doi: 10.17221/276/2015-HORTSCI

Determination of antioxidant activities of some apple cultivars

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Abstract

Pirlak L., Ünüvar G., Ersoy N. (2017): Determination of antioxidant activities of some apple cultivars. Hort. Sci. (Prague), 44: 120–125.

This study was conducted in order to determine the antioxidant activities of certain apple varieties. 'Galaxy Gala', 'Scarlet Spur', 'Fuji', 'Pink Lady' and 'Granny Smith' were characterized in this study. The antioxidant activities among the varieties were determined via three different procedures. Antiradical activity figures of the apple varieties were between 0.592 ('Galaxy Gala') and 0.802 ('Scarlet Spur'). Inhibition levels of chelating activity with Fe^{+2} varied between 40.20–55.50%; 'Scarlet Spur' and 'Pink Lady' extracts had the highest inhibition with 55.50% and 'Granny Smith' extract had the lowest with the level. Among the extracts showing H_2O_2 clearance effect, Granny Smith (62.54%) and Scarlet Spur (44.67%) extracts had the highest inhibition levels. In this study conducted on apple varieties, total phenolic matter levels were determined in 'Scarlet Spur', 'Pink Lady', 'Fuji', 'Galaxy Gala' and 'Granny Smith'.

Keywords: antiradical activity; phenolics; DPPH

Apple trees have been grown in all continents except Antarctic and are well suited to the temperate climate regions and tropical regions with high altitudes (Korban, Skirvin 1984). Turkey is accepted as the genetic centre for other agricultural crops as well as fruit plants and is rich in different types of flora and cultivars of fruit trees. Turkey is the original growth country of most fruit plants as well as apple tree.

In recent years, healthy food consumption has gained great importance for human beings. Grapes, among the fruits, play a very important role due to high contents of flavonoids, phenolic acids of which some are anti-cancer, or have anti-mutagenic and antioxidant components. Antioxidants eliminate the molecules called 'free radicals' that occur in the human body after the metabolic activities. In the case of excess free radical production, they have a harmful effect on cell, and cancer may occur from the action of some enzymes. Antioxidants are not produced only by body cells, but also are taken from food. The fundamental natural antioxidants that are present in food that protect the human body from the harmful free radicals are vitamins (C, E and A), flavonoids, carotenoids and polyphenols. There was found an inverse relationship between the consumption of fruit and vegetable, and cancer or heart attack (Rice-Ivens et al. 1997). Therefore, it was suggested that such risk could be reduced by consumption of food with high antioxidant content (Sandra 2004).

Apple fruit is a very important food resource for humans and most apple fruits are consumed fresh. In some places, they are also consumed as dry fruit, sliced in cans, syrup, marmalades and jam. In recent years, they have been used for pasta, fruit juice and vinegar sectors. Apple fruit has mineral salts and vitamins so it is a very important source of food for human. It may be helpful in reduction of risks of some diseases such as some cancers, heart problems, asthma and diabetes due to its content of rich chemical compounds. Laboratory tests indicated that apple fruit consumption inhibited expansion of cancer cells and reduced the lipid oxi-

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dation and cholesterols (Leontowicz et al. 2002). After blueberry, it has the highest antioxidant activities (Boyer, Liu 2004).

A number of epidemiologic studies showed that there is a negative relationship between heart-vessel or cancer diseases and fruit-vegetable consumption (Block et al. 1992; Liu 2003). In recent years, consumption of food with high antioxidant capacity has been suggested (Halliwell 2001); it is known that fruits, very important for healthy life, have great amounts of antioxidants (Vinson et al. 2005).

There are different amounts and types of phenolic compounds (secondary metabolites), which protect the crops from some harmful materials. Phenolic compounds found in food are classified into two group namely phenolic acids (phenolic carbonic acids) and flavonoids (flavone derivatives). Phenolic compounds are very important since they are a substrate resulting in black colouring in fruit and vegetables (Eskin et al. 1976), they react with metal ions causing colour changes (Herrmann 1976), they cause undesired tastes in food (Lea 1984), and they react with proteins leading to precipitation (Oh, Hoff 1987). Apple is the fruit with the second highest phenolics content after blueberry. In preventing lung cancer, it is ranked third after blueberry and lemon. Apple also ranked the second in total concentration of phenolic compounds, and perhaps more importantly, apples had the highest portion of free phenolics when compared to other fruits (Sun et al. 2002).

To solve the health problems especially in developing countries, plant products were used widespread due to the large amount of budget requirement of pharmacological products. There are many plants used for different purposes by the public in Turkey but their scientific activities are still unknown. There are some studies showing antioxidant effectiveness of plant extracts and plant products (Çoban et al. 2003; Couladis et al. 2003; Tepe et al. 2006; Altun et al. 2007; Kartal et al. 2007). For this reason, apple cultivars found in Turkey were studied.

MATERIAL AND METHODS

'Galaxy Gala', 'Granny Smith', 'Pink Lady', 'Scarlet Spur' and 'Fuji' apple cultivars grafted onto M9 rootstock were used. The fruit samples were picked at the ideal commercial maturity from the 'Galaxy Gala', 'Granny Smith', 'Pink Lady', 'Scarlet Spur' and 'Fuji' apple cultivars at the time of harvest. The maturity is based on the determination of firmness, refractometric value and starch conversion. Antioxidant analysis was performed at the laboratory of Department of Food Engineering, Afyon Kocatepe University. The study design comprised three replicates with ten fruits for each analysis. For investigations, apple fruits were thinly sectioned from skin to the fruit centre.

Determination of antioxidant activities

Sample preparation. 2.5 g fruit tissue from each dried plant sample were extracted by homogenizing in a mixer (Ultra Turrax; IKA-Werke GmbH & Co., Germany) tube disperser (IKA, Staufen, Germany) with 50 ml solvent (50% water-methanol). Extracts were filtered thorough paper (Filter-Lab; Inoia Filters, S.A., Spain), then centrifuged at 4,000 g × 3 min at 4°C. 50 ml of supernatant was further filtered using blue band, No. 589 filter paper (Schleicher and Schuell, Green ribbon; Schleicher and Schuell & GmbH, Germany).

Determination of free radical scavenging activity. To measure the free radical scavenging activity of 1.1-diphenyl-1-picrylhydrazyl (DPPH) modified assay of Brand-Williams et al. (1995) was used. For this purpose, dilutions in the range of between 0.4 and 4 mg/ml were prepared from the juice extractions. The dilutions between 0.4 to 4 mg/ml were prepared with methanol. 3.9 ml DPPH solution prepared with 6×10^{-5}M (molar) methanol was added to each 0.1 ml of dilution and was shaken well. Those samples were kept 60 min in the dark room under room temperature. Absorbance was then measured against a methanol standard at 515 nm. Samples without DPPH were used as control treatment. All analyses were carried out in duplicate.

The activity was calculated utilizing a standard curve. The linear regression equation was derived by measuring the absorption in 515 nm from seven different concentrations of DPPH (6×10^{-5}M):

$$A(515\ nm) = 15.412\ (C\ DPPH) - 0.0171\ (R^2 = 0.961)$$

The residual DPPH concentrations as % were calculated as:

$$\%\ Residual\ DPPH = (DPPH)_{sample}/(DPPH)$$

The regression equation was developed by dividing residual DPPH* to DPPH* of sample under test

doi: 10.17221/276/2015-HORTSCI

media. By use of that equation, sample concentration reducing 50% of initial DPPH* concentration, (efficient concentration = EC_{50}), was obtained for crop samples. By dividing EC_{50}, $1/EC_{50}$, antiradical activity (AE) was calculated.

Calculation of Fe^{+2} chelating activity. Chelating activity of apple samples were measured by methods modified by RIVAL et al. (2001). 1 ml of extract with different concentrations between 6–45 mg/ml and 3.7 ml deionized water were mixed. A 0.1 ml 2mM $FeCl_2$ solution was added, shaken and kept in the dark at room temperatures for 70 min. After that, 0.2 ml 5mM ferrozin was added and mixed again. The resulting Fe^{+2} – ferrozin complex absorbance at 562 nm were measured after 10 minutes. In the control, 1 ml water was used instead of sample. The chelating capacity of samples was calculated as in Yen and Wu (1999):

$$\% \text{ chelating capacity} = [1 - (\text{sample absorbance}/\text{control absorbance})] \times 100$$

Measurement of H_2O_2 scavenging. H_2O_2 scavenging capability of crops and herbs can be diagnosed spectrometrically (Ruch et al. 1989). For that, 1 ml (2, 6 and 10 mg/ml) sample, 3.4 ml 0.1M phosphate buffer solution (pH 7.4) and 0.6 ml 43mM H_2O_2 were mixed and after 60 min the absorbance of the mixture was measured at 230 nm. Control samples containing no H_2O_2 solution were prepared for each sample.

