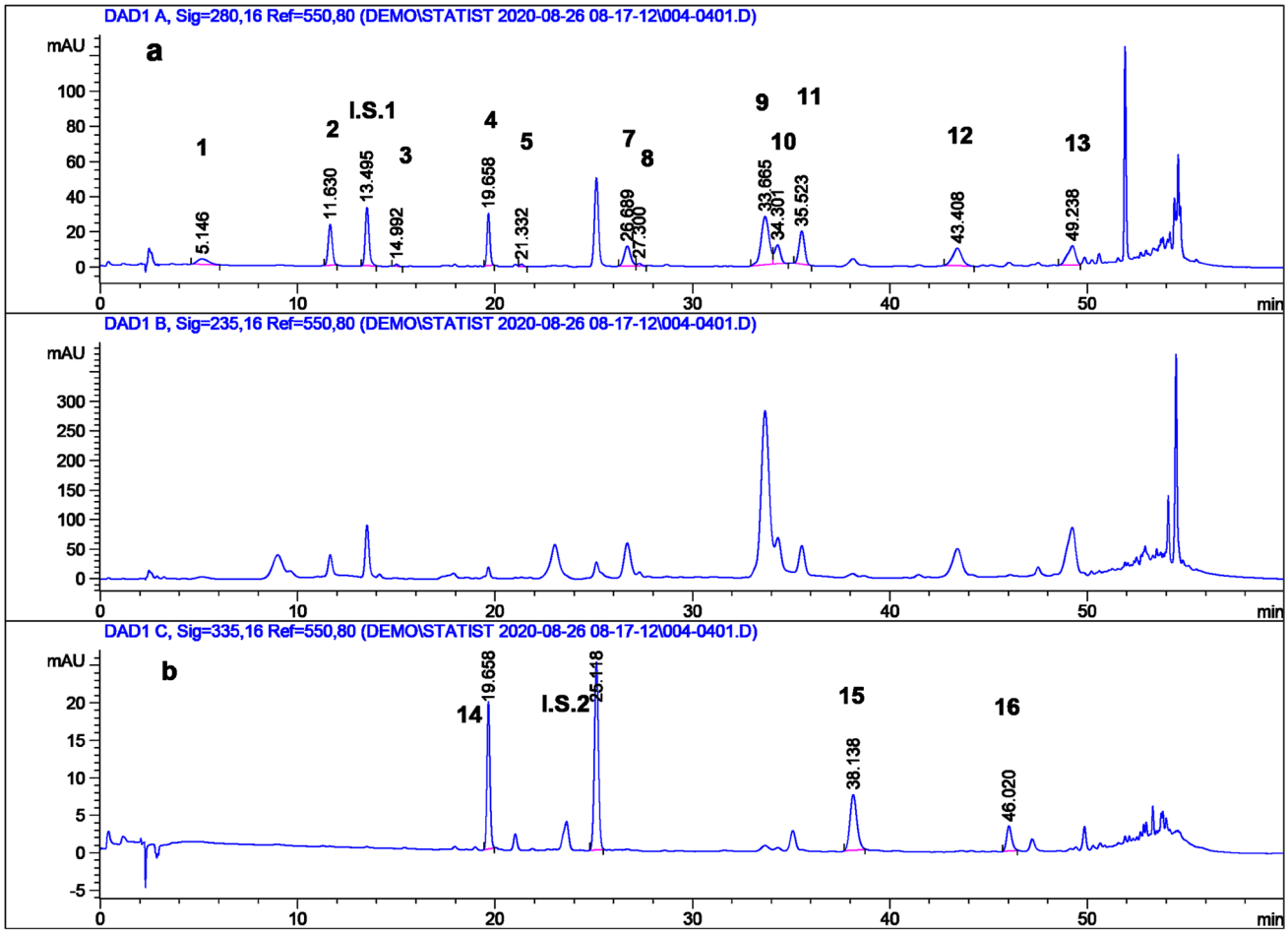

Fig. 4 **a** Profile of the phenolic compounds extracted from olive oil and detected by HPLC at 280 nm. Peaks: (1) hydroxytyrosol, (2) tyrosol, (I.S) p-hydroxyphenylacetic acid (internal standard), (3) vanillic acid, (4) vanillin, (5) p-coumaric, (6) ferulic acid, (7) dialdehydic form decarboxymethyl oleuropein aglycon, (8) tyrosyl acetate, (9) dialdehydic form decarboxymethyl ligstroside aglycon, (10) pinoresinol, (11) 1-acetoxypinoresinol, (12) aldehydic form oleuropein aglycon, and (13) aldehydic form of oleuropein aglycone. **b** Profile of the phenolic compounds extracted from olive oil and detected by HPLC at 335 nm. Peaks: (14) ferulic acid, o-coumaric acid (I.S.2) (15) luteolin, (16) apigenin

Table 4 The main phenolic compounds (mg/kg) present in the olive oil samples harvested on 1/10–30/10

Peak	Compound	K18/Azraq	Nabali Mohasen/Azraq	Nabali Baladi/ Azraq	Nabali Baladi/ Az Zarqa	Nabali Mohasen/ Ajloun	Nabali Baladi/ Ma'an	Rumi/Irbid (1)	Rumi/Irbid (2)
