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Bazı Hurma Çeşitlerinde Yaygın Olarak Kullanılan Pestisitlerin Belirlenmesi, Geri Kazanımı ve Antioksidan özelliklerinin Araştırılması

Güldeniz Yazıcı[1], Mustafa AKIN[2], Neslihan ŞAKİ[1*]

Öne Çıkanlar:

- Hurma meyvesinin antioksidan aktivitesi ve toplam fenol içeriği ekstraksiyon yöntemlerinden etkilendi
- Hurma ekstraktlarında çok sayıda kalıntı pestisit tespit edildi
- Bazı pestisit kalıntılarının belirlenen limitlerin üzerinde olduğu belirlendi

Anahtar Kelimeler:

- Antioksidan
- Pestisit
- Geri Kazanım
- Hurma
- LC-MS-MS

ÖZET:

Bu çalışmada, hurmaların antioksidan aktivite özelliklerini belirlemek için İran, Medine, Tunus, Kudüs ve Bağdat olmak üzere farklı ülkelerden ithal edilen beş çeşit hurma seçilmiştir. Ayrıca hurma bitkilerinde kullanılan 10 adet pestisit kalıntısı analiz edilmiş ve pestisit kalıntılarının geri kazanım kullanımına etkileri araştırılmıştır. Hurma ekstraktlarının antioksidan aktiviteleri DPPH ve Folin–Ciocalteu fenol reaktif deneyleri kullanılarak belirlendi. Pestisit kalıntı çalışmaları LC-MS-MS tekniği kullanılarak, geri kazanım çalışmaları ise AOAC.2007.01 ve 15662 Quechers yöntemleri kullanılarak gerçekleştirilmiştir. Hurma meyvesinin antioksidan aktivitesi ve toplam fenol içeriği lokasyon, genetik değişkenlik, çevresel özellikler, olgunlaşma aşamaları ve ekstraksiyon yöntemlerinden etkilenmiştir. Ekstraktlarda çok sayıda kalıntı pestisit tespit edilmiştir. Medine hurma meyvesinde bulunan Dioxacarb kalıntısının tolerans limitinin üzerinde olduğu belirlendi. AOAC 2007.01 Quechers yöntemine göre en yüksek Chlorpyrifos Methylin geri kazanımı Medine hurmasında 57.069 olarak bulunmuştur. Yapılan çalışmada hurma ekstratlarının standart antioksidanlara oranla daha düşük antioksidan aktivite gösterdiği tespit edildi. Hurmalarda yapılan pestisit analizlerinde birçok pestisit kalıntısı tespit edildi ve bazılarının belirlenen limitlerin üzerinde olduğu tespit edildi. Gıda maddelerindeki pestisit kalıntı miktarlarının daha önceden tespit edilip tolerans sınırlarını geçmemesi gerek tüketici sağlığı açısından ve gerekse ihraç gıda ürünlerinin geri dönmemesi açısından büyük öneme sahiptir. Çalışma kapsamında Hurma meyvesinde yaygın kullanılan pestisit kalıntıları analiz edilerek belirlenmiştir.

Determination, Recovery and Investigation of Antioxidant Properties of Commonly Used Pesticides in Some Types of Date Fruits

Highlights:

- Antioxidant activity and total phenol content of date fruit were affected by extraction methods
- Many residual pesticides were detected in date extracts
- It was determined that some pesticide residues were above the specified limits

Keywords:

- Antioxidant
- Pesticide
- Recovery
- Date fruit
- LC-MS-MS

ABSTRACT:

In this study, five types of dates imported from different countries, namely Iran, Medina, Tunisia, Jerusalem, and Baghdad, were selected to determine the antioxidant activity properties of dates. In addition, 10 pesticide residues used in date plants were analyzed and the effects of pesticide residues on recovery using were investigated. Antioxidant activities of the date extracts were determined by using DPPH and Folin–Ciocalteu phenol reagent assays. Pesticide residue studies were performed by using LC-MS-MS technique and recovery studies were carried out by using AOAC.2007.01 and 15662 Quechers methods Antioxidant activity and total phenol contents of date fruit were affected by location, genetic variability, environmental characteristics, maturation stages, and extraction methods. A lot of residual pesticides were determined in the extracts. It was determined that the Dioxacarb residue in the Medina date fruit was above the tolerance limit. According to AOAC 2007.01 Quechers method, the highest recovery of Chlorpyrifos Methylin was found as 57.069 in Medina date. In the study, it was determined that date extracts showed lower antioxidant activity compared to standard antioxidants. Many pesticide residues were detected in the pesticide analyzes made on dates, and some of them were found to be above the specified limits. It is of great importance that the pesticide residue amounts in foodstuffs are determined beforehand and not exceed the tolerance limits, both in terms of consumer health and in terms of not returning the exported food products. Within the scope of the study, pesticide residues commonly used in Date fruit were analyzed and determined.

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Bu çalışma Güldeniz Yazıcı'nın Yüksek Lisans tezinden üretilmiştir.

INTRODUCTION

Date fruit is one of the well-known plants in human history and has been consumed as food for about 6000 years due to their taste and concentrated nutrients. It has been played a major role both nutritionally and economically in the Middle East and North Africa regions for many years (Chao & Krueger, 2007). Date fruit, which has hundreds of varieties with different taste, color, and appearance, belongs to Phoenix dactylifera L. familias (Elshibli, 2009; Abul-Soad et al., 2017). The variety of dates is based on the valuable properties of the fruit; It is mainly rich in dietary abundant soluble sugar, dietary fiber, various phenolic compounds, and antioxidants (Dransfield, et al., 2005; Asmussen, et al., 2006). Date fruit varieties are spread over a wide geography starting from Canary Islands and including Middle East Countries. The most important factor affecting their growth is the warm climate, but differences in soil structure and plant genetic characteristics in the climatic regions where dates are grown cause changes in the antioxidant levels of dates (Al-Yahyai & Manickavasagan, 2012; Hifnawy et al., 2016). As it is known, antioxidants are important and unique compounds for animals, and their function is to prevent or slow down the activities of free radicals, unstable molecules produced by the body in response to metabolic activities and other pressures that damage cells (Gutowski & Kowalczyk, 2013; Phaniendra et al., 2015; Dinesh, 2021). Free radicals are defined as by-products formed during normal metabolic activity of cells. Both Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) collectively form free radicals and other non-radical reactive species. ROS/RNS play a dual role as compounds both beneficial and toxic to living systems (Pham-Huy et al., 2008; Zhang et al., 2013). At moderate or low levels, this ratio plays a role in cell immune tasks, several cellular signaling pathways, and various physiological functions such as mitogenic response and redox regulation. However, when present in higher concentrations, they produce oxidative and nitrosative stress and cause potential damage to biomolecules. High rates of ROS can also damage the integrity of various biomolecules, including lipids, proteins, and DNA (Taysi et al., 2018; Sharma et al., 2019).

Antioxidants are sometimes called "free radical scavengers" and can be produced naturally or artificially (Lorenz et al., 2003; Ahmadinejad et al., 2017). Natural antioxidants are mainly substances such as phenolic compounds, vitamin C, carotenoids and selenium derived from plants. Additionally, it can be given as examples of phenolic compounds originating from plant flavonoid compounds, cinnamic acid derivatives, coumarin and tocopherol (Gil et al., 2002; Siddeeg et al., 2019). Many studies prove that date fruits are rich in phenolic antioxidants (Brand-Williams et al., 1995; Cserhati et al., 2004), thus, have high antioxidant potential, and their content has changed depending on the diversity of dates, agricultural and environmental conditions (Pinelo et al., 2005; Zargoosh et al., 2019). The anti-inflammatory effect of dates is also attributed to polyphenol compounds that scavenge free radicals produced during the inflammatory process and act as antioxidants that prevent unwanted biochemical reactions. Date increases the activity of superoxide dismutase and catalase enzymes, indicating that dates modulate enzymatic behavior and thus trigger a signal chain of the antioxidant defense system as an anti-inflammatory. As a result of the fractionation and isolation of different extracts of dates, phenolic compounds, flavonoids and flavanols have been identified in its content. These subclasses of polyphenols are seen as antimicrobials and powerful antioxidants and are attributed to structural interactions between phenolic compounds and microorganisms (Puupponen-Pimia et al., 2001; Biglari et al., 2008).

Pesticides are the general name given to substances applied to kill or deter organisms (pests) that threaten the health and welfare of humans and animals or harm plants. While pesticides are supposed to be effective on insects, they can also poison humans and animals (Agrawal et al., 2010). While some of the pesticides do not cause any toxicological damage, some of them have been found to be toxic, mutagenic and even carcinogenic on the nervous system. Therefore, it is very important to keep pesticides away from the waste stream. They can leak into water channels and contaminate the soil so organic and synthetic pesticides must be disposed of in accordance with the rules. While pesticides and insecticides are technically non-recyclable, it is vital to dispose of them in an environmentally friendly way (Kaur et al., 2019; Narenderan et al., 2020). Nowadays, insecticides such as insecticides, herbicides and fungicides are widely used to obtain productive crops during cultivation. The reasons for use are to obtain a more controlled and improved food product by eliminating harmful organisms that will affect the yield of the crop. However, many pesticides which were used in agricultural applications have caused accumulation in the adipose tissues of people (Ntzani et al., 2017). In addition, pesticides pollute the water and soil and damage the environment. In our age, the use of some harmful pesticides has been banned and the use of some pesticides has been limited (Carlile, 2006). Nevertheless, this is not enough, and an urgent action plan needs to be determined and implemented. From the production of pesticides to the use of their packaging and disposal, the necessary protocols to protect the environment and health must be implemented effectively. The course of pesticides after application should be controlled in detail in terms of the importance of consuming pesticide-free foods in national and international consumption and preventing environmental contamination caused by pesticides. The indicator of countries' commitment to this issue is the number of qualified pesticide residue studies (Tiryaki, 2016).

In this study, five types of dates imported from different countries, namely Iran, Medina, Tunisia, Jerusalem, and Baghdad, were selected, and DPPH radical scavenging activity and total phenol content were examined to determine the antioxidant activity properties of dates. In addition, 10 pesticide residues used in date plants were analyzed by LC/MS/MS instrument. The effects of pesticide residues on recovery using AOAC.2007.01 and 15662 Quechers methods were compared.

MATERIALS AND METHODS

Chemicals and reagents

Folin–Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, ascorbic acid and analytical grade acetone were purchased from Sigma-Aldrich, and used as received without further purification. Sodium carbonate (Na_2CO_3), analytical grade methanol, acetonitrile, glacial acetic acid and formic acid were purchased from Merck. Disodium hydrogen citrate syrup ($Na_2C_6H_6O_7$), trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$), Primary Secondary Amine (PSA), magnesium sulphate kit ($MgSO_4$), sodium acetate kit (CH_3COONa) and sodium chloride (NaCl) were purchased from Agilent. Pesticide mix. standard was purchased from Accu-Standard. Millipore water (Milli-Q, 18,2 MΩ cm-1) was used to prepare all the samples and solutions throughout the experiments.

Preparation of date fruit extracts

Iran, Tunisia, Medina, Jerusalem, and Baghdad dates used in the experiments were purchased from herbalists in Kocaeli province. 1 kg of each type of date was purchased in packaged form. Then the damaged dates are separated. The remaining dates were weighed one by one and those with ± 10% difference in weight were selected and used in the experiments. They were separately grinded in the blender and each type of date was weighed to be 100 grams in 500 mL closed flasks. 200 mL of acetone and acetonitrile solvents were added to each date fruit, and they were stirred for 24 hours at room temperature for extraction. Acetonitrile and acetone extracts were filtered through Whatman 2 filter paper then the solvents were evaporated at 40°C. Stock date fruit extract solutions (30 mg/mL) were prepared by dissolving with the solvents used for the extraction and stored in a refrigerator for further use (Chaira et al., 2009; Al-Harrasi et al., 2014).

DPPH radical scavenging activity

DPPH free radical scavenging activity was determined according to literature (Gezer et al., 2006). Fruit extracts were prepared at different concentrations (100, 200, 300, 400 and 500 μg/mL) and 2.0 mL of each of the date fruit extract at different concentrations were taken into the test tubes and 1.0 mL of 1.0 mM DPPH solution was added on it. After the solutions were mixed with vortex, they were incubated at room temperature for 30 minutes in the dark. In addition, 2.0 mL acetone and acetonitrile and 1.0 mL DPPH solution were taken as control samples. After the incubation was completed, the absorbances of the dates, which were examined at different concentrations in two different solvents, were measured at 517 nm in a UV-Vis spectrophotometer and ascorbic acid was used as a standard for measurements. The % inhibition values according to the absorbance values of the extracts were calculated with the formula given below. All tests were repeated three times and inhibition values were calculated by using the mean values.

