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Bioactive Contents of Commercial Cultivars and Local Genotypes of Walnut (*Juglans regia* L.)

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Abstract. Total phenolic content and antioxidant activity (ferric reducing antioxidant power, FRAP and trolox equivalent antioxidant capacity, TEAC assays) of either commercial (Sebin, Kaman, and Bilecik) or regional walnut genotypes (Kag-1, Kag-2, Kag-3, Kag-4, Kag-5, Kag-6, Kag-7, Kag-8, Kag-9 and Kag-10) were determined. Walnut fruits were harvested in 2009 from East Anatolia region in Turkey. Total phenolic content of commercial cultivars ranged from 1142 to 1861 mg of GAE/ 100 g fresh weight basis and 954 to 2106 mg of GAE/100 g fresh weight basis among regional genotypes. The regional genotype Kag-2 had the highest antioxidant activity in both assays while Kag-10 had the lowest activity. Total phenolic content highly correlated with both antioxidant methods.

Keywords: walnut, antioxidant activity, biological activity

1. Introduction

Edible tree nuts including walnut, almond, hazelnut and pistachios are globally popular and valued for their sensory, nutritional, and health attributes [1].

According to recent studies on nuts for benefit of human health, walnuts (*Juglans regia* L.) declared the best one from the Medical points of view because it contains healthy ingredients which are very beneficial for human health. A valuable edible nuts produced by walnut trees are well appreciated because they are enriched with unsaturated fat (linoleic, oleic acid). They also contain other beneficial components like plant protein (e.g. arginine, leucine), carbohydrates (e.g. dietary fibre), vitamins (e.g. vitamin A, E), pectic substances, minerals (magnesium, potassium, phosphorus, sulphur, copper, iron), plant sterols, phytochemicals (phenolicacids, flavonoids, etc.) etc. Its nuts are also highly appreciated for its unique organoleptic characteristics for food industry [1].

More recent studies showed that bioactive ingredients which are present in walnut fruits in reducing the risk of cardiovascular disease [2, 3], type 2 diabetes [4] and some types of cancer [5] so it should be included necessary in routine meals.

Turkey is one of the most important walnut producers in the world after China, USA and Iran [6] and walnut cultivation spread through and well adapted to the most of temperate parts of the country, from which 10 % of the world production is obtained. In Turkey walnut trees are an important crop, due to both its fruits and also its timber of high commercial value. There was a great diversity among regional walnut genotypes grown in Turkey that mostly obtained from seeds for a long time. Moreover there were national and also introduced cultivars as well. Therefore semi domesticated, regional cultivars and introduced international cultivars are grow together in Turkey. [7].

Reactive oxygen species (ROS) are chemically-reactive molecules containing oxygen. Examples include oxygen ions and peroxides. Reactive oxygen species are highly reactive due to the presence of unpaired

valence shell electrons. ROS form as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically [8]. This may result in significant damage to cell structures. This cumulates into a situation known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation.

Under normal circumstances, cells are able to defend themselves against ROS damage with enzymes such as superoxide dismutases, catalases, lactoperoxidases, glutathione peroxidases and peroxiredoxins. Antioxidants presented in plant foodstuffs such as ascorbic acid (vitamin C), carotenoids, tocopherol (vitamin E), phenolic acids etc. play important roles as cellular antioxidants. In similar manner, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals. Dietary antioxidants provide protection against oxidative attack by decreasing oxygen concentration, intercepting singlet oxygen, preventing first-chain initiation by scavenging initial radicals, binding of metal ion catalysts, decomposing primary products of oxidation to nonradical compounds and chain breaking to prevent continuous hydrogen removal from substrates [9].

Apart from their role as health benefactors, antioxidants are also added to food to prevent or delay its oxidation, normally initiated by free radicals formed during the food's exposure to environmental factors such as air, light and temperature [10]. At present most of the antioxidants used for this are manufactured synthetically. The main disadvantage with the synthetic antioxidants is their side effects when taken *in vivo* [11]. Strict governmental rules regarding the safety of the food has necessitated the search for alternatives as food preservatives [12]. In this point plant antioxidants are also important not only for humans but also for food safety.

As far as we searched, it seems few reports of bioactive contents of walnut genotypes and cultivars. Therefore the aim of this study was to determine total phenolic content and antioxidant activity of regional genotypes comparing commercial national cultivars in Eastern Anatolia region in Turkey.

2. Material and methods

2.1. Walnut samples

Raw walnut fruits harvested in 2009, from Kagizman district in East Anatolia region in Turkey. In total, 200 fruits from 10 regional genotypes (Kag-1, Kag-2, Kag-3, Kag-4, Kag-5, Kag-6, Kag-7, Kag-8, Kag-9 and Kag-10) and 3 commercial cultivars (Cebin, Bilecik, Kaman) were collected from the orchards in the district. For each cultivar, the mass of 200 g walnuts was prepared for total phenolic and antioxidant analysis.

2.2. Analytical procedures

The samples were chopped in a coffee mill. Total phenolics were measured by the Folin–Ciocalteu-reagent mentioned by Linkens and Jackson [13]. A total phenolic extract was prepared by a lightly modified procedure of Anderson et al. [14]. The chopped nuts were extracted with a solution of 75% acetone and 25% of 526 μmol/L sodium metabisulfite. The supernatant was pipetted and centrifuged. Then, the extraction solution was evaporated and extracted with hexane. The water soluble phase was used for determining the concentration of total phenolics and antioxidant activity.

The total phenol content of walnut extract was determined according to Singleton et al. [15] with results expressed as mg gallic acid equivalents (GAE) per 100 g raw walnut. The TEAC (trolox equivalent antioxidant capacity) and FRAP (ferric reducing antioxidant power) assays assessed to estimate total antioxidant capacity [16, 17]. FRAP reducing power is expressed as μmol Fe^{2+} per g walnuts and TEAC expressed as μmol TE per g walnuts.

2.3. Statistics and data analysis

For all experiments, samples were analysed in duplicate. Statistical significance was determined by ANOVA and Duncan Multiple Range Test. Pearson's correlation analysis was performed using GraphPad Prism v 5.01 (GraphPad Software Inc., La Jolla, CA). Differences were considered significant at $P \leq 0.01$.

3. Results and discussions

3.1. Total phenolic content

Table 1 shows total phenolic concentrations obtained in the 10 regional genotypes and 3 commercial walnut cultivars. Folin-Ciocalteau method allows the estimation of all the flavonoids, anthocyanins and nonflavonoid phenolic compounds that is, of all the phenolics present in the samples.

After an overview of the results, total phenolic content in commercial national cultivars and regional genotypes varied from 1142 mg (cv. Sebin) to 1861 mg GAE per 100 g nut (cv. Birecik) and ranged from 954 mg (Kag-10) to 2106 mg (Kag-2) GAE per 100 g nut, respectively (Table 1). Surprisingly, Kag-2, a regional genotype, revealed the highest content in phenol compounds (2106 mg/g). We found strong positive correlation between total phenolic content and antioxidant activity (R^2=0.96 for between total phenolics and FRAP and R^2=0.78 between total phenolics and TEAC). Some authors have reported a direct correlation between antioxidant activity and total phenolic content in different plants [18, 19]. The antioxidant activity of phenolics may be related to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors, their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [20]. Thus, natural antioxidants function as free-radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation [21]. Previously total phenolic content of walnut cultivars from different countries were reported between 1071-2370 mg GAE/100 g nut (14, 22, 23) which indicates good accordance with our study. Walnut phenolic compounds are composed from tannins (24). Tannins in walnut fruit are responsible astringency taste of fruit and color of skins (25). The total phenols in nuts are within the range of fruit such as blueberries (531 mg GAE/100 g), plums (367 mg GAE/100 g), and raisins (1065 mg GAE/100 g) (26).

