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Bioactive content and antioxidant characteristics of wild (*Fragaria vesca* L.) and cultivated strawberry (*Fragaria × ananassa* Duch.) fruits from Turkey

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Summary

In this study, some biochemical properties (total soluble solid content, pH, acidity, antioxidant activity) and contents of biological compounds (vitamin C, total phenolics, total ellagic acid and concentration of anthocyanins) of fifteen wild strawberry accessions (*Fragaria vesca* L.) and one commercial strawberry cultivar Camarosa (*Fragaria × ananassa* Duch.) sampled in Northeastern Turkey were determined. Antioxidant activity of fruit samples was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric reducing antioxidant power) assays. Notable differences were found both among wild strawberries (*Fragaria vesca*) and also between wild strawberries and cv. Camarosa (*Fragaria x ananassa*). Among the strