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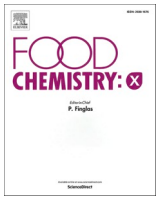

Potential of fruit seeds: Exploring bioactives and ensuring food safety for sustainable management of food waste

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ABSTRACT

Sweet cherry pits, date seeds, and grape seeds are abundant fruit by-products in the Mediterranean region. Assessing their antioxidant capacity is crucial for their valorization. Grape and date seeds exhibited higher concentrations of total phenolic and flavonoid contents, and significant antioxidant capacity. Epicatechin was the main flavonoid in sweet cherry pits and date seeds (29–85 mg/g), while vanillic acid was the predominant phenolic acid across all by-products (5–23 mg/g). However, some sweet cherry pit varieties exceeded Maximum Residue Levels (MRL) for five pesticides, while grape seeds contained thirteen fungicide residues, all below MRL. Ochratoxin A was detected in one date seed but below the limit of quantification. Additionally, grape seeds showed an Al content of approximately 130 mg/kg, along with levels of As, Cd, and Pb. Date seeds exhibited high potential for food and pharmaceutical applications, pending evaluation for chemical contaminants.

1. Introduction

Fruits are consumed worldwide because they are an excellent source of vitamins, minerals, dietary fibers, and polyphenolic compounds which help protect against several chronic diseases (Blumfield et al., 2022).

Sweet cherries (*Prunus avium* L.) and dates (*Phoenix dactylifera* L.) are stone fruits typical of the Mediterranean region. They consist of a hard stone (endocarp) that encloses the seed in the center of the fruit, covered by edible flesh (mesocarp) and a thin outer layer (epicarp) (Hong et al., 2021; Lara et al., 2020). The stone is a non-edible part of stone fruits (Fig. 1), being removed for direct consumption or during industrial processing, thus being classified as food waste. In cherries and dates, the seed represents an average of 10–15% of the whole fruit mass (Soares Mateus et al., 2023). The huge production and industrial transformation of these fruits, around 9.7 million tonnes of dates and 2.7 million tonnes of cherries (FAOSTAT, 2023), unavoidably leads to a significant production of fruit waste.

Grapes (*Vitis vinifera*) are one of the most produced crops worldwide, with 74 million tonnes produced (FAOSTAT, 2023). Primarily

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recognized for their role in winemaking, grapes generate substantial by-products (namely seeds - Fig. 1, skin, and pulp), commonly referred to as grape pomace.

One of the biggest global concerns is food waste, due to its negative impact on the economy and environment (FAO, 2014; Osorio et al., 2021). Currently, several works reported the high potential of fruit waste due to their richness in bioactive compounds, with antioxidant and antimicrobial properties (Socas-Rodríguez et al., 2021).

Therefore, there is a lot of interest in using these by-products as ingredients for the development of functional foods with positive health effects for consumers using these by-products. For instance, sweet cherries were used as an ingredient in meat burgers (Martín-Mateos et al., 2022), date seed and grape seed flours as a partial substitute for wheat flour in muffins (Ambigaipalan & Shahidi, 2015; Yalcin et al., 2022), and dietary fibers from date seeds in chocolate spreads (Bouaziz et al., 2017).

Although many studies have used these seeds and other by-products in food production, the food safety of these by-products has not been fully evaluated (Bouaziz et al., 2017; Martín-Mateos et al., 2022). A few studies evaluated the safety of fruit waste regarding residues of pesticides (Celeiro et al., 2014; Moncalvo et al., 2016; Nieto-García et al., 2015; Rose et al., 2009; Sójka et al., 2015) while the content of mycotoxins was only assessed by Moncalvo et al. (2016) in grape skins. Due to the Human health implications of food contaminants is of high importance to evaluate, besides bioactive compounds, also these compounds when using food by-products in a circular economy approach.

Pesticides are chemical compounds applied in agriculture to safeguard fruit crops against insects, fungi, weeds, and other pests. Nonetheless, their presence, even at residual levels, in food products raise significant health concerns due to their potential toxicity effects on humans, namely at neurological level (Casida & Bryant, 2017; Mir et al., 2022; Richardson et al., 2019). Mycotoxins are secondary metabolites produced by fungi that may contaminate fruit crops. These natural chemical compounds have several consequences on human health, many of which are carcinogenic and represent a health concern to consumers (Altomare et al., 2021; Mihalache et al., 2023; Mukhtar et al., 2023). If these two classes of chemical contaminants are present in fresh whole fruits, it is important to ensure that their by-products are safe for application in food for human consumption.

Some of these by-products are already reused. For example, the cherry kernel is used, with apricot kernel, to produce a popular liqueur in Italy named "Amaretto", while date seeds are used to produce a drink similar to coffee with no caffeine (Fikry et al., 2019; Senica et al., 2016). Grape seeds are used for the production of grape seed oil, which is rich in bioactive compounds, and has potential uses in the food, cosmetic, and pharmaceutical industries (Yang et al., 2021).

This study aims to provide a dual perspective on fruit seeds from the Mediterranean area, encompassing sweet cherries, dates and grapes, being a pioneering effort in evaluating both antioxidant properties and food safety of food waste, with the goal of their safe application according to circular economy practices. On the one hand, the objective was to evaluate the antioxidant properties, and characterize the individual phenolic compounds. On the other hand, this study aimed to determine three important chemical contaminants, namely residues of pesticides, mycotoxins and heavy metals.

2. Materials and methods

2.1. Fruit seeds

The fruit by-products were kindly supplied by different food industries from the Mediterranean area. Sweet cherry pits of three different varieties (*Campo Corso*, *Ferrovia,* and *Imperiali*) were kindly supplied by a company located in southern Italy. Date seeds of three different varieties: *Alig* (DA), *Deglet Nour* (DDN), and *Kentichi* (DK), were kindly provided by a Tunisian business. From France, grape seeds of the *Ugni blanc* variety were generous supplied. Sweet cherry pits and date seed samples were lyophilized while fresh grape seeds were ground. All samples were preserved at −20 °C until further processing.

2.2. Preparation of fruit seed extracts

The extracts were obtained from fresh and freeze-dried by-products. Briefly, 5 g of each sample was mixed with 50 mL of absolute ethanol. Ethanol was selected as the extraction solvent since it is a food-grade solvent, compliant with Directive 2009/32/EC and its amendments (European Commission, 2009). The samples were subjected to an ultrasonic bath for 15 min and were then stirred for 30 min on a horizontal shaker (Kottermann 4010, Labexchange, Burladingen, Germany). Subsequently, the samples were centrifuged (Megafuge 1.0R, Heraeus, Thermo Scientific, Massachusetts, EUA) at 2862*g*, for 10 min at 4 °C. The supernatant was filtered with Whatman® No. 4 filter papers, and the