A linear regression equation was developed to determine the H_2O_2 (mM) concentration. For that, 0.6 ml 10, 15, 25, 43 and 50mM H_2O_2 were added to the 3.4 phosphate buffer solution and their absorbance was measured at 230 nm. The following linear regression equation was developed:

$$A(230) = 0.0125 \times C\ (H_2O_2, \text{mM}) + 0.08541 \quad (R^2 = 0.961)$$

Removing H_2O_2 capacity of samples was calculated as:

$$H_2O_2 \text{ removing capacity (\%)} = [1 - (H_2O_2 \text{ of sample}/H_2O_2 \text{ of control})] \times 100$$

Calculation of total phenolic content. The total phenolic content of samples was analysed by using the Folin Ciocalteu colorimetric method (Singleton, Rossi 1965). Readings were performed spectrophotometrically at 765 nm. Values were calculated as mg GA/g. Total phenolic matter content was presented as gallic acid (GA) equivalent.

Statistical analysis. The results were evaluated using the R- program with the Duncan test at 5% significance level in accordance with the Randomized Parcel Trial design (Anonymous 2013).

RESULTS AND DISCUSSION

The antioxidant capacity of five apple cultivars grown in Turkey was measured by four different assays to determine the relative level of potentially health-beneficial aspects of each of the cultivars. Each assay measured a different aspect of capacity from being able to reduce free radicals to total phenolic content.

Determination of antioxidant activity

Free radical scavenging capacity. Antioxidant capacity was measured by the amount of sample capable to reduce the DPPH concentration to 50% of the initial amount with lower values being an indicator of high antioxidant capacity. From that value the antiradical efficiency or antiradical activity was calculated (Molyneux 2004).

The present study showed that antiradical activity, AE, was in increasing amounts: 0.545% for 'Granny Smith', 0.592% for 'Galaxy Gala', 0.675% for 'Fuji', 0.737% for 'Pink Lady' and 0.802% for 'Scarlet Spur' (Fig. 1).

Chelating activity of Fe^{+2}

Chelating activity for metal can be evaluated by the competitions between plant extracts and ferrozin for binding Fe^{+2} ions. The inhibition graph showing the metal chelate potential as a percentage of plant extract obtained from all the apple cultivars is shown in Fig. 2. The highest values were obtained from 'Scarlet Spur' at 55.20% and 'Pink Lady' at 53.18% while the lowest value was calculated as 40.20% from 'Granny Smith'.

H_2O_2 scavenging

The oxidation of H_2O_2 by the plant extracts was determined by using the method as suggested by Ruch et al. (1989). Although none of the extracts

doi: 10.17221/276/2015-HORTSCI

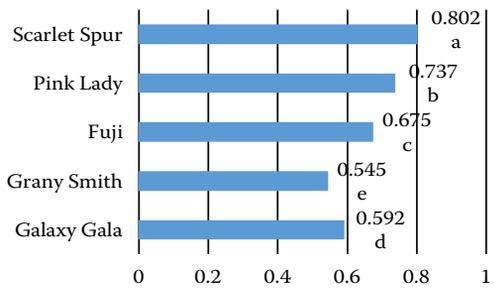

Fig.1. Antiradical activity for different apple cultivars (%) (LSD: 0.1683)

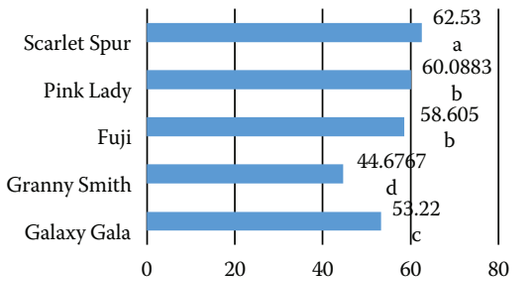

Fig. 3. H_2O_2 scavenging (%) (LSD: 1.32053)

completely oxidized the H_2O_2, they did range from 44.67% to 62.53% (Fig. 3).