Table 1. Total phenolic content, trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) of walnut cultivars and genotypes

Cultivars/Genotypes	Total Phenolic Content mgGAE/100 g FW	TEAC µmol TE/g FW	FRAP µmol Fe^{2+}/g FW
Sebin	**1142h**	**116e**	**401fg**
Kaman	**1413fg**	**135d**	**407fg**
Bilecik	**1861c**	**164ab**	**515ab**
Kag-1	**1704d**	**152bc**	**467de**
Kag-2	**2106a**	**171a**	**522a**
Kag-3	**2015b**	**164ab**	**507b**
Kag-4	**1642de**	**143c**	**442e**
Kag-5	**1503f**	**154bc**	**419f**
Kag-6	**1081i**	**109e**	**397g**
Kag-7	**1840c**	**161b**	**484c**
Kag-8	**980ijk**	**102ef**	**370gh**
Kag-9	**1018ij**	**110efg**	**385g**
Kag-10	**954k**	**97f**	**359h**
Minimum	**954**	**97**	**359**
Mean	**1481**	**137**	**437**
Maximum	**2106**	**171**	**522**

Values in same columns bearing different letters differ tested by Anova and Duncan $p<0.01$

3.2. Antioxidant activity

Besides anthocyanins, other flavonoids, phenolic acids and vitamins can contribute to the protective effect against oxidative damage to cells. Since the antioxidant capacity of individual dietary compounds cannot always be evaluated, the determination of the total antioxidant capacity allows a more realist evaluation of the potential protective effect of a food [27].

Several *in vitro* methods have been developed to assess the total antioxidant capacity of fruits, vegetables and beverages. FRAP and TEAC are widely used methods to determine total antioxidant capacity of plant materials [27, 28]. The FRAP assay is a simple, convenient and reproducible method that was initially

developed to measure the plasma antioxidant capacity, but is now widely employed in the antioxidant studies of other biological samples, such as food, plant extracts, juices and beverages, etc. [29].

The ferric reducing ability (FRAP) of walnut fruit extracts was in the range of 359-522 μm of Fe^{2+} per g fresh weight (Table 1). Similar to their total phenolic content, regional genotype, Kag-2 walnuts had the highest antioxidant acitivity with FRAP value of 522 μmol Fe^{2+} per g FW. Previously FRAP value of walnuts was found 454 μmol Fe^{2+} per g FW [17] supporting our present results.

The trolox equivalent antioxidant capacity (TEAC) of walnut fruit extracts was in the range of 97-171 μm of TE per g fresh weight (Table 1). Similar to their total phenolic content, regional genotype, Kag-2 walnuts had the highest antioxidant acitivity with TEAC value of 171 μmol TE per g FW. Previously TEAC value of walnuts was found 137 μmol TE per g FW [17] which in accordance with our results.

The TEAC method (trolox equivalent antioxidant capacity) is one of the most used methods for quantifying radicals which can be scavenged by some antioxidant. It is based on scavenging of the cation radical originated by the one-electron oxidation of the synthetic chromophore 2,2‘-azinobis (3-ethylbenzothiazoline-6-sulfonate(ABTS•) to ABTS•+ [30].

We found a big diversity among used genotypes and cultivars in terms of antioxidant capacity. It is known that genetics, harvest season, origin, environmental conditions, soil composition, maturity level and the methods of cultivation highly influence the composition of nuts [17, 27, 29].

The results of this study indicated that regional and commercial walnut cultivars originated from Turkey has a high antioxidant activity and in particular regional genotype Kag-2 possesses good medicinal potential. A positive relationship between antioxidant activities and total phenolic contents was also observed. The high level of total phenolic in Kag-2, Kag-3, Kag-7 and cv. Bilecik indicated high antioxidant activities. This relationship was also reported in previous studies on other fruits [17, 29]. Human health and nutrition are still one of the most studied and interesting topics. Natural compounds, including those coming from plants, are nowadays under detailed investigation due to their potentially beneficial effects. Further work is required to establish the components in phenolics and flavonoids that may have contributed to the high antioxidant activities so far observed.

4. References

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