% Inhibition = [100 x (A control - A sample) / A control] (1)

A control: absorbance of the control

A sample absorbance of the sample

Total phenolic content

Total phenolic content was performed according to the method specified in the literature at different concentrations (Singleton et al., 1999). 1.0 mL of Folin–Ciocalteu phenol reagent was added to 1.0 mL of sample and mixed for 5 minutes. Then, by adding 1.0 mL of 10.0 % Na_2CO_3, it was filled to 10.0 mL with distilled water and incubated for 2 hours at room temperature. Finally, the absorbance was spectrophotometrically measured with a UV/Vis spectrometer at 760 nm, and the results were shown in mg of gallic acid equivalents per volume of sample (mg GAE/g). A calibration curve was created in the range of 50.0-600.0 μg/mL of gallic acid as a standard, and the results were recorded against curve. Tests were carried out triplicate.

LC-MS-MS for pesticide residue analysis

Pesticide residue and recovery studies were carried out on date fruits by using Orbitrap LC/MS/MS instrument of Kocaeli Food Control Laboratory Directorate which is accredited. An LC system consisting of a Thermo Ultimate 3000 liquid chromatography (Thermofisher, Waltham, MA, USA) and Thermo Accuroce QC18 column (Lot: 15341), (100 x 2.1mm, particle size 2.6 μm) was used for separation and maintained at 35°C. The mobile phase was composed of water (eluent A: H2O) and methanol (eluent B: MeOH), auto sampler temperature was 15˚C and flow rate was 0.3 mL/min and injection volume was 10.0 μL.

MS/MS experiments were performed using Q-Exactive Focus (Thermofisher, Waltham, MA, USA) equipped with a heated electrospray ion source (HESI). Positive ion analysis was performed in the multiple reaction monitoring (MRM) mode. The collision gas pressure was 0.2 Pa (nitrogen purity of 99.9995%), while the sheath and auxiliary gas pressures (99.9% purity) were set to 40 and 10 Pa, respectively. The vaporizer and capillary temperatures were set to 350°C and 320°C respectively, with a spray voltage of +4 kV. In the PRM experiments, the normalized collision energy (NCE) was obtained from the inclusion list. The data were collected at a resolution (R) = 17500 @m/z 195.0882.

AOAC 2007.01 Quechers method

AOAC 2007.01 Quechers method was applied to determine pesticide residue in date fruits according to the method specified in the references (Çetinkaya Açar, 2015; Lawal et al., 2018, Varela-Martínez et al., 2019). From the dates that were grinded in a blender and homogenized, 5.0 grams were weighed into 50.0 mL centrifuge tubes. 10.0 mL of distilled water was added on them. According to the document numbered SANTE 11813/2017, the amount of knitting may have to be reduced in products such as flour, dried fruit, honey, and spices with less than 25 % water content, and the amount is completed to 15.0 grams with the addition of water (Petrović et al., 2017). Hence, firstly, acetic acid was dissolved in 15.0 mL of 1 % acetonitrile, then added to the Quechers salt (6.0 g $MgSO_4$, and 1.5 g CH_3COONa). The centrifuge tube was shaken for 2 minutes to allow the pesticides present in the sample to pass into the solvent. 8.0 mL of the upper phase of the date fruits which were centrifuged at 4000 rpm for 2 minutes was taken and transferred to a 15.0 mL centrifuge tube containing Quechers salts (1200 mg $MgSO_4$ and 400 mg PSA). After 30 seconds of agitation, it was centrifuged at 4000 rpm and taken from the upper phases with a 2.0 mL injector and passed through a 0.45 μm filter and 500.0 μl was taken into 1.5 mL vials. Then, 6.7 mM formic acid was added to the vial. Calibration standards for pesticide screening were likewise prepared in the form of 500 μl spike sample (15.0 g weighed sample) and 500 μl formic acid. Therefore, as the dates were studied by weighing 5.0 g, threefold dilution was made in the analysis and the results were evaluated by considering the dilution coefficient. Methanol and water were used as the mobile phase.

Recovery by AOAC 2007.01 Quechers method

According to the SANTE/11813/2017 Food and Feed Pesticide document, the date fruit matrix, which is included in the group of products with high sugar and low water content was homogenized and approximately 7.5 g was weighed into 50.0 mL centrifuge tubes and 7.5 mL distilled water was added. 750 μl (50,0 ppb) from the 500.0 ppb Accu mix pesticide standard were spiked to the dates, then acetonitrile which containing 1.0 % acetic acid was added. In the extraction stage, 6.0 g $MgSO_4$ and 1.5 g CH_3COONa salts were added. Centrifuge tubes were shaken in a shaker for 2 minutes, then centrifuged at 4000 rpm and 8.0 mL of the upper phase was transferred to the 15.0 mL centrifuge tubes which had Quechers second stage salts (1200.0 mg and 400.0 mg PSA) (Costa et al., 2014). After 30 seconds agitation, the solution was centrifuged 2 minutes at 4000 rpm. The upper phases were passed through a 0.45 μm filter with a 2.0 mL injector and 500.0 μL sample and 500 μL formic acid solution (6.7 mM) were added to the vials. Calibration was also performed with a date matrix, so the results were evaluated without multiplying by the dilution coefficient.

Recovery by EN 15662 Quechers method

In this method, as in the AOAC 2007.01 Quechers method, the same amount of weighing from the homogeneous date fruits and water were added to fortification with 750 μl pesticide standard. Unlike the AOAC 2007.01 Quechers method, in the extraction step, after the solvent addition, 4.0 g magnesium sulphate, 1.0 g sodium chloride, 1.0 g trisodium citrate dihydrate, 0.5 g disodium hydrogen citrate extraction salts were added. In the next stage, the analysis was carried out by following the steps in the AOAC 2007.01 Quechers method (Cieslik et al., 2011).

Quechers recycling methods with a different extraction salt were studied using MeOH (methanol) and H2O (water) mobile phases in the LC/MS/MS instrument of the dates to understand differences of two versions of Quechers. These solutions, called mobile phases A and B, were prepared as follows. Mobile Phase A: To identify the types of pesticides contained in date fruits, distilled water was added to a 1000 mL flask and 4.0 mL of ammonium formamide and 1,0 mL of formic acid were added, and the final volume was completed to 1.0 L with distilled water.

Mobile Phase B: To identify the types of pesticides contained in date fruits, methanol was added to 1000 mL volumetric flask and 4.0 mL of ammonium formamide and 1.0 mL of formic acid were added, and the final volume was completed to 1.0 L with methanol.