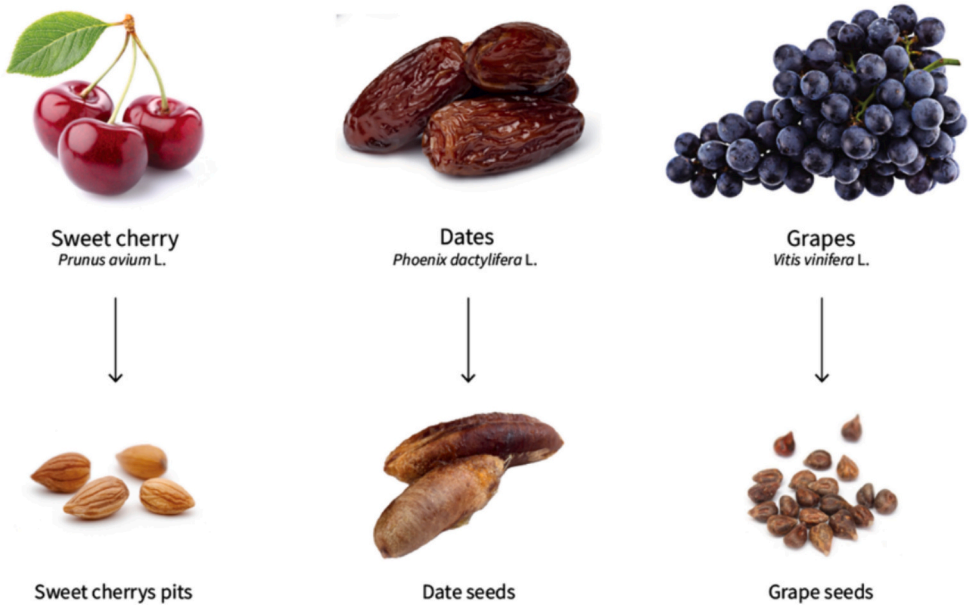

Fig. 1. Sweet cherries (*Prunus avium* L.), dates (*Phoenix dactylifera* L.) and grapes (*Vitis vinifera* L.) and their seeds.

ethanol was then completely evaporated on a rotary evaporator (Rotavapor R-114, Büchi, Barcelona, Spain) at 35 °C. The extract was removed with a spatula and kept at −20 °C, protected from light, until further use.

2.3. Determination of phenolic compounds and antioxidant capacity

The antioxidant capacity was determined in fruit seed extracts by the β-carotene bleaching assay and the DPPH radical scavenging assay. Additionally, the Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were determined. These assays were conducted using ethanolic solutions of each extract at 10 mg/mL. A UV–Vis Spectrophotometer (U-2810, Hitachi, Digilab, Sydney, NSW, Australia) was used to measure the samples' absorbance for each of the four experiments.

2.3.1. Total phenolic content (TPC)

TPC was determined using Erkan et al. (2008) methodology. Briefly, 1 mL of the sample was mixed with 7.5 mL of Folin–Ciocâlteu reagent (10%, *v*/v) (Sigma-Aldrich) for 5 min. Then, 7.5 mL of sodium carbonate aqueous solution (60 mg/mL) (Sigma-Aldrich) was added. Following homogenization, the solutions were incubated for two hours at 23 ± 0.2 °C in the dark. Absorbance was measured at 725 nm. A calibration curve was constructed using gallic acid (Sigma-Aldrich) as the standard ($y = 0.0059\times$ - 0.1391, $r^2 = 0.9933$), with a working range of 25–150 g/mL. Results were expressed in milligrams of gallic acid equivalent (GAE) per gram of extract.

2.3.2. Total flavonoid content (TFC)

The method described by Yoo et al. (2008) was used to determine the TFC. The assay was performed with 1 mL of ethanolic solution of extract. First, 300 μL of a 5% (*w/v*) aqueous solution of sodium nitrite (Supelco) was added and homogenized. After 5 min of incubation, 600 μL of 10% (w/v) aqueous aluminum chloride solution (Sigma-Aldrich) was added and stood for 6 min. Finally, 2 mL of 1 M sodium hydroxide aqueous solution (Sigma-Aldrich) was added and allowed to stand for 6 min. Finally, 2 mL of 1 M sodium hydroxide aqueous solution (Sigma-Aldrich) was added, and, after homogenization, the absorbance was measured at 510 nm. For the TFC assay, a calibration curve ($y = 0.0018$ x + 0.0181, $r^2 = 0.9954$) was constructed by plotting increased epicatechin (Sigma-Aldrich) concentrations (5–200 μg/mL). The results were expressed as milligrams of epicatechin equivalent (EE) per gram of extract.

2.3.3. DPPH radical scavenging activity assay

The protocol described by Moure et al. (2001) was applied while performing the DPPH ((2,2-diphenyl-1-picryl-hydrazyl) radical assay. In brief, 50 μL of the sample was mixed with 2 mL of a methanolic solution of DPPH (Sigma-Aldrich) at 14.2 μg/mL. The solutions were then incubated for 30 min in the dark at 23 ± 0.2 °C. The absorbance was measured at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich) was used at various concentrations to generate a calibration curve ($y = 0.3086\times + 0.1894$, $r^2 = 0.9983$) within a working range of (5–250 μg/mL). Trolox equivalent (TE) was used to express the results. Eq. (1) was used to calculate the percentage inhibition (IP%) of DPPH, where A_C is the absorbance of the control (ethanol) and A_S is the absorbance of the sample following incubation.

$$IP\ (\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

2.3.4. β-carotene bleaching assay

The β-carotene bleaching assay was carried out in accordance with Miller (1971). In brief, 5 mL of the β-carotene emulsion was added to 200 μL of each sample, and the samples were incubated for 120 min at 50 ± 0.2 °C in a heating bath (B-491, Büchi, Barcelona, Spain). The β-carotene emulsion was prepared by adding 2 mL of the β-carotene solution (2 mg/mL, in chloroform, Sigma-Aldrich) to 40 mg of linoleic acid (Sigma-Aldrich) and 400 mg of Tween® 40 (Sigma-Aldrich). After evaporating the chloroform at 40 ± 0.2 °C using a rotary evaporator (Büchi Rotavapor R-114), 100 mL of ultrapure water was added and strongly agitated to form an emulsion. The Antioxidant Activity Coefficient (AAC) was calculated using eq. (2), where As_1 is the absorbance of the sample at 120 min, Ac_1 is the absorbance of the control at 120 min, and Ac_0 is the absorbance of the control at 0 min. The control sample was prepared using 200 μL of ethanol. The absorbance was measured at 470 nm.

$$AAC = \frac{As_1 - Ac_1}{Ac_0 - Ac_1} \times 1000 \quad (2)$$

2.4. Determination of individual phenolic compounds

Individual phenolic compounds in samples were identified and quantified by Ultra-High Performance Liquid Chromatography (Nexera X2, Shimadzu, Kyoto, Japan) combined with Time-of-Flight Mass Spectrometry (SCIEX, Foster City, CA, USA), equipped with a Turbo Ion Spray electrospray ionization source working in positive mode (ESI +), following the method described by Teixeira et al. (2023).

The method was previously validated for 28 phenolic compounds, including flavonoids and phenolic acids (**Table S1** of the supplementary material). By comparing the extracted mass of isotope in PeakView™ 2.2 software (with a tolerance of 5 ppm) and the retention time (with a maximum relative retention time deviation (ΔRRT) of 2.5%) using MultiQuant™ 3.0 software, the identification of individual phenolic compounds was confirmed. The calibration curves were conducted using pure analytical standards of phenolic compounds.

The standards included protocatechuic acid, 4-hydroxybenzoic acid, gallic acid, gentisic acid, vanillic acid, syringic acid, caffeic acid, *p*-coumaric acid, *o*-coumaric acid, *trans*-ferrulic acid, sinapic acid, ellagic acid, chlorogenic acid, neochlorogenic acid, epicatechin, catechin, quercetin, isoquercitrin, quercitrin, rutin, naringenin, eriodyctiol, sakuranetin, eriocitrin, hesperidin, apigenin, luteolin and phloridzin, which were purchased from Sigma-Aldrich (Missouri, EUA) and Extrasynthese (Genay, France).

2.4.1. Extraction of phenolic compounds

The extraction procedure was optimized in fresh samples and was based on a double solid-liquid extraction with 10 mL of 50% (*v*/v) MeOH acidified with 0.1% (v/v) formic acid. The samples were sonicated for 15 min, followed by shaking for 30 min in a horizontal shaker at 450 rpm, and then centrifuged at 2862*g* for 10 min at 4 °C. The supernatant was homogenized and stored at −20 ± 0.2 °C.

2.4.2. UHPLC-ToF-MS conditions

In terms of chromatographic conditions, water [A] and acetonitrile [B], both acidified with 0.1% formic acid, constituted the mobile phase. The gradient program used, with a total time of 11 min, was as follows: 0–0.5 at 10% [B]; 0.50–8 min from 10% to 80% [B]; and maintained for 2 min; and back to 10% [B] in 1 min, with a flow rate of 0.3 mL/min. The separation of compounds was carried out through an Acquity UPLC BEH C18 (2.1 mm × 100 mm, 1.7 μm) column at 30 °C. The volume of injection was 20 μL, while the autosampler was held at 4 °C. The acquisition was carried out in full scan from 100 to 750 Da using the following mass spectrometry parameters: ion source voltage: 5500 V; source temperature: 575 °C; curtain gas (CUR): 30 psi; gas 1 and gas 2: 55 psi; and declustering potential (DP): 100 V. The software used for this analysis was Analyst® TF (SCIEX, Foster City, CA, USA, version 1.7). To ensure suitable mass resolution, the ToF-MS detector was calibrated every seven injections in the mass range addressed by the method.

2.5. Determination of pesticide residues

The identification and quantification of pesticide residues was performed based on the methodology described by Melo et al. (2019). The pesticide residues were determined through High-Performance Liquid Chromatography (Nexera X2,Shimadzu, Kyoto, Japan) coupled with a triple quadrupole instrument (QTRAP 5500+) MS/MS detector (SCIEX, Foster City, CA, USA), equipped with an electrospray ionization (ESI) source.

The previous validation was performed in peaches and grapes, as representative of stone fruits and grapes, respectively. According to SANTE 11312 (2021), validating the method in one matrix demonstrates its applicability to other matrices within the same commodity group. Sweet cherries, dates, plums, peaches, and apricots belong to the high-water-content commodity group, while grapes belong to the high acid content and high-water content group, which also includes citrus fruits, small fruit, and berries. The validation demonstrated the applicability of the method for 141 compounds in peach and 102 compounds in grapes, fulfilling the validation criteria for quantitative methods ((European Commission, 2021) with limits of quantification (LOQ) set at 5 μg/kg. Triphenylphosphate (TPP) and dinitrocarbanilide (DNC) were used as internal standards. Samples and standards were corrected for internal standard (IS) response. In total, 159 pesticide residues were included in the validation.

2.5.1. Extraction of pesticide residues

The analysis of pesticide residues was performed with quick, easy, cheap, effective, rugged, and safe (QuEChERS) method. First, 10.0 ± 0.01 g of the sample was weighed into 50 mL Falcon tubes, and 10 mL of acetonitrile was added. Then, a liquid–liquid partitioning step was performed by adding 0.65 g of a mixture of salts (including magnesium sulfate, sodium chloride, trisodium citrate dihydrate, and disodium hydrogen citrate sesquihydrate at 4:10:1:0.5 *w*/w/*w*/w). After centrifugation at 2191*g* for 10 min at 4 ± 2 °C, 6 mL of the extract underwent clean-up by dispersive solid-phase extraction (d-SPE) with 900 mg of anhydrous magnesium sulfate mixed with 150 mg primary secondary amine (PSA) sorbent. Following centrifugation, 220 μL acetonitrile was added to 1 mL of the cleaned extract. Finally, 25 μL of the internal standards solution (1 ng/μL) was added to the extract, which was then filtered through a PVDF mini-uniprep™ and analyzed using HPLC-MS/MS.

2.5.2. HPLC-MS/MS conditions

Regarding chromatographic conditions, a Synergi Fusion-RP 80 A (50 mm × 2 mm, 4 μm) column (Phenomenex, Torrance, CA, USA) was used for the separation of residues of pesticides. The column was kept at 35 °C and the autosampler was maintained at 10 °C to refrigerate the samples. A volume of 10 μL of sample extract was injected into the column. Water [A] and methanol [B], both acidified with 0.1% formic acid, were used as the mobile phase with a flow rate of 0.25 mL/min. The gradient elution program was started with 5% of [B] for 0.5 min. Then, it was increased to 90% [B] from 0.5 to 8 min and was kept at that concentration for 5 min. Finally, the concentration returned to 5% [B] in 2 min and was kept at 5% [B] until the end of the run (total of 18 min).

Using the Analyst® TF (SCIEX, Foster City, CA, USA) software and the following parameters, the mass spectrometry acquisition was carried out in Multiple reaction monitoring (MRM) mode from 100 to 750 Da: ion spray voltage of 4500 V; source temperature of 600 °C; curtain gas (CUR) at 35 psi; and gas 1 and gas 2 at 40 and 60 psi, respectively. The ESI source worked simultaneously in both positive and negative modes (ESI + and ESI −). For each pesticide residue, two ion transitions were therefore chosen: a qualifier and a quantifier.

2.6. Determination of mycotoxins

Nine mycotoxins, including aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), ochratoxin A (OTA), zearalenone (ZEA), toxin T2 (T2) and fumonisins (FB_1 and FB_2) were determined by UHPLC-ToF-MS equipped with a Turbo Ion Spray electrospray ionization source working in positive mode (ESI +), following the method described by Silva et al. (2019).

2.6.1. Extraction of mycotoxins

Solid-liquid extraction (SLE) was implemented to extract mycotoxins. Briefly, 2.0 ± 0.1 g of sample was extracted with 10 mL of acetonitrile 80% (*v*/v) for 1 h at 110 rpm, at room temperature, using a horizontal shaker. After centrifugation at 2191*g* for 10 min, the supernatant was transferred to another Falcon tube, and samples were re-extracted with the same volume of acetonitrile 80% (v/v) for 1 h. After centrifugation, the supernatants were collected. Then, two different procedures were followed depending on the type of mycotoxins: (1) for analysis of fumonisins, 1 mL of the extract was diluted with 1 mL of ultra-pure water or (2) for the other mycotoxins, 8 mL of the extract was evaporated to dryness under a gentle stream of nitrogen at 40 °C and the residue was redissolved with 1 mL of acetonitrile 40% (v/v). The final extracts, in both cases, were filtered through a PVDF mini-uniprep™ and injected into the UHPLC-ToF-MS system.

2.6.2. UHPLC-ToF-MS conditions

Regarding the chromatographic conditions, the separation was carried out in the Zorbax Eclipse Plus C18 (2.1 × 50 mm, 1.8 μm) column at 30 °C with gradient elution at 0.5 mL/min. A volume of 20 μL of sample extract was injected into the column. The mobile phases consisted of 0.1% formic acid [A] and acetonitrile [B]. The following gradient program was used as the mobile phase: 0–12 min from 10% to 70% [B]; 12–13 min from 70% to 90% [B] and kept until 14 min; back to 10% [B] from 14 to 15 min and kept at 10% [B] until 17 min. The acquisition of mass spectrometry with 5600+ ToF-MS detector (SCIEX, Foster City, CA) was performed in full-scan from 100 to 750 Da, using the Analyst® TF 1.7 (SCIEX, Foster City, CA) software and with the following settings: ion source voltage of 5500 V; source temperature 575 °C; curtain gas (CUR) 30 psi; Gas 1 and Gas 2 of 55 psi; declustering potential (DP) 100 V. To ensure accurate mass resolution, the ToF-MS detector was calibrated within the method's mass range every seven injections.