Phenolic content

Phenolic contents as the equivalent of gallic acid in research apples were calculated. As seen in Fig. 4, the highest phenolic contents were found in 'Scarlet Spur' at 26.86% and, again, 'Granny Smith' had the lowest level at 18.29%.

The phenolic and flavonoid contents highly depend on apple cultivars. Therefore, different apple cultivars have different antioxidant activity (Boyer, Liu 2004). Apples, like other fruits, vary in chemical composition even within the same variety, depending on maturity, location produced, and agricultural practices, as well as numerous other environmental factors (Lee et al. 2003). Indeed, significant variations in phenolic content and antioxidant activity were observed among cultivars and even among different fruits in the same cultivar (Imeh, Khokhar 2002).

Flavonoid contents of 'Golden Delicious', 'Reineta', 'Red Delicious' and 'Granny Smith' apple cultivars were compared and 'Golden Delicious' had the lowest, and 'Reineta' apple cultivar had the highest flavonoid content followed by 'Granny Smith' and 'Red Delicious' apple cultivars (Escarpa and Gonzales, 1998).

Drogoudi et al. (2008) measured the phenolic and ascorbic acid contents of 'Fuji', 'Golden Delicious', 'Granny Smith', 'Jonagored', 'Mutsu', 'Starkrimson' and 'Fyriki' (local cultivar of Greece) in apple skin. The highest antioxidant capacity of 35.7 mg/g and phenolic matter content of 19.9 mg/g were obtained from 'Starkrimson' and was followed by 'Jonagored', 'Mutsu', 'Fyriki' and 'Fuji'. The skin of 'Golden Delicious' (13.8 mg/g) and 'Granny Smith' (8.4 mg/g) had the lowest antioxidant capacity. In the analysis of fruit flesh, the highest antioxidant capacity was obtained from 'Fyriki' as 11.9 mg/g or 9.8 mg/g and that was followed by 'Jonagored', 'Mutsu' and 'Starkrimson' 5.6 mg/g and 5.4 mg/g. The lowest antioxidant capacity in fruit flesh was found in 'Fuji', 'Golden Delicious' and 'Granny Smith' (between 3.7 mg/g and 3.5 mg/g). The other important results are that the antioxidant capacity of fruit skin was three fold higher than the fruit flesh and the highest ascorbic acid content among the apple cultivars was obtained from 'Fyriki' (4.4 mg/g).

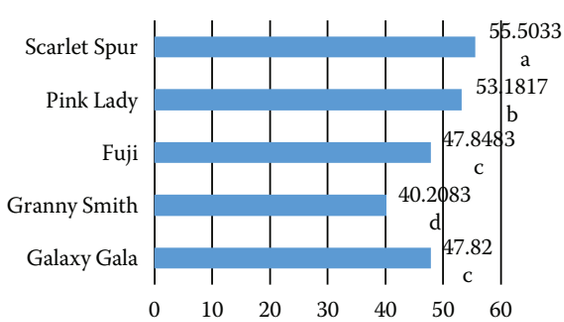

Fig. 2. Chelating activity of Fe^{+2} (%) (LSD: 1.00701)

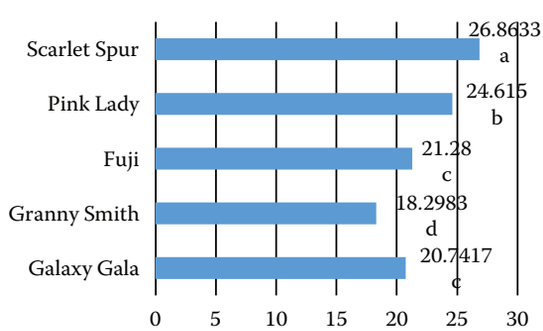

Fig. 4. Phenolic content (mg GA/g) (LSD: 0.61798

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In the present study, the antioxidant capacity was found in a decreasing order in 'Scarlet Spur', 'Pink Lady', 'Fuji', 'Galaxy Gala' and 'Granny Smith' apple cultivars. All four measurements were in agreement with this order. Apple is rich in anthocyanins and phenolic matter content among the fruits and vegetables. Thus, apple fruit should be added to the diet menu to protect the human body from the oxidative stress. In addition, apple plants are grown very widely in Turkey and it is thought that apple fruits have some other possible useful effects for our health. That issue should be researched in details in order to minimize the public health budget.

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Received for publication December 24, 2015
Accepted after corrections August 8, 2016