1	Hydroxytyrosol	7.40 ± 0.24[a]	0.00[b]	10.44 ± 0.47[c]	7.09 ± 0.10[a]	7.96 ± 0.19[d]	3.51 ± 0.87[e]	40.55 ± 2.96[f]	11.17 ± 1.26[g]
2	Tyrosol	11.30 ± 0.42[a]	3.82 ± 0.02[b]	15.34 ± 0.93[c]	10.65 ± 0.10[d]	4.99 ± 0.22[e]	4.17 ± 0.52[e]	101.88 ± 3.69[f]	43.91 ± 4.35[g]
3	Vanillic acid	1.90 ± 0.24[a]	0.35 ± 0.00[b]	0.98 ± 0.34[c]	0.00[d]	0.07 ± 0.00[d]	0.33 ± 0.30[e]	0.57 ± 0.81[e]	0.62 ± 0.08[e]
4	Vanillin	0.00[a]	0.89 ± 0.01[b]	0.72 ± 0.72[b]	0.75 ± 0.10[b]	0.37 ± 0.01[c]	0.11 ± 0.03[d]	0.37 ± 0.52[c]	1.49 ± 0.12[e]
5	p-Coumaric acid	0.00[a]	0.09 ± 0.01[a]	0.00[a]	0.14 ± 0.10[b]	0.49 ± 0.03[c]	0.04 ± 0.01[a]	0.00[a]	0.09 ± 0.01[a]
7	DDOA[a]	74.80 ± 3.40[a]	2.59 ± 0.75[b]	64.17 ± 6.95[c]	59.07 ± 1.00[d]	101.73 ± 2.96[e]	34.58 ± 6.05[f]	27.32 ± 3.11[g]	1.29 ± 0.09[h]
8	Tyrosyl acetate	17.60 ± 1.30[a]	0.00[b]	2.40 ± 1.31[c]	0.00[b]	8.65 ± 0.21[d]	7.40 ± 1.67[e]	4.40 ± 1.79[f]	1.36 ± 0.24[g]
9	DDLA[b]	119.20 ± 5.40[a]	3.08 ± 0.03[b]	125.03 ± 8.09[a]	148.07 ± 1.00[c]	119.51 ± 5.55[a]	66.89 ± 3.59[d]	41.09 ± 0.59[e]	17.62 ± 1.34[f]