2.7. Determination of heavy metals & other metals of safety concern

Heavy metals, including arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb) were determined through Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) (Agilent 7900 x, Hewlett-Packard, Waldbronn, Germany) equipped with a sample introduction system consisting of a Micromist glass low-flow nebulizer, a double-pass glass spray chamber with a Peltier system (2 °C) and a quartz torch, as described by Luna et al. (2019). Furthermore, other metals related to hypersensitivities such as cobalt (Co), nickel (Ni) and aluminum (Al) due to its widespread environmental presence and neurotoxic effects were determined by the method. The operating conditions have been recorded in **Table S2** of the supplementary material. Apple leaves NIST 1515, Rice flour NIST 1569b and ERM BB422 – Fish Muscle were also analyzed as certified food standards. Heavy metals quantification was performed by external standard calibration, injected in the same conditions. Rh^{103} was used as internal standard. The calibration curves of the seven determined elements are presented in Table S3.

2.7.1. Extraction of heavy metals & other metallic elements

Heavy metals content was extracted in triplicate by acid digestion through microwaves (ultraWave, Milestone Src Technology, Sorisole, BG, Italy). Hence, 300 mg of sample were digested with 3 mL HNO_3 69% (*v*/*v*) and 1 mL of H_2O for 1 h using the conditions shown in Table S4 of the supplementary material. After cooling, samples were properly diluted with MilliQ water to a final volume of 50 mL.

2.8. Statistical analysis

The statistical analysis of the data was analyzed with IBM® SPSS® Statistics, version 28.0.1.1. (Chicago, IL, USA), employing one-way analysis of variance (ANOVA), when the normality of data and homogeneity of variances were validated. The Tukey test was applied to examine the disparities among average values. Significance was defined at $p < 0.05$. Results concerning the statistical evaluation were expressed as mean value plus the standard deviation (SD) of three replicates.

3. Results and discussion

3.1. Determination of antioxidant capacity

Date seeds showed the highest values for TPC (mean = 467.84 μg GAE/g extract) and TFC (mean = 518.87 μg EE/g extract), followed by grape seeds (24.60 ± 0.38 μg GAE/g extract and 160.23 ± 1.26 μg EE/g extract) (Table 1).

Sweet cherry pits presented the lowest TPC (mean = 11.04 μg GAE/g extract) and TFC (mean = 2.4 μg EE/g extract). Among sweet cherry varieties, the *Campo Corso* variety exhibited notably high TPC and TFC ($p < 0.05$). No other study assessed the antioxidant properties of these pits. The antioxidant capacity of sweet cherry pits was previously determined only by Afonso et al. (2020). Among four varieties, *Early Bigi NC* had the higher phenolic content, with a TPC of 2.76 mg GAE/g dried weight (DW) and 2.59 mg catechin equivalents (CE)/g DW for TFC. However, the extraction procedure is not comparable.

Regarding date seeds, the *Alig* variety showed the highest phenolic content, with significant superiority ($p < 0.05$). However, among eight varieties of date seeds studied by Shi et al. (2024), the *Deglet Nour* variety had the highest TPC (27.87 mg GAE/g fresh weight (FW)) and TFC (5.03 mg quercetin equivalents (QE)/ g FW). No other studies have been carried out on date seeds from the *Alig* or *Kentichi* varieties.

In a recent study by Krasteva et al. (2023), grape seed powder derived from three red varieties exhibited elevated levels of phenolics, with a mean content of 100.89 mg GAE/g DW, and flavonoids, with a mean content of 49.82 mg QE/g DW. Also, one white variety of grape seed was assessed and showed the lowest content of TPC and TFC (79.06 mg GAE/g DW and 40.05 mg QE/g DW, respectively).

Table 1
Antioxidant capacity, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of fruit seeds: sweet cherry pits, date seeds, and grape seeds extracts.

Seeds	*vr.*	TPC μg GAE/g extract	TFC μg EE/g extract	DPPH μg TE/g extract	β-carotene AAC
Sweet Cherry Pits	*Campo Corso*	15.91 ± 0.12^{c}	3.68 ± 0.33^{b}	8.59 ± 0.52^{b}	751.37 ± 137.50^{a}
	Ferrovia	10.38 ± 0.03^{b}	1.92 ± 0.18^{a}	5.43 ± 0.22^{a}	703.30 ± 200.38^{a}
	Imperiali	6.84 ± 0.02^{a}	1.60 ± 0.10^{a}	4.22 ± 0.10^{a}	634.62 ± 164.62^{a}
Date Seeds	*Alig*	483.31 ± 2.65^{C}	538.31 ± 11.97^{B}	14.54 ± 0.14^{B}	2181.82 ± 0.00 ^{A}
	Deglet Nour	453.41 ± 1.81 ^{A}	511.03 ± 13.30 ^{A}	14.06 ± 0.09 ^{A}	2242.42 ± 85.71^{B}
	Kentichi	466.79 ± 3.41^{B}	507.27 ± 13.30 ^{A}	14.36 ± 0.08^{B}	2303.03 ± 0.00 ^{A}
Grape Seeds		24.60 ± 0.38	160.23 ± 1.26	753.92 ± 8.38	5121.21 ± 128.56

The results are expressed as mean ± standard deviation ($n = 3$).
The superscript letters indicate the statistical analysis. Different letters indicate statistically significant differences ($p < 0.05$). Lowercase letters indicate statistically significant differences between cherry stone varieties. Uppercase letters indicate statistically significant differences between date seeds varieties.
vr. – variety; TPC - Total Phenolic Content; TFC - Total Flavonoid Content; GAE – gallic acid equivalent: EE – epicatechin equivalent; TE – Trolox equivalent; AAC - Antioxidant Activity Coefficient.

In date and grape seeds, TFC is higher than TPC. Since flavonoids are a class of phenolic compounds, it was expected that TFC would be lower than TPC. However, the units of measurement used to express these phenolic compounds are different (gallic acid equivalents for TPC and epicatechin equivalents for TFC) making direct comparison impossible. Previously, Gómez-Mejía et al. (2022) had reported a higher TFC (13.4 mg quercetin equivalents (QE)/g DW) in grape seeds, compared to TPC (1.89 mg GAE/g DW), using different standards for TFC.

In antioxidant assays, grape seeds exhibited the highest antioxidant capacity in both DPPH radical (753.92 μg TE/g extract) and β-carotene (AAC = 5121.21) assays, followed by date seeds. Sweet cherry pits presented the lowest antioxidant capacity, with *Campo Corso being* the variety with the highest antioxidant capacity in both assays, with significant difference ($p < 0.05$). Comparing the results of DPPH radical and β-carotene bleaching assays, both tests are in agreement regarding the antioxidant capacity of fruit stones.

Our research findings indicate that the β-carotene bleaching assay is not a widely used assay to assess fruit seeds' antioxidant capacity. This assessment was only conducted by Afonso et al. (2020) on cherry pits, revealing that cherry pits of various cherry varieties exhibited a comparable percentage inhibition, approximately 91.5%.

On the contrary, the DPPH radical assay is widely applied. For example, grape seeds evaluated by Abouelenein et al. (2023) showed a high antioxidant capacity by DPPH radical assay (303.36 mg TE/g DW), showing a correlation with the TPC. Likewise, grape seeds powder derived from three red varieties demonstrated a substantial antioxidant capacity, averaging 537.27 μM TE/g DW (Krasteva et al., 2023). For date seeds, Shi et al. (2024) found 15.99 mg TE/g FW. Due to the diverse approaches are used for assessing antioxidant capacity and the distinct extraction processes employed by the authors, it is challenging to compare the limited results available in the literature.

3.2. Determination of individual phenolic compounds

The individual phenolic compounds identified and quantified in sweet cherry pits are summarized in Table 2. Out of the 28 phenolic compounds validated by the method, 14 were identified and 11 were quantified in sweet cherry pits. Cherries of *Ferrovia* variety is one of the most representative varieties from Italy, being the most commercially important and extensively studied (De Leo et al., 2021). However, according to our results, seed derived from *Ferrovia vr.* presented the lowest amount of total phenolic compounds (160.90 mg/g). Similarly, when comparing pulps from six varieties of sweet cherry, *Ferrovia vr.* exhibited one of the lower amounts of phenolic compounds, with cyanidin-3-*O*-rutinoside (14.0 mg/100 g FW) and rutin (2.3 mg/100 g FW) being the most abundant (De Leo et al., 2021).

Imperiale and *Campo Corso* are relatively understudied varieties, as there are no data assessing the phenolic compounds content in these specific varieties. However, these varieties showed the highest content of total phenolic compounds, with 263.71 mg/g and 243.62 mg/g, respectively. The *Imperiale* variety is a white sweet cherry and was the only variety where caffeic acid and *p*-coumaric acid were determined. Similarly, Senica et al. (2016) have also identified hydroxycinnamic and hydroxybenzoic acids in sweet cherry pits, including *p*-coumaric acid (9.49 μg/g) and ellagic acid pentoside (50.09 μg/g). Vanillic acid was the major phenolic acid in all varieties, with 11.16 mg/g for the *Ferrovia* vr and 23.24 mg/g for the *Imperiale* variety. The content of vanillic acid in *Imperiale* variety was significant ($p < 0.05$) higher than the content on *Campo Corso* and *Ferrovia* varieties. No other study has reported the presence of this phenolic compound in cherry pits.

Flavonoids were the predominant class of phenolic compounds present in the greatest quantity. Rutin emerged as the major phenolic compound in sweet cherry pits across all varieties (96.77 to 110.48 mg/g), followed by epicatechin (33.82 to 85.17 mg/g) and sakuranetin, all from the flavonoid class. Contrarily, previous studies have reported catechin as the major phenolic compound in sweet cherry kernels

Table 2
Phenolic compounds (mg/g) in three different varieties of sweet cherry pits from Italy.

	Sweet Cherry Pits (mg/g)		
vr.	*Campo Corso*	*Ferrovia*	*Imperiale*
Phenolic acids			
Benzoic acids derivatives			
Vanillic acid	13.59 ± 5.29^{a}	11.16 ± 0.87^{a}	23.24 ± 0.55^{b}
Hydroxycinnamic acids derivatives			
Caffeic acid	*nd*	*nd*	1.10 ± 0.22
p-coumaric acid	*nd*	*nd*	14.54 ± 0.37
trans -ferulic acid	0.64 ± 0.18^{a}	2.60 ± 0.01^{b}	8.21 ± 0.21^{c}
Flavonoids			
Flavan-3-ols			
Epicatechin	85.17 ± 2.41^{b}	33.82 ± 0.68^{a}	83.30 ± 1.53^{b}
Catechin	6.77 ± 0.87^{b}	2.24 ± 0.26^{a}	*nd*
Flavonols			
Quercetin	0.946 ± 0.031^{b}	3.37 ± 0.025^{c}	0.113 ± 0.007^{a}
Isoquercetin	4.33 ± 0.054^{c}	3.04 ± 0.037^{a}	3.27 ± 0.15^{b}
Quercitrin	3.35 ± 0.014^{b}	2.81 ± 0.165^{a}	3.56 ± 0.11^{c}
Rutin	98.66 ± 2.73^{a}	96.77 ± 0.18^{a}	110.48 ± 0.71^{b}
Flavanone			
Naringenin	0.218 ± 0.011^{a}	0.369 ± 0.010^{b}	0.710 ± 0.014^{c}
Eriodyctiol	*nd*	< LOQ	< LOQ
Sakuranetin	29.95 ± 0.095^{c}	4.72 ± 0.098^{a}	15.19 ± 0.054^{b}
Flavone			
Apigenin	< LOQ	< LOQ	< LOQ
Σ	**243.62**	**160.90**	**263.71**

The results are expressed as mean ± standard deviation (n = 3).
The superscript letters indicate the statistical analysis. Different letters indicate statistically significant differences (p < 0.05) on phenolic compound content between the sweet cherry pit's varieties.
vr. – *variety;* LOQ – Limit of Quantification; *nd* – not detected; Σ - sum of phenolic compounds.

Table 3
Phenolic compounds (mg/g FW) in three different varieties of date seeds from Tunisia.

	Date seeds (mg/g)		
vr.	*Alig*	*Deglet Nour*	*Kentichi*
Phenolic acids			
Benzoic acids derivatives			
Vanillic acid	4.94 ± 0.18^{a}	7.42 ± 1.62^{c}	5.61 ± 1.12^{b}
Syringic acid	1.67 ± 0.01^{a}	2.39 ± 0.36^{b}	2.65 ± 0.19^{b}
Hydroxycinnamic acids derivatives			
Caffeic acid	14.59 ± 0.79^{b}	*nd*	9.99 ± 0.35^{a}
p-coumaric acid	3.21 ± 0.07^{b}	3.41 ± 0.13^{b}	2.29 ± 0.19^{a}
trans -ferulic acid	1.15 ± 0.06^{c}	0.74 ± 0.07^{b}	0.46 ± 0.09^{a}
Sinapic acid	0.99 ± 0.11^{b}	0.24 ± 0.01^{a}	0.27 ± 0.05^{a}
Flavonoids			
Flavan-3-ols			
Epicatechin	28.80 ± 2.67^{a}	63.15 ± 0.74^{b}	29.44 ± 2.14^{a}
Catechin	31.90 ± 0.90^{a}	57.91 ± 0.88^{c}	37.66 ± 1.58^{b}
Flavonols			
Quercetin	0.25 ± 0.02^{b}	0.64 ± 0.07^{c}	0.15 ± 0.05^{a}
Isoquercetin	2.90 ± 0.01^{b}	6.68 ± 0.33^{c}	1.81 ± 0.07^{a}
Flavanone			
Naringenin	0.20 ± 0.02^{b}	0.21 ± 0.09^{b}	0.10 ± 0.01^{a}
Flavone			
Apigenin	0.014 ± 0.002^{b}	0.005 ± 0.002^{a}	0.046 ± 0.001^{c}
Luteolin	0.010 ± 0.006^{a}	< LOQ	0.006 ± 0.002^{a}
Σ	**90.624**	**128.60**	**90.48**

The results are expressed as mean ± standard deviation (n = 3).
The superscript letters indicate the statistical analysis. Different letters indicate statistically significant differences (p < 0.05) on phenolic compound content between the date seeds' varieties.
vr. – *variety;* LOQ – Limit of Quantification; *nd* – not detected; Σ - sum of phenolic compounds.

(Afonso et al., 2020; Chezanoglou et al., 2024). Other authors reported the presence of quercetin and their derivates, namely isoquercitrin, quercitrin, and rutin, among which rutin was the predominant flavonol (Senica et al., 2016). Among sweet cherry, the rutin content in *Imperiale* variety was significantly ($p < 0.05$) higher than the content in other two varieties, while the epicatechin content was significantly ($p < 0.05$) higher in *Campo Corso* variety.

Sakuranetin is a typical flavanone in the *Prunus* family, but it is mostly found in the form of glycosides (sakuranin). Chemically, sakuranetin is the *O*-methylated derivative of naringenin, the best-known citrus flavanone (Stompor, 2020). No other study has reported the presence of sakuranetin in sweet cherry pits. This flavanone was the main compound found in the pits, with a significant ($p < 0.05$) higher amount in *Campo Corso* vr. *(*29.95 mg/g*)* and lower amount in *Ferrovia* vr. (4.72 mg/g).

The individual phenolic compounds identified and quantified in date seeds are summarized in Table 3. Out of the 28 phenolic compounds validated by the method, 13 were identified and quantified in date seeds. *Deglet Nour* was the date seed variety that showed the highest amount of total phenolic compounds. Similarly, among eight varieties of date seeds studied by Shi et al. (2024), *Deglet Nour* and *Medjool* varieties emerged as rich sources of phenolic compounds.

Flavan-3-ols was the class of phenolic compounds present in the greatest quantity, including catechin and epicatechin, which accounted for 94.1% of the total phenolic compounds in *Deglet Nour vr.* The amount of epicatechin and catechin were significant superior ($p < 0.05$) in the *Deglet Nour vr.* In *Alig* and *Kentichi vr.* the amount of flavan-3-ols was lower, 67% and 74%, respectively.

Caffeic acid was the main phenolic acid found in the *Alig* and *Kentichi* varieties, followed by vanillic acid and *p*-coumaric acid. The *Alig vr.* was considerably ($p < 0.05$) richer in caffeic acid. However, caffeic acid was not detected in *the Deglet Nour* variety. The content of vanillic acid was significant superior ($p < 0.05$) in this variety. Our findings are consistent with those of Djaoudene et al. (2021), who identified ferulic and vanillic acids as the major phenolic compounds in eight different varieties of date seeds, ranging from 1.104 to 3.802 mg/g DW and from 0.326 to 0.627 mg/g DW, respectively. Other studies have reported that *p*-coumaric acid is the most found phenolic compound in date seeds followed by rutin, caffeic, and ferulic acids (Bouhlali et al., 2020). Although rutin was not detected in our date seeds, they all contained quercetin and were particularly rich in its derivative isoquercetin. *Deglet Nour* variety was considerably ($p < 0.05$) richer in quercetin and isoquercetin.

Regarding grape seeds, none of the 28 validated phenolic compounds were detected. This could be related to the fact that the analysis of individual phenolic compounds was performed in fresh grape seeds, where the phenolic compounds could be present at low concentrations, below our LODs. Another possible cause might be that the phenolic compounds that provide grape seed extracts their antioxidant activity are not in the scope of our method.

Nevertheless, several studies have reported that catechin is the most abundant flavan-3-ol (Abouelenein et al., 2023; Andrade et al., 2019; Gómez-Mejía et al., 2022; Krasteva et al., 2023). Additionally, quercetin derivatives were quantified, including isoquercetin, quercitrin, and rutin, have been quantified (Abouelenein et al., 2023). Regarding phenolic acids, both benzoic acid derivatives (gallic acid, vanillic acid, syringic acid and ellagic acid) and hydroxycinnamic acids derivatives (chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid and 3,5-dicaffeoylquinic acid) have been detected in grape seeds (Abouelenein et al., 2023; Gómez-Mejía et al., 2022).

Variations in the number and quantity of phenolic compounds can be associated with genotypic diversity, edaphoclimatic conditions, and fruit maturity, as well as differences in extraction and analysis techniques (Soares Mateus et al., 2023). Additionally, phenolic compounds are often bound to glucosides, whereas our method primarily targets free phenolics. For example, Senica et al. (2016) identified apigenin hexoside in cherry pits, whereas and our levels of apigenin are below the LOQ (0.250 μg/g).

Furthermore, the number of phenolic compounds is countless, and the determination of phenolic compounds is limited by the availability

of commercial standards. A given matrix may contain additional phenolic compounds, but confirming their presence requires comparison to commercial standards for quantification. Consequently, it is challenging to compare data across studies because various authors determine various phenolic compounds in each matrix and report their findings using different units.

Comparing all the varieties of sweet cherry pits and date seeds, cherry pits presented high amounts of phenolic compounds, which contrasts with our results regarding antioxidant capacity (Section 2.2.). Our findings suggest that sweet cherry pits and date seeds are significant phenolic and flavonoid sources known as natural antioxidant compounds, namely catechin, epicatechin, vanillic, caffeic, and *p*-coumaric acids, that could potentially be used in food and nutraceutical formulation.

3.3. Determination of pesticide residues

Regarding the presence of pesticides in the evaluated fruit seeds, none of the 141 residues of pesticides evaluated in the method was found in date seeds. To our knowledge, there are no other studies determining pesticide residues specifically in date seeds. However, other authors have determined residues of pesticides in date fruits, for instance, Jafarian Asl et al. (2023) quantified chlorpyrifos, malathion, hexachlorocyclohexane, and metribuzin in date fruits of *Mazafati* variety.

Nevertheless, some residues of pesticides were found in three varieties of sweet cherry pits and grape seeds. It was not possible to obtain the list of the pesticide treatments for the collected sweet cherry pits and grape seeds to better understand the chemical contamination of the samples. The results of the determination of pesticide residues in those matrices are presented Table 4.

Table 4 also indicates the Maximum Residue Levels (MRLs) for each chemical compound in the edible portion of the corresponding matrices, namely sweet cherries and wine grapes, according to Regulation (EC) No 396/2005 and its amendments (European Commission, 2005). Every pesticide, depending on its degree of toxicity, has a maximum authorized value for application, which should not be exceeded to minimize negative effects on human health. The MRLs are reported for the whole fruit, since the official analysis of pesticide residues in fresh fruit is carried out in whole fruit, including the edible parts of the fruits and parts that are not normally consumed (European Commission, 2002).

Grape seeds exhibited the highest frequency of detected pesticide residues, mainly fungicides. Thirteen of the 102 residues of pesticides validated by the method were found in grape seeds. The pesticide residue levels varied between 0.011 ± 0.001 mg/kg for mandipropamid and 0.137 ± 0.001 mg/kg for pyrimethanil. According to Gava et al. (2021), cyprodinil is one of the most frequently found pesticide residues in grapes and grape derivatives (juice and wine), followed by acetamiprid and boscalid. The authors also indicated that fenhexamid, fludioxonil, dimethomorph, pyrimethanil, and tebuconazole were other relevant fungicides with residues found in grapes and derivatives, which is in line with our results, these pesticides having been detected also in our grape seeds.

Although the high number of pesticide residues detected in grape seeds, all the residues were below the MRLs established for wine grapes, present in Table 4, according to Regulation (EC) No 396/2005 and its amendments (European Commission, 2005). Our results confirmed the data found in the few studies found in the literature on pesticide residues in grape by-products. For instance, Celeiro et al. (2014) determined 11 fungicides, including cyprodinil, fenhexamid, tebuconazole, and dimethomorph, in white grape bagasse, using gas chromatography coupled with mass spectrometry (GC–MS/MS). The results showed that tebuconazole and dimethomorph were the most frequently found pesticides. Although the high levels detected of some fungicides, such as fenhexamid (1.427 mg/Kg) and dimethomorph (1.698 mg/Kg), all of them were below the MRLs for wine grapes, with the exception of cyprodinil with a value of 3.858 mg/Kg (MRL = 3.0 mg/Kg). Grape seeds are used as a source of resveratrol in dietary supplements. Nieto-García et al. (2015) determined >130 pesticides in dietary supplements with grape seed extracts by GC- MS/MS. The authors found malathion, chlorothalonil, bifenthrin, and terbutryn in samples, at concentrations ranging from 2.4 to 20.6 μg/kg. These chemical compounds are not included in the list of pesticides considered in our work, except malathion which was not found in our grape seed sample.

Early, Rose et al. (2009) evaluated the presence of pesticides in grapes, grape pomace, grape seeds, and grape seed oil, using gas

Table 4
Results of determination of pesticide residues (mg/kg) in sweet cherry pits and grape seeds by HPLC-MS/MS and the Maximum Residue Levels (MRLs) (European Commission, 2005).

	Sweet Cherry Pits (mg/kg)			Grape Seeds	MRLs (mg/kg)	
vr.	*Campo corso*	*Ferrovia*	*Imperiale*		Sweet Cherry	Wine Grapes
Insecticide						
Acetamiprid	0.192 ± 0.007	0.021 ± 0.001	*nd*	*nd*	1.5	0.5
Imidacloprid	*nd*	*nd*	0.029 ± 0.008	*nd*	0.01	0.7
Phosmet	0.439 ± 0.031	0.023 ± 0.004	*nd*	*nd*	0.01	0.01
Insecticide; Acaricide						
Dimethoate	*nd*	0.008 ± 0.001	0.976 ± 0.131	*nd*	0.01	0.01
Omethoate	*nd*	*nd*	0.187 ± 0.023	*nd*	0.01	0.01
Fungicide						
Boscalid	*nd*	*nd*	*nd*	0.132 ± 0.037	5	5
Cyprodinil	0.014 ± 0.004	0.012 ± 0.001	0.695 ± 0.018	0.042 ± 0.001	2	3
Difenoconazole	*nd*	*nd*	*nd*	0.021 ± 0.001	0.3	3
Dimethomorph	*nd*	*nd*	*nd*	0.050 ± 0.001	0.01	3
Fenhexamid	*nd*	*nd*	*nd*	0.013 ± 0.001	7	15
Fludioxonil	0.050 ± 0.019	< LOQ	0.575 ± 0.002	0.032 ± 0.002	5	4
Mandipropamid	*nd*	*nd*	*nd*	0.011 ± 0.001	0.01	2
Pyrimethanil	< LOQ	*nd*	*nd*	0.137 ± 0.001	4	5
Tebuconazole	0.338 ± 0.014	0.045 ± 0.011	0.075 ± 0.008	0.036 ± 0.005	1	1
Tetraconazole	0.016 ± 0.008	< LOQ	< LOQ	0.051 ± 0.001	0.01	0.07
Trifloxystrobin	*nd*	*nd*	*nd*	0.033 ± 0.001	3	3
Zoxamide	*nd*	*nd*	*nd*	0.036 ± 0.003	0.02	5
Fungicide; Nematicide						
Fluopyram	0.282 ± 0.001	0.030 ± 0.004	0.007 ± 0.001	0.110 ± 0.001	2	1.5
Σ	1.331	0.139	2.544	0.704		

The results are expressed as mean ± standard deviation (n = 2).
vr. – *variety;* LOQ – Limit of Quantification; *nd* – not detected; MRLs – Maximum Residue Level.

chromatography coupled with a nitrogen phosphorous detector and LC-MS/MS for determination of fungicides and insecticides ($n = 6$ and $n = 12$, respectively), reporting a higher content in the seeds than in the pomace. Fungicides and insecticides are applied to wine grapes before harvest and the authors concluded that those pesticides are concentrated in the grape seed oil, especially procymidone and cyprodinil, both used to control grey mold. In our study, cyprodinil was also found in grape seeds at a concentration of 0.042 ± 0.001 mg/kg, lower than that found by Rose et al. (2009) of 0.48 mg/kg after 72 days of application of pesticides.

The samples containing the highest concentrations of pesticide residues were sweet cherries pits. They contained seven of the 141 pesticide residues that the procedure validated. The *Imperiale* variety had the highest level of total pesticide residues of the three varieties investigated in this study. The highest residue levels were for dimethoate, cyprodinil, and fludioxonil, with 0.976 ± 0.131 mg/kg, 0.695 ± 0.018 mg/kg, and 0.575 ± 0.002 mg/kg, respectively. To the best of our knowledge, there are no other studies determining pesticide residues in sweet cherry pits.

It has been determined that at least one pesticide residue in sweet cherry pits exceeds the MRL. The levels of imidacloprid (0.029 ± 0.008 mg/kg), dimethoate (0.976 ± 0.131 mg/kg), and omethoate (0.187 ± 0.023 mg/kg) in the *Imperiale* variety were higher than the MRLs allowed by EU regulations for the corresponding residues (0.01 mg/kg) (European Commission, 2005). Furthermore, the residue of phosmete found in *Campo Corso* and *Ferrovia* varieties exceeds the MRLs. These residues are chemical compounds used for the control of insects. In the *Campo Corso* variety, the residue of tetraconazole was slightly higher than the MRL (0.016 ± 0.008 mg/kg).

Among the identified active principles, cyprodinil, tebuconazole, and fluopyram were the most found residues in the samples. These substances are usually used to control a range of fungal diseases in fruit crops (Carrasco Cabrera et al., 2023; Santos-Miranda et al., 2022). Additionally, fluopyram is also a nematicide (Schleker et al., 2022).

3.4. Determination of mycotoxins

Regarding the presence of mycotoxins in evaluated the fruit seeds investigates, none of the nine mycotoxins (AFs, OTA, ZEA, T_2, and FBs) evaluated in the method were detected in sweet cherry pits or grape seeds. To the best of our knowledge, there have been no other studies determining mycotoxins in sweet cherry by-products. Nevertheless, Moncalvo et al. (2016) assessed the presence of OTA in grape pomace powders and extracts. Skin powders were found to contain OTA in small quantities, with the highest concentration was 0.32 μg/kg, which falls below the maximum residue levels allowed by EU legislation for OTA in dry grapes (8 μg/kg)), as outlined in Commission Regulation No 2023/915 (European Commission, 2023).

However, OTA was detected in date seed from the *Alig* variety but at a level below the method's limit of quantification (LOQ = 1.5 μg/kg). OTA, which is mainly produced by *Aspergillus Nigri*, is recognized for its nephrotoxic effects and has been categorized as a potential human carcinogen (category 2B) by the International Agency for Research on Cancer (IARC) (Schrenk, Bodin, et al., 2020). While no specific studies have focused on date seeds, the presence of OTA and AFs has been confirmed in date fruits (González-Curbelo & Kabak, 2023; RASFF, 2023).

Despite the absence of regulated mycotoxins in the fruit seeds tested, it is essential to note that the method only targeted regulated mycotoxins. Emerging mycotoxins, which are not regulated but are increasingly prevalent in various fruit matrices, were not included in the assessment. These emerging mycotoxins, such as citrin (CIT), enniatins (ENNs), beauvericin (BEA) and *Alternaria* toxins, such as alternariol (AOH), alternariol methyl ether (AME), tenuazonic acid (TeA), and tentoxin (TEN), have been documented in fruits (Mihalache et al., 2023; Soares Mateus et al., 2021). For that reason, a comprehensive assessment should encompass a wide range of regulated and unregulated mycotoxins.

3.5. Determination of metals of safety concern

The concentration of the determination of seven metals in fruit by-products is displayed in Table 5. Regarding the arsenic residual content in samples, just grape seeds demonstrated a concentration higher than the LOD of the contaminant. Milićević et al. (2018) have evaluated the presence of different minerals in grapevine parts (leaf, skin, pulp, and seed) and wine (mg/L) and reported ~ 90% lower concentrations of As, Cd and Pb in grape seed samples. According to the Commission Regulation (UE) 2023/915 on maximum levels for certain contaminants in food (European Commission, 2023), the level found in the present study has not exceeded the maximum established for total Arsenic.