Acetonitrile and water are the mobile phases of EN 15662 Quechers method. To examine the differences of the mobile phase how to effect on recovery in the Quechers method, date fruits matrices were carried out with the EN 15662 Quechers method. This time, acetonitrile and water were used as a mobile phase at LC/MS/MS. Thus, the same extraction salts were used, and the mobile phases were replaced due to the recycling study was carried out with the same method (Alder et al., 2006).

RESULTS AND DISCUSSION

DPPH radical scavenging assay

The DPPH method evaluates the capacity of compounds in date fruit extract to reduce DPPH radical. Figures 1 and 2 shows the DPPH free radical scavenging activities of date fruit extracts determined at different µg/mL in acetonitrile and acetone, at 517 nm (Marinova et al., 2011). According to these results, Medina acetone extract had the highest DPPH free radical scavenging activity with 43.57% ± 0.37%, while the lowest activity was found in Tunisian date acetonitrile extract with 3.37% ± 0.14%. In our study, the DPPH radical scavenging activity of Medina date fruit was highest in acetone extracts (43.57% ± 0.37%), followed by Tunisian date (24.44% ± 0.12%), Jerusalem date (17.04% ± 0.38%), Baghdad date (15.42% ± 0.53%) and Iranian date (9.70% ± 0.12%). In acetonitrile extracts DPPH free radical scavenging activity result respectively were found that Medina date fruit (18.24% ± 0.95 %), Jerusalem date fruit (13.65% ± 1.64%), Iran date fruit (11.66% ± 0.41%), Baghdad date fruit (9.07% ± 3.32) and Tunisian date (3.37% ± 0.14%). The results of this study showed that date fruit grown in Medina can be a good source of antioxidants as it has higher DPPH scavenging properties than ascorbic acid used as a standard antioxidant. In a study in the literature, the highest DPPH scavenging activity was determined in Allig date extract and the lowest activity in Deglet Nour date extracts. DPPH radical scavenging of Allig, Bejo, and Deglet Nour date extracts were found to be 58.77%, 40.78%, and 23.98%, respectively (Abbes et al., 2013). Another study indicates that the DPPH removal activity of Tunisian date extract was found to be 1.53%. However, in our study, the DPPH removal activity of acetone extract of Tunisian date was detected higher than the literature results (Saafi et al., 2009).

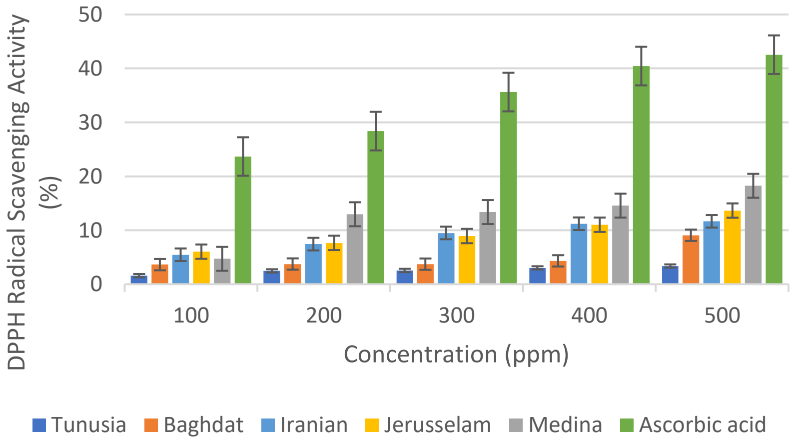

Figure 1. DPPH free radical scavenging activity of acetonitirile extracts (%)

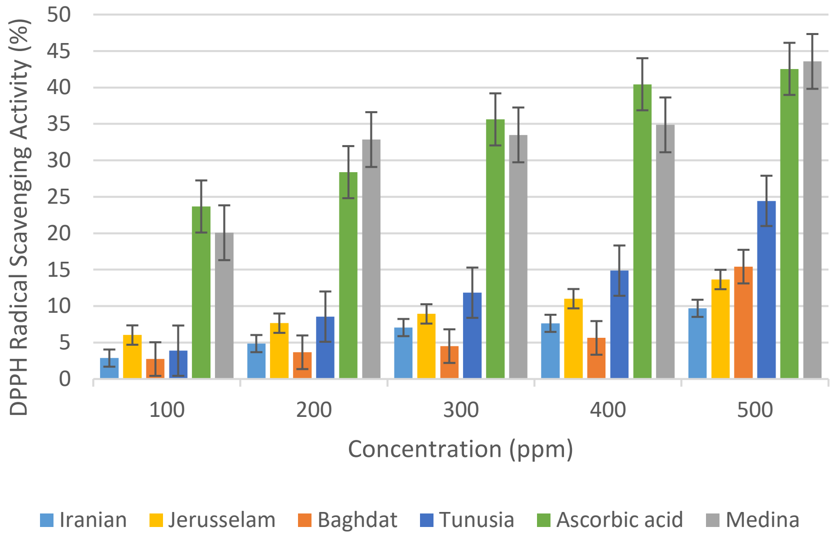

Figure 2. DPPH free radical scavenging activity of acetone extracts (%)

Total phenolic content

To determine total phenol content in date fruit extracts, a graph of gallic acid used as a standard compound was prepared (Singleton et al., 1965). Total amount of gallic acid in all extracts was calculated from the formula obtained from the standard graph (r2: 0.9739). Table 1 shows the total phenol contents of Baghdad (Zahidi), Tunisia (Berni), Jerusalem (Medjoul), Iran (Mazafati) and Medina (Hudri) Phoenix dactylifera L/ 100 g dates. It is seen that the total phenolic contents increase depending on the concentration and the total phenolic contents of the dates give different results. Among varieties, the total phenol contents in 500 ppm of acetonitrile extracts of dates are; Tunisia (15.86 ± 0.09 mg of GAE/100 g), Baghdad (12.89 ± 0.23 mg of GAE/100 g), Jerusalem (8.63 ± 0.07 mg of GAE/100 g), Medina (7.72 ± 0.16 mg of GAE/100 g) and Iran (7.13 ± 0.10 mg of GAE/100 g). The total phenol content of date fruit acetone extracts at 500 ppm; Baghdad (29.24 ± 0.07 mg of GAE/100 g), Iran (16.79 ± 0.17 mg of GAE/100 g), Tunisia (14.81 ± 0.07 mg of GAE/100 g), Jerusalem (13.92 ± 0.18 mg of GAE/100 g), Medina (12.27 ± 0.16 mg of GAE/100 g). While the highest total phenol content was in the Baghdad date acetone extract, the lowest phenolic content was obtained in the Iranian date acetonitrile extract. According to the literature, the total phenolic contents of Allig, Deglet Nour, and Bejo dates were calculated as 505.49 ± 3.36 mg, 240.38 ± 1.12 mg, and 391.94 ± 5.18 mg, respectively. Furthermore, the total phenolic substance content in the dates was determined as 147.14 mg GAE / 100.0 g, while the phenolic substance content was the highest in the history of Iranian date (Rabbi) 250.62 mg GAE / 100 g, the lowest 35.21 mg GAE / 100 g found in the Iranian (Rutab) date (Kchaou et al., 2016). In another study, when the phenolic substance contents in Algerian dates were examined, results showed that the highest phenolic substance was 954.59 mg GAE / 100 g in the Ghazi dates and 225.57 mg GAE / 100 g on Deglet Nour dates (Benmeddour et al., 2013). In the study conducted in 2005, phenolic content of Algerian dates between 2.49-8.36 mg GAE / 100 g were found (Mansouri et al., 2005). All these works clearly demonstrate that the amounts of phenolic compounds and the antioxidant capacities of dates were affected by location, genetic variability, environmental characteristics, maturation stages, and extraction methods.

Table 1. % Total phenolic content of date extract (mg GAE/100 g date).

Extracts	Concentration (µg/mL)	Total Phenolic Content (mg GAE/100 g date)	Extracts	Concentration (µg/mL)	Total Phenolic Content (mg GAE/100 g date)
Iranian Date Acetonitrile Extract	100	5.91±0.11	Iranian Date Acetone Extract	100	6.61±0.15
	200	6.18±0.09		200	7.00±0.15
	300	6.34±0.07		300	7.79±0.17
	400	6.61±0.14		400	8.41±0.19
	500	7.13±0.10		500	16.79±0.17
Baghdad Date Acetonitrile Extract	100	9.19±0.27	Baghdad Date Acetone Extract	100	18.89±0.29
	200	9.76±0.21		200	19.44±0.10
	300	11.08±0.29		300	20.06±0.09
	400	12.09±0.17		400	24.17±0.08
	500	12.89±0.23		500	29.24±0.07
Medina Date Acetonitrile Extract	100	6.29±0.11	Medina Date Acetone Extract	100	8.47±0.12
	200	6.61±0.10		200	9.02±0.15
	300	6.98±0.15		300	10.79±0.13
	400	7.45±0.06		400	11.84±0.12
	500	7.72±0.16		500	12.27±0.16
Jerusalem Date Acetonitrile Extract	100	7.49±0.08	Jerusalem Date Acetone Extract	100	11.25±0.13
	200	7.70±0.11		200	11.56±0.11
	300	8.04±0.09		300	12.66±0.26
	400	8.30±0.10		400	13.03±0.09
	500	8.63±0.07		500	13.92±0.18
Tunisia Date Acetonitrile Extract	100	7.61±0.13	Tunisia Date Acetone Extract	100	8.08±0.19
	200	8.14±0.26		200	11.71±0.07
	300	9.11±0.12		300	11.95±0.11
	400	9.50±0.07		400	12.64±0.21
	500	15.86±0.09		500	14.81±0.07

*Data are expressed as mean ± standard deviation; Values within each type of date fruit extract marked with the same letter in the same column are not significantly different.

According to the antioxidant activity results, non-parallel results were obtained when the DPPH and Total phenol content methods were compared. Although DPPH removal activity was high in both solevnt extractions in Medina dates, the date extract with the highest total phenol content was obtained in Tunisia and Baghdad dates. The reason for this is that -OH molecules in the structure are active in the method of determining the total phenol content, while in the DPPH method, the reaction takes place by electron transfer. In the studies carried out, it was determined that there was no correlation between the DPPH method and the total phenol content (Yongchou et al., 2014).

Screening of pesticide residues

Table 2 shows the substances detected in date extracts and residual pesticide concentrations. No pesticide residue was found in the Iranian date. Malathion Oxon (0.222 ± 0.111 µg / kg) in Baghdad dates, Cyprodinil (0.189 ± 0.094 µg / kg) and Fenpropimorph (0.423 ± 0.212 µg / kg) in Jerusalem dates, Cyprodinil (0.165 ± 0.082 µg / kg), Dioxacarb (36.384 ± 18.192 µg / kg), Fenpropimorph (0.213 ± 0.106 µg / kg), Malathion Oxon (0.249 ± 0.124 µg / kg) and Terbufos (7.224 ± 3.612 µg / kg) in Medina dates, Cyprodinil (0.249 ± 0.124 µg / kg) and Fenpropimorph (0.189 ± 0.094) in Tunisia dates, residues were detected. Dioxacarb residue was found above the tolerance limit in the Medina dates, and other pesticides are below the tolerance values. Chromatograms of pesticide residues are given in Figure 3.