10	Pinoresinol	3.80 ± 0.16[a]	1.17 ± 0.02[b]	4.99 ± 0.29[c]	4.12 ± 0.10[a]	3.85 ± 0.19[a]	4.52 ± 0.52[a]	2.92 ± 0.10[d]	1.69 ± 0.13[b]
11	Acetoxypinoresinol	15.20 ± 0.75[a]	4.18 ± 0.04[b]	30.17 ± 1.83[c]	3.69 ± 0.10[b]	19.19 ± 4.57[d]	12.81 ± 1.62[e]	8.33 ± 0.47[f]	18.09 ± 1.97[g]
12	AOA[c]	116.80 ± 3.4[a]	3.43 ± 0.45[b]	94.96 ± 0.28[c]	69.82 ± 1.00[d]	111.26 ± 3.09[a]	18.27 ± 2.99[e]	72.46 ± 5.57[f]	59.25 ± 7.17[g]
13	AOL[d]	68.30 ± 3.4[a]	1.64 ± 0.15[b]	71.91 ± 5.49[c]	88.41 ± 1.00[d]	47.75 ± 2.03[e]	15.88 ± 2.28[f]	60.80 ± 3.66[a]	70.88 ± 8.14[c]
14	Ferulic acid	0.30 ± 0.05[a]	2.49 ± 0.04[b]	6.39 ± 0.27[c]	1.76 ± 0.10[d]	0.72 ± 0.03[e]	0.23 ± 0.01[e]	2.01 ± 0.08[b]	6.97 ± 4.24[c]
15	Luteolin	7.90 ± 0.51[a]	7.49 ± 0.61[a]	19.57 ± 0.32[b]	2.45 ± 0.10[c]	7.12 ± 0.17[d]	9.55 ± 1.45[e]	7.51 ± 0.41[d]	5.11 ± 0.57[f]
16	Apigenin	3.40 ± 0.15[a]	3.27 ± 0.07[a]	7.57 ± 0.41[b]	0.71 ± 0.10[c]	3.35 ± 0.06[a]	6.76 ± 0.94[b]	2.97 ± 0.04[d]	2.51 ± 0.25[d]
Total phenols (ppm)		448.00 ± 6.24[a]	34.49 ± 1.15[b]	454.65 ± 10.25[c]	396.72 ± 4.00[d]	437.02 ± 19.33[a]	185.08 ± 17.69[e]	373.19 ± 14.56[f]	242.08 ± 29.77[g]

[a]Dialdehydic form decarboxymethyl oleuropein aglycon; [b]dialdehydic form decarboxymethyl ligstroside aglycon; [c]aldehydic form oleuropein aglycon; [d]aldehydic form ligstroside aglycon. Values are means ± standard deviation ($n = 2$). Different letters within a row are significantly different ($p < 0.05$)

Table 5 The main phenolic compounds (mg/kg) present in the olive oil samples harvested on 1/11–30/12

Peak	Compound	K18/Azraq	Nabali Mohasen/ Azraq	Nabali Baladi/ Azraq	Nabali Baladi/ Az Zarqa	Nabali Mohasen/ Ajloun	Nabali Baladi/ Ma'an	Rumi/Irbid (1)	Rumi/Irbid (2)
1	Hydroxytyrosol	4.80 ± 0.14[a]	0.00[b]	2.45 ± 0.10[c]	1.42 ± 0.29[d]	24.97 ± 3.93[e]	8.46 ± 1.21[f]	15.93 ± 1.83[g]	6.45 ± 0.91[h]
2	Tyrosol	15.70 ± 0.42[a]	5.61 ± 0.24[b]	15.36 ± 1.00[a]	21.53 ± 1.08[c]	80.03 ± 1.38[d]	4.90 ± 0.84[e]	28.30 ± 2.44[f]	30.97 ± 2.96[g]
3	Vanillic acid	0.20 ± 0.0[a]	0.51 ± 0.02[b]	0.00[c]	0.09 ± 0.01[d]	0.36 ± 0.01[e]	0.00[c]	0.62 ± 0.03[b]	0.25 ± 0.04[e]
4	Vanillin	2.25 ± 0.07[a]	1.63 ± 0.04[b]	1.71 ± 0.10[b]	0.17 ± 0.02[c]	1.22 ± 0.11[d]	0.10 ± 0.02[c]	0.31 ± 0.14[e]	0.08 ± 0.00[c]
5	p-Coumaric acid	0.10 ± 0.0[a]	0.12 ± 0.00[a]	0.09 ± 0.1[a]	0.00[b]	0.00[b]	0.00[b]	1.03 ± 0.09[c]	0.26 ± 0.03[d]
7	DDOA[a]	17.25 ± 1.77[a]	1.13 ± 0.13[b]	8.38 ± 1.00[c]	1.28 ± 0.36[b]	3.29 ± 1.66[d]	220.61 ± 38.51[e]	4.11 ± 0.09[f]	0.84 ± 0.27[g]
8	Tyrosyl acetate	1.40 ± 0.0[a]	0.00[b]	1.51 ± 0.10[a]	0.72 ± 0.03[b]	0.00[b]	16.79 ± 2.79[c]	0.81 ± 0.25[d]	0.40 ± 0.15[e]
9	DDLA[b]	78.60 ± 0.57[a]	5.29 ± 0.22[b]	55.84 ± 1.00[c]	9.59 ± 0.12[d]	3.89 ± 0.72[e]	178.81 ± 27.84[f]	10.32 ± 0.77[g]	1.05 ± 0.09[h]
10	Pinoresinol	2.45 ± 0.07[a]	0.87 ± 0.03[b]	1.76 ± 0.10[c]	0.32 ± 0.00[d]	1.64 ± 0.11[e]	4.85 ± 0.01[f]	2.39 ± 0.17[g]	0.83 ± 0.02[h]
11	Acetoxypinoresinol	11.65 ± 0.21[a]	6.82 ± 0.51[b]	0.00[c]	2.59 ± 0.06[d]	6.48 ± 0.05[e]	7.47 ± 0.69[f]	30.59 ± 2.69[g]	8.56 ± 0.25[h]
12	AOA[c]	28.20 ± 2.12[a]	0.00[b]	25.20 ± 1.00[c]	5.05 ± 0.52[d]	29.03 ± 2.23[e]	91.33 ± 16.45[f]	51.09 ± 6.88[g]	9.62 ± 2.63[h]
13	AOL[d]	36.65 ± 0.92[a]	5.75 ± 0.32[b]	10.25 ± 1.00[c]	13.56 ± 1.11[d]	12.67 ± 0.25[e]	31.55 ± 5.72[f]	47.32 ± 4.09[g]	17.86 ± 2.58[h]
14	Ferulic acid	6.25 ± 0.21[a]	4.45 ± 0.12[b]	4.79 ± 0.10[b]	9.93 ± 0.54[c]	3.32 ± 0.19[d]	0.37 ± 0.19[e]	1.98 ± 0.13[f]	1.19 ± 0.09[g]
15	Luteolin	8.10 ± 0.14[a]	8.56 ± 0.99[a]	2.11 ± 0.10[b]	2.43 ± 0.22[b]	31.94 ± 1.22[c]	18.78 ± 3.69[d]	9.26 ± 0.51[e]	4.89 ± 0.11[f]
16	Apigenin	2.75 ± 0.07[a]	4.12 ± 0.61[b]	1.04 ± 0.10[c]	1.08 ± 0.18[c]	10.97 ± 1.50[d]	7.79 ± 1.58[e]	4.14 ± 0.34[f]	2.46 ± 0.23[g]
Total phenols (ppm)		216.35 ± 3.18[a]	44.87 ± 2.97[b]	130.50 ± 3.00[c]	69.75 ± 4.52[d]	209.83 ± 4.35[e]	591.84 ± 98.17[f]	208.20 ± 20.12[g]	58.73 ± 8.44[h]

[a]Dialdehydic form decarboxymethyl oleuropein aglycon; [b]dialdehydic form decarboxymethyl ligstroside aglycon; [c]aldehydic form oleuropein aglycon; [d]aldehydic form ligstroside aglycon. Values are means ± standard deviation ($n = 2$). Different letters within a row are significantly different ($p < 0.05$)

Table 6 Total phenolic compound content of olive oil samples analyzed by solid phase extraction and acid hydrolysis

Variety/location	Harvesting time	Total phenols by SPE (mg/kg)	Total phenols by acid hydrolysis (mg/kg)
K18/Azraq	1/10–30/10	448.00 ± 6.20^{a}	421.70 ± 4.90^{b}
Nabali Mohasen/Azraq	1/10–30/10	34.50 ± 1.20^{a}	33.20 ± 1.20^{a}
Nabali Baladi/ Azraq	1/10–30/10	454.70 ± 10.30^{a}	254.50 ± 3.50^{b}
Nabali Baladi/Az Zarqa	1/10–30/10	396.70 ± 4.00^{a}	199.10 ± 1.10^{b}
Nabali Mohasen/Ajloun	1/10–30/10	437.00 ± 19.30^{a}	244.80 ± 1.50^{b}
Nabali Baladi/Ma'an	1/10–30/10	185.10 ± 17.70^{a}	128.40 ± 3.00^{b}
Rumi/Irbid (1)	1/10–30/10	373.20 ± 14.60^{a}	318.20 ± 4.30^{b}
Rumi/Irbid (2)	1/10–30/10	242.10 ± 29.80^{a}	137.80 ± 4.40^{b}
K18/Azraq	1/11–30/12	216.40 ± 3.20^{a}	138.00 ± 1.50^{b}
Nabali Mohasen/Azraq	1/11–30/12	44.90 ± 3.00^{a}	22.60 ± 1.00^{b}
Nabali Baladi/ Azraq	1/11–30/12	130.50 ± 3.00^{a}	138.30 ± 3.00^{a}
Nabali Baladi/Az Zarqa	1/11–30/12	69.80 ± 4.50^{a}	65.00 ± 2.10^{b}
Nabali Mohasen/Ajloun	1/11–30/12	209.80 ± 4.40^{a}	158.40 ± 1.80^{b}
Nabali Baladi/Ma'an	1/11–30/12	591.90 ± 98.20^{a}	308.30 ± 8.30^{b}
Rumi/Irbid (1)	1/11–30/12	208.20 ± 20.10^{a}	170.90 ± 5.30^{b}
Rumi/Irbid (2)	1/11–30/12	58.70 ± 8.40^{a}	74.30 ± 3.90^{b}

Values are means ± standard deviation ($n = 2$); values bearing different lowercase letters as superscripts for each sample and the same analyte determined by the two techniques are statistically different at $p < 0.05$ (paired *t*-test)

The phenolic compounds and their amounts identified in the Jordanian olive oil samples being harvested on (1/10–30/10) and (1/11–30/12) are presented in Tables 4 and 5, respectively. The main identified phenols in olive oil samples were Htyr and Tyr (20–142 mg/kg) and their derivatives (157–540 mg/kg) (Table 4). These compounds belong to the secoiridoid group, which are the most characteristic compounds in olives and extra virgin olive oil. The other phenolic compounds such as vanilic acid, vanillin, *p*-coumaric acid, flavones (luteolin and apigenin), and lignans (pinoresinol and 1-acetoxypinoresinol) were also present in all samples, but in lower concentrations (less than 20 mg/kg). The phenolic compounds' profile for the collected Jordanian olive oil samples was closed to the Greek extra virgin olive oil, which recorded a total phenolic compounds in the range of 250.0–950.0 mg/kg. These compounds included 35.68–579.02 mg/kg simple phenolic compounds (Tyr and Htyr) and 153.99–402.07 mg/kg for their derivatives (Kotsiou and Tasioula-Margari, 2016). On the other hand, the Italian autochthonous varieties Tonda di Villacidro, Tonda di Cagliari, Semidana, and Bosana showed different phenolic compound profiles with less total phenol range 180.00–335.00 mg/kg. The main identified phenols were secoiridoids, dominating in Bosana oil, such as decarboxymethyl ligstroside aglycone (p-HPEA-EDA, 35.80 ± 19.9 mg/kg) and oleuropein aglycone (3,4-HPEA-EA up to 84.70 mg/kg) (Tuberoso et al. 2016). The total phenolic compounds calculated from individual compounds using SPE method showed a clear negative correlation with increasing the harvesting date (Tables 4 and 5). The main compounds controlling the total phenolic content were the secoiridoid compounds and the concentration of these compounds decreased significantly during fruit maturation, whereas the amounts of other phenolic compounds such as flavonoids and lignans remained constant or showed slight variations at different harvesting times. These results match the results reported for olive oil varieties from different countries (De Torres et al. 2018; Yu et al. 2021).

The total phenolic compounds extracted from the Jordanian olive oil samples using SPE method and acid hydrolysis method are compared in Table 6. Generally, the concentration of phenols extracted by SPE was higher than the concentration of these target compounds obtained from the acid hydrolysis method for substantial number of samples such as Nabali Baladi/Azraq, Nabali Baladi/Az Zarqa, Nabali Mohasen/Ajloun, and Rumi/Irbid. The higher yields of total phenolic compounds obtained by the SPE refer to its high efficiency to extract complex phenols such as secoiridoid derivatives, lignans, and flavonoids that could not be hydrolyzed efficiently by acid hydrolysis method (Reboredo-Rodríguez et al. 2016). When the analytical results obtained from solid phase extraction and acid hydrolysis methods were compared, the two methods exhibited significant difference at $p < 0.05$ (paired samples *t*-test) (Table 6) (Harvey, 2000). The differences in the determination of phenolic compounds in olive oil using various methodologies were observed practically by many different published methodologies, such as LLE protocol followed by LC–MS and the global methodology (Folin–Ciocalteau (FC) colorimetric

assay) and the International Olive Council (IOC) method (Olmo-García et al. 2019).

Conclusions

In this study, a simple, rapid, and low-cost acid hydrolysis method was developed and validated for routine analysis of phenols in olive oil. The method enables the determination of the most abundant phenolic compounds (hydroxytyrosol and tyrosol) by direct acid hydrolysis of secoiridoid aglycons in virgin and extra virgin olive oil. The conditions for hydrolysis and extraction of phenolic compounds such as organic solvents, acid type/concentration, time, and temperature were studied and optimized. The developed method exhibits significantly higher recovery of total phenolic content (> 94.0%) comparing to other acid hydrolysis extraction previously described in the literature. The applicability and reliability of the validated method have been confirmed by the analysis real Jordanian olive oil samples. The analyzed samples showed a large range of variability in the total phenolic compounds contents, suggesting the influence of olive variety, environment area, and harvesting time on the phenol content. The phenolic extracts, obtained from different Jordanian olive oil samples, yielded similar HPLC profiles with olive oil extracts from Mediterranean region. Phenolic compounds were also identified by SPE method. When the total phenolic compounds obtained by the validated acid hydrolysis were compared to their yields obtained by SPE methods, the SPE method gave higher total phenolic compound yield, suggesting its suitability for this purpose. However, the acid hydrolysis method has the advantage to be simpler and environmentally accepted due to the low solvent consumption. In addition, the acid hydrolysis method showed high repeatability and reproducibility in the obtained results. This suggest that the acid hydrolysis method is a promising choice for the extraction of phenolic compounds from olive oils, especially for routine analysis in industrial and scientific laboratories. The simple and accurate measurement of the amount of phenolic compounds in olive oil will be useful for the application of the "olive oil polyphenols" health claim in the markets, allowing the consumers to recognize the highest quality of virgin and extra virgin olive oils.

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Declarations

Competing interests The authors declare no competing interests.

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Conflict of interest Diya Alsafadi declares that he has no conflict of interest. Jameel Aljariri Alhesan declares that he has no conflict of interest. Aya Mansour declares that she has no conflict of interest. Saba Oqdeh declares that she has no conflict of interest.

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