Cadmium has attained quantifiable concentrations in all fruit by-products, but just in some varieties. Looking at sweet cherry pits, the *Imperiale* variety has shown a level of Cd lower than all those established in the European Regulation and found by Lazović et al. (2022). The *Kentichi* was the only date seed variety that has presented Cd, without surpassing any of the concentrations defined for the different seeds in the Regulation.

It has not been found any concentrations of Hg in any of the seed samples.

Concerning Pb presence in fruit seeds, all of them have demonstrated a residual concentration, except for *Kentichi* and *Alig* date varieties. *Deglet Nour* variety has shown a concentration of 0.027 mg/kg, lower than all the specific maximums established in the Regulation, apart from the infant formulas. Abdrabo et al. (2015) did not find Pb in Tunisian *Deglet Nour* date seeds, but similar for other varieties cultivated in Spain, Iran or Israel. The lead quantities measured in sweet cherry varieties equal those reported by Lazović et al. (2022), exhibit no statistical differences, and are below all safe limits. Grape seed had the most outstanding concentration, at least 88% higher than the other seeds studied.

Ni-sensitiveness causes eczematous reactions. Ahlström et al. (2019) have described a prevalence ranging from 8% to 19% in the general population. Therefore, EFSA has established the lowest observed adverse effect level (LOAEL) of 4.3 μg Ni/kg body weight (bw) per day, hence, a 60 kg person should not exceed an intake of 0.25 mg (Schrenk, Bignami, et al., 2020). A hypothetical consumption of 500 g of the studied by-products, the *Deglet Nour* variety of date seeds and grape seeds would overcome the recommended quantity per day. The *Kentichi* and *Alig* varieties showed higher concentration levels than other date seed varieties studied by Abdrabo et al. (2015). On the other hand, sweet cherry pits have presented the lowest concentration of Ni, far from reaching the maximum specified by EFSA.

Cobalt is another mineral reported to cause hypersensitivities. Because of that, EFSA has established a maximum level intake of 1 mg/kg bw per day, whose valor is notably superior to that found in all samples under a regular intake. Even so, grape seed values have been found in higher concentrations compared to the results obtained by Milićević et al. (2018). Cobalt found in date varieties, especially *Deglet Nour* are in concordance with the reported by Abdrabo et al. (2015).

Different levels of aluminum are reported in different foodstuffs. Fruits have a mean value of 2.7 mg/kg fresh weight and nuts have been reported with a mean value of 4.1 mg/kg in France and 5.7 mg/kg in the UK (Aguilar et al., 2008). The aluminum concentration recovered from sweet cherry pits was correlated to aluminum contents found in other fruit seeds (Krstić et al., 2019) and it would mean a consumption of 1 kg per week to not exceed the Tolerable Weekly Intake (TWI) of 1 mg/kg bw established by EFSA. Grape seeds have shown the highest concentration of Al (130 mg/kg) and the *Deglet Nour* variety has displayed the lowest level of Al, being just 0.27 mg/kg.

More studies should be performed to establish the safest levels of heavy metals in seed by-products, considering their potential applicability in the food industry as new ingredients.

Table 5
Results of determination of heavy metals and other metals of safety concern (mg/kg) in fruit seeds by ICP-MS and the corresponding Maximum Levels (MLs) applied to the fresh weight.

	Metals						
	Al	As	Cd	Co	Hg	Ni	Pb
Sweet Cherry seeds							
Campo corso	9.6448 ± 0.1361 ^{A}	< LOD	< LOD	0.0058 ± 0.0002^{B}	< LOD	0.1198 ± 0.0082 ^{A}	0.0121 ± 0.005004
Ferrovia	6.439 ± 0.3206^{B}	< LOD	< LOD	0.0185 ± 0.0023 ^{A}	< LOD	0.0729 ± 0.019^{B}	0.0118 ± 0.000b
Imperiale	9.4072 ± 0.382 ^{A}	< LOD	0.0009 ± 0.0005	0.0047 ± 0.0005^{B}	< LOD	0.0432 ± 0.0096^{B}	0.01105 ± 0.0022
Date seeds							
Alig	< LOD	< LOD	< LOD	0.0018 ± 0.0001^{B}	< LOD	0.1705 ± 0.0082^{B}	< LOD
Deglet Nour	0.2762 ± 0.0557	< LOD	< LOD	0.0277 ± 0.0047 ^{A}	< LOD	1.5441 ± 0.3928 ^{A}	0.0273 ± 0.00403
Kentichi	< LOD	< LOD	0.0151 ± 0.0002	0.0037 ± 0.0003^{B}	< LOD	0.3166 ± 0.0062^{B}	< LOD
Grape seeds							
Ugni blanc	130.565 ± 21.0602	0.1992 ± 0.0099	0.0084 ± 0.0005	0.0971 ± 0.0205	< LOD	1.4724 ± 0.3369	0.2396 ± 0.0239
ML (mg/kg)	n.a.	0.020*	0.020	n.a.	n.a.	n.a.	0.10

The results are expressed as mean ± standard deviation (SD), from three replicates. Different capital letters indicate the significant difference ($p < 0.05$) among cultivars.
LOD – Limit of Detection**; n.a.** - not applicable.
* The ML for arsenic present in table is for fruit juices, concentrated fruit juices as reconstituted and fruit nectars and the ML is defined for inorganic arsenic (sum of As(III) and As(V)).

4. Conclusions

This research concludes that sweet cherry pits, date seeds, and grape seeds contain varying amounts of antioxidant compounds, with grape and date seeds exhibiting the highest antioxidant capacities and phenolic content. Catechin and epicatechin were the main flavonoids found in sweet cherry pits and date seeds, while vanillic acid was the predominant phenolic acid. The study highlights the critical importance of food safety in valorizing fruit by-products for human consumption within the circular economy framework. Chemical contaminant analysis revealed that grape seeds had the highest number of pesticide residues, all below MRLs, whereas sweet cherry pits had high levels of insecticides and acaricides exceeding MRLs. Date seeds were free of pesticide residues but contained OTA below the LOQ, and Pb was detected in all sweet cherry pits, with As, Cd, and Pb in grape seeds. Considering all data, date seeds exhibit high potential for utilization in the food industry, given their high phenolic content and safe levels of chemical contaminants. However, attention should also be given to the presence of other toxic compounds, such as cyanogenic glycosides, in fruit seeds, to avoid or mitigate any potential adverse effects deriving from the consumption of foods containing fruit seeds.

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CRediT authorship contribution statement

Ana Rita Soares Mateus: Writing – original draft, Visualization, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Sílvia Cruz Barros:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Sandra Mariño Cortegoso:** Writing – original draft, Investigation, Formal analysis, Data curation. **Raquel Sendón:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Letrícia Barbosa-Pereira:** Writing – review & editing, Visualization, Resources, Project administration, Methodology, Funding acquisition. **Khaoula Khwaldia:** Writing – review & editing, Resources, Investigation. **Gianpiero Pataro:** Writing – review & editing, Visualization, Resources, Investigation. **Giovanna Ferrari:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Marion Breniaux:** Writing – review & editing, Visualization, Resources. **Remy Ghidossi:** Writing – review & editing, Validation, Supervision, Resources. **Angelina Pena:** Writing – review & editing, Validation, Supervision. **Ana Sanches-Silva:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101718.

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