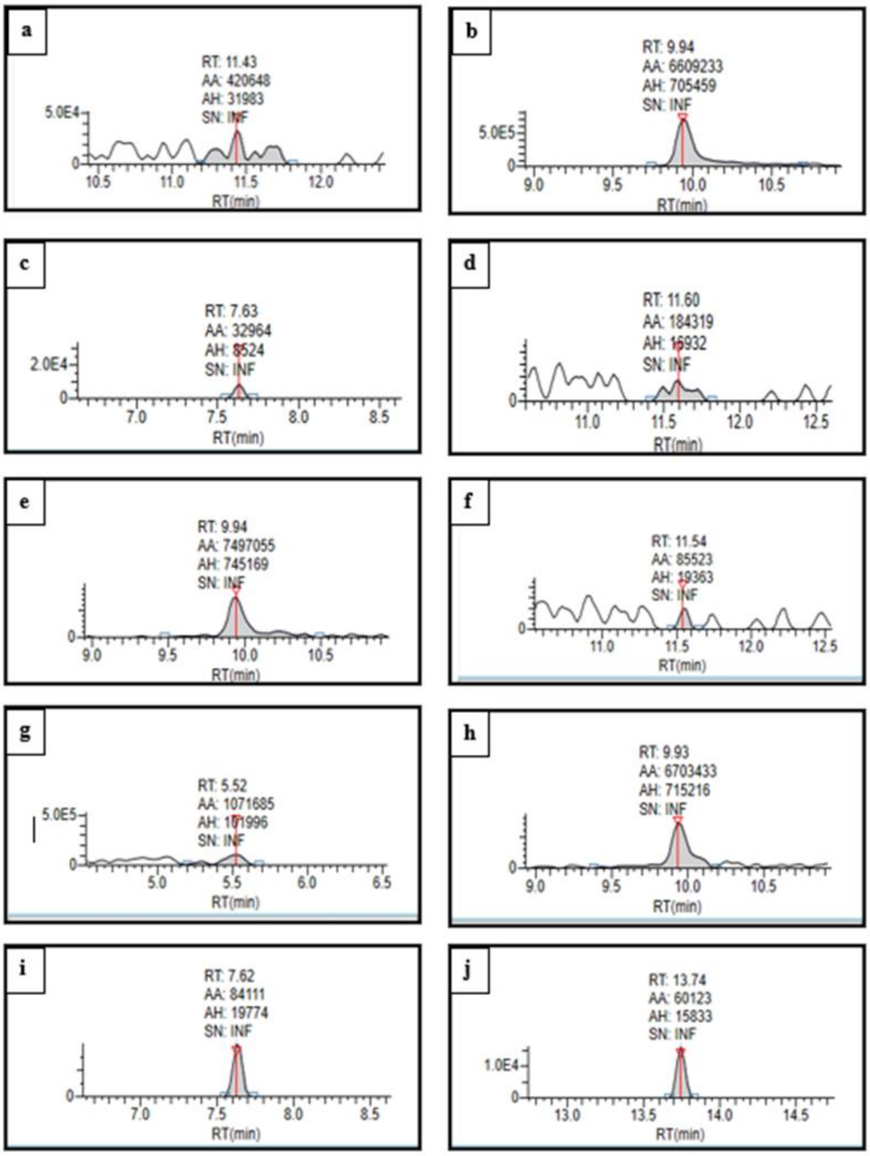

Figure 3. Chromatograms of pesticide residues. a) Tunisian cyprodinil b) Tunisian Fenpropimorph, c) Baghdad Malathion Oxon, d) Jerusalem cyprodinil, e) Jerusalem Fenpropimorph, f) Medina cyprodinil, g) Medina Dioxacarb, h) Medina Fenpropimorph, i) Medina Malathion Oxon, j) Medina Terbufos

The residual amounts detected in dates were calculated by using the peak areas. The results have been evaluated considering the European Commission's Food Safety Maximum Residue Limits list and the Turkish Food Codex Pesticides Maximum Residue Limits Regulation. The maximum residual amounts (MRL) of pesticides that can be found in the date fruit sample are given as ppm (mg/kg) in the TFC regulation annexes. Furthermore, these values are used in Table 2 given by ppb (µg/kg) to better interpret the results. Dates pesticide residues were approved according to the Turkish Food Codex Pesticides Maximum Residue Limit (MRL) regulation. However, the active ingredient Dioxacarb, which is one of the banned pesticides in the Medina date, was found to be above the allowable MRL value (10.0 µg/kg) with a residual amount of 36.384 ± 18.192 µg/kg.

Table 2. Pesticide residue concentrations determined in date samples and their evaluation according to The Turkish Food Codex (TFC).

Dates	Detected Compound	Residue Amount (µg/kg)	Analysis Method / Instrument	TFC Tolerant Values (µg/kg)
Iranian	---	ND	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	---
Baghdad	**Malathion Oxon**	0.222±0.111	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	20*
Jerusalem	**Cyprodinil**	0.189±0.094	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	20*
	Fenpropimorph	0.423±0.212	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	50*
Medina	**Cyprodinil**	0.165±0.082	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	20*
	Dioxacarb	36.384±18.192	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	10*
	Fenpropimorph	0.213±0.106	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	50*
	Malathion Oxon	0.249±0.124	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	20*
	Terbufos	7.224±3.612	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	10*
Tunisian	**Cyprodinil**	0.249±0.124	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	20*
	Fenpropimorph	0.189±0.094	AOAC 2007.01 Quechers Orbitrap LC/MS/MS	50*

*ND: Not detected. Values with * LOD (Maximum Permitted Detection Limit) The LOD for non-MRL pesticides is specified in Annex 5 of the Turkish Food Codex (TFC) regulations.

Recovery of pesticides

The recovery determination of Chlorpyrifos Ethyl, Dimethoate, Fenazaquin, Fenpyroximate, Fipronil, Hexythiazox, Imidacloprid, Malathion, Pyriproxyfen and Spinosad (Spinosyn A + Spinosyn D) pesticides in dates were carried out AOAC 2007.01 and EN 15662 Quechers method. According to AOAC 2007.01 Quechers method, recovery of Chlorpyrifos Methylene was found as 57.069 in Medina date, 56.430 in Tunisia date, 55.576 in Baghdad date, 50.176 in Iran date and 45.046 in Jerusalem date. Dimethoate recovery 56.689, Fenpyroximate recovery 56.008, Pyriproxyfen recovery 58.896 and Spinosad (A + D) total recovery 53.453 in Medina, Fenazaquin recovery 57.618 in Baghdad, Fipronil recovery 46.384, Hexythiazion recovery 57.328, Malathion recovery 58.503 in Tunisia dates and Imidacloprid recovery was determined as 58.512 in Iran dates. The data of the values are shown in Table 3 below.

Table 3. Recovery values of pesticides by AOAC 2007.01 Quechers method.

Pesticides	Spike Concentration (µg/kg)	Recovery Values of Dates				
		Iran	Jerusalem	Medina	Baghdad	Tunisia
Chlorpyrifos Methyl	50	50.176	45.046	57.069	55.576	56.430
Dimethoate	50	54.905	53.057	56.689	53.943	55.714
Fenazaquin	50	53.984	50.071	54.752	57.618	54.718
Fenpyroximate	50	54.538	50.827	56.008	54.709	55.278
Fipronil	40	40.442	40.184	43.399	42.965	46.384
Hexythiazox	50	56.181	51.136	56.342	56.060	57.328
Imidacloprid	50	58.512	53.976	58.098	56.924	54.569
Malathion	50	54.552	55.670	58.226	58.195	58.503
Pyriproxyfen	50	52.119	53.772	58.896	56.412	55.054
Total Spinosad (A+D)	50	48.362	49.064	53.453	52.238	52.663

* Results were averaged over 3 repetitions. (n = 3) Measurements were taken from the Orbitrap LC/MS/MS instrument in accordance with the AOAC 2007.01. Quechers method.

The recovery determination results of Chlorpyrifos Ethyl, Dimethoate, Fenazaquin, Fenpyroximate, Fipronil, Hexythiazox, Imidacloprid, Malathion, Pyriproxyfen and Spinosad (spinosyn

A + spinosyn D) pesticides in dates with EN 15662 Quechers method are given in Table 4. Chlorpyrifos Methyl recovery was found in Tunisian dates (55.507), Dimethoate (60.256), Fipronil (47.032), Malathion (55.941) in Medina dates, Fenazaquin (55.488), Fenpyroximate (55.958), Hexythiazox (57.829), Pyriproxyfen (57.16). Spinosad (A + D) (53.904) were detected in the Tunisia dates, and Imidacloprid (57.079) pesticide residue was detected in the Iran dates.

Table 4. Recovery values of pesticides by EN 15662 Quechers method.

Pesticides	Spike Concentration (µg/kg)	Recovery Values of Dates Iran	Jerusalem	Medina	Baghdad	Tunisia
Chlorpyrifos Methyl	50	40.361	49.326	53.716	44.042	55.507
Dimethoate	50	54.518	54.512	60.256	55.458	54.776
Fenazaquin	50	39.694	52.046	52.488	45.726	55.488
Fenpyroximate	50	43.947	46.757	53.409	45.652	55.958
Fipronil	40	31.954	38.296	47.032	36.845	43.550
Hexythiazox	50	44.229	50.845	56.994	51.480	57.829
Imidacloprid	50	57.079	53.117	56.468	55.794	55.687
Malathion	50	44.468	50.050	55.941	48.511	53.612
Pyriproxyfen	50	40.032	48.364	55.399	45.126	57.164
Spinosad (A+D)	50	39.238	46.398	52.305	42.668	53.904

* Results were averaged over 3 repetitions. (n = 3) Measurements were taken from the Orbitrap LC/MS/MS instrument in accordance with the EN 15662 Quechers method.

CONCLUSION

In this study, Medina, Tunisia, Iran, Baghdad, and Jerusalem date fruits were studied. Their antioxidant properties were tested by DPPH free radical scavenging activity and total phenolic content assays. Quechers methods were used to specify total residual pesticide of date fruits. Moreover, Quechers method were used to recovery determinations of Chlorpyrifos Methyl, Dimethoate, Fenazaquin, Fenpyroximate, Fipronil, Hexythiazox, Imidacloprid, Malathion, Pyriproxyfen and Spinosad (spinosyn A + spinosyn D) pesticides. Antioxidant activity and total phenol contents of date fruit were affected by location, genetic variability, environmental characteristics, maturation stages, and extraction methods. A lot of residual pesticides were determined in the extracts. It was determined that the Dioxacarb residue in the Medina date fruit was above the tolerance limit. According to AOAC 2007.01 Quechers method, the highest recovery of Chlorpyrifos Methylin was found as 57.069 in Medina date.

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Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article**.**

REFERENCES

Abbès, F., Kchaou, W., Blecker, C., Ongena, M., Lognay, G., Attia, H. & Besbes, S. (2013). Effect of processing conditions on phenolic compounds and antioxidant properties of date syrup. *Ind. Crops Prod.*, 44, 634–642.

Abul-Soad, A. A., Jain, S. M., Jatoi, M. A. (2017). Biodiversity and conservation of woody plants. *Sustainable development and biodiversity*,17, 313–353.

Agrawal, A., Pandey, R. S., Sharma, B. (2010). Water Pollution with Special Reference to Pesticide Contamination in India. *J. Water Resour. Prot*., 02(05), 432–448.

Ahmadinejad, F., Møller, S. G., Hashemzadeh-Chaleshtori, M., Bidkhori, G., Jami, M. S. (2017). Molecular mechanisms behind free radical scavengers function against oxidative stress. *Antioxidants,* 2017, 6(3), 51.

Alder, L., Greulich, K., Kempe, G., Vieth, B. (2006). Residue analysis of 500 high priority pesticides: Better by GC-MS or LC-MS/MS. *Mass Spectrom. Rev*., 25(6), 838–865.

Al-Harrasi, A., Rehman, N. U., Hussain, J., Khan, A. L., Al-Rawahi, A., Gilani, S. A., Al-Broumi, M., Ali, L. (2014). Nutritional assessment and antioxidant analysis of 22 date palm (Phoenix dactylifera) varieties growing in Sultanate of Oman. *Asian Pac. J. Trop. Med*., 7(S1), 591–S598.

Al-Yahyai, R., Manickavasagan, A. (2012). Dates production, processing, food, and medicinal values an Overview. *Date Palm Prod*., 24(5), 1–10.

Asmussen, C. B., Dransfield, J., Deickmann, V., Barfod, A. S., Pintaud, J. C., Baker, W. J. (2016). In A new subfamily classification of the palm family (Arecaceae): Evidence from plastid DNA phylogeny. *Botanical Journal of the Linnean Society*, 151(1), 15–38.

Benmeddour, Z., Mehinagic, E., Meurlay, D., Louaileche, H. (2013). Phenolic composition and antioxidant capacities of ten Algerian date (Phoenix dactylifera L.) cultivars: A comparative study. *J. Funct. Foods.,* 5(1), 346–354.

Biglari, F., AlKarkhi, A. F. M., Easa, A. M. (2008). Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran. *Food Chem*., 107(4), 1636–1641.

Brand-Williams, W., Cuvelier, M. E., Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology,* 28(1), 25–30.

Carlile, B. (2006). Pesticide selectivity, health and the environment. Cambridge University Press, doi:10.1017/CBO9780511617874.

Chaira, N., Mrabet, A., Ferchichi, A. (2009). Evaluation of antioxidant activity, Phenolics, sugar and mineral contents in date palm fruits. *J. Food Biochem*., 33(3), 390–403.

Cieślik, E., Sadowska-Rociek, A., Ruiz, J. M. M., Surma-Zadora, M. (2011). Evaluation of QuEChERS method for the determination of organochlorine pesticide residues in selected groups of fruits. *Food Chem.*, 125(2), 773–778.

Costa, F. P., Caldas, S. S., Primel, E. G. (2014). Comparison of QuEChERS sample preparation methods for the analysis of pesticide residues in canned and fresh peach. *Food Chem*., 165(16), 587–593.

Cserháti, T., Forgács, E., Deyl, Z., Miksik, I., Eckhardt, A. (2004). Chromatographic determination of herbicide residues in various matrices. *Biomedical Chromatography*. 18(6), 350–359.

Çetinkaya, A. Ö. (2015). Pestisit Analizleri Eğitim Notu. *T.C. Gida Tarim Ve Hayvanc. Bakanl. Ulus. Gida Ref. Laboratuvari.,* 32, 13.

Dinesh, R. (2021). The role of antioxidants and ROS scavenging machinery in wild mushrooms. In New and Future Developments in Microbial Biotechnology and Bioengineering. *Elsevier*, 245–251.

Dransfield, J., Uhl, N. W., Asmussen, C. B., Baker, W. J., Harley, M. M. (2005). A new phylogenetic classification of the palm family, Arecaceae. *Kew Bull*., 60(4), 559–569.

Elshibli, S. (2009). *Genetic Diversity and Adaptation of Date Palm (Phoenix dactylifera L .),* Faculty of Agriculture and Forestry, University of Helsinki, for public criticism in Viikki, Auditorium B5 on December, 1–77.

Gezer, K., Duru, M. E., Kivrak, I., Turkoglu, A., Mercan, N., Turkoglu, H., Gulcan, S. (2006). Free-radical scavenging capacity and antimicrobial activity of wild edible mushroom from Turkey. *African J. Biotechnol*., 5(20), 1924–1928.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Kader, A. A. (2012). Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. *J. Agric. Food Chem*., 50(17), 4976–4982.

Gutowski, M., Kowalczyk, S. (2013). A study of free radical chemistry: Their role and pathophysiological significance. *Acta Biochimica Polonica*, 60(1), 1–16.

Hifnawy, M. S., Mahrous, A. M. K., Ashour, R. M. S. (2016). Phytochemical investigation of Phoenix canariensis Hort. ex Chabaud leaves and pollen grains. *J. Appl. Pharm. Sci.*, 6(12), 103–109.

Kaur, R., Mavi, G. K., Raghav, S., Khan, I. (2019). Pesticides Classification and its Impact on Environment. Int. J. Curr. Microbiol. Appl. Sci., 2019, no. 8(03), pp. 1889–1897.

Kchaou, W., Abbès, F., Mansour, R., Blecker, C., Attia, H., Besbes, S. (2016). Phenolic profile, antibacterial and cytotoxic properties of second grade date extract from Tunisian cultivars (Phoenix dactylifera L.). *Food Chem*., 194, 1048–1055.

Lawal, A., Wong, R. C. S., Tan, G. H., Abdulra'Uf, L. B., Alsharif, A. M. A. (2018). Recent modifications and validation of QuEChERS-dSPE coupled to LC-MS and GC-MS instruments for determination of pesticide/agrochemical residues in fruits and vegetables: Review. *J. Chromatogr. Sci*., 56(7), 656–669.

Lorenz, P., Roychowdhury, S., Engelmann, M., Wolf, G., Horn, T. F. W. (2003). Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: Effect on nitrosative and oxidative stress derived from microglial cells. Nitric Oxide - *Biol. Chem*., 9(2), 64–76.

Mansouri, A., Embarek, G., Kokkalou, E., Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (Phoenix dactylifera). *Food Chem*., 89(3), 411–420.

Marinova, G., Batchvarov, V. (2011). Methods DPPH. *Bulg. J. Agric. Sci*., 17(1), 11–24.

Narenderan, S. T., Meyyanathan, S. N., Babu, B. (2020). Review of pesticide residue analysis in fruits and vegetables. Pre-treatment, extraction and detection techniques. *Food Research International*., 133, 109-141.

Ntzani, E. E., Ntritsos, G. C. M., Evangelou, G. E., Tzoulaki, I. (2017). Literature review on epidemiological studies linking exposure to pesticides and health effects. *EFSA Support. Publ.*, 10(10), 497.

Petrović, A., Marinković, D., Zeremski, T. (2019). A simplified LC/MS-MS method for the detection, identification and quantification of over 100 pesticides in sour cherries as a complex matrix. *The Proceedings, ISEM8 October,* 2-5, Budva, Montenegro.

Pham-Huy, L. A., He, H., Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*, 4(2), 89–96.

Phaniendra, A., Jestadi, D. B., Periyasamy, L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian Journal of Clinical Biochemistry*., 30(1), 11–26.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J. Agric. Food Chem*., 53(6), 2111–2117.

Puupponen-Pimiä, R., Nohynek, L., Meier, C. (2001). Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol*., 90(4), 494–507.

Saafi, E. B., Arem, A., Issaoui, M., Hammami, M., Achour, L. (2009). Phenolic content and antioxidant activity of four date palm (Phoenix dactylifera L.) fruit varieties grown in Tunisia. *Int. J. Food Sci. Technol*., 44(11), 2314–2319.

Sharma, A., Gupta, P., Prabhakar, P. K. (2019). Endogenous Repair System of Oxidative Damage of DNA. *Curr. Chem. Biol.*, 13(2), 110–119.

Siddeeg, A., Zeng, X. A., Ammar, A. F., Han, Z. (2019). Sugar profile, volatile compounds, composition and antioxidant activity of Sukkari date palm fruit. *J. Food Sci. Technol*. 56(2), 754–762.

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic*., 16(3), 144.

Singleton, V. L., Orthofer, R., Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol*., 299, 152–178.

Taysi, S., Tascan, A. S., Ugur, M. G., Demir, M. (2018). Radicals, Oxidative/Nitrosative Stress and Preeclampsia. *Mini-Reviews Med. Chem*., 19(3), 178–193.

Tiryaki, O. (2017). Türkiye'de yapılan pestisit kalıntı analiz ve çalışmaları. *Erciyes Üniversitesi Fen Bilim. Enstitüsü Fen Bilim. Derg.,* 32(1), 72–80.

Younchou, Z. (2014). Relationship of Total Phenolic Contents, DPPH Activities and Anti-Lipid-Oxidation Capabilities of Different Bioactive Beverages and Phenolic Antioxidants. B.S., Shanghai Institute of Technology.

Zargoosh, Z., Ghavam, M., Bacchetta, G., Tavili, A. (2019). Effects of ecological factors on the antioxidant potential and total phenol content of Scrophularia striata Boiss. *Sci. Rep*., 9(1), 1–15.

Zhang, C. R., Aldosari, S. A., Vidyasagar, P. S. P. V., Nair, K. M., Nair, M. G. (2013). Antioxidant and anti-inflammatory assays confirm bioactive compounds in ajwa date fruit. *J. Agric. Food Chem*., 61(24), 5834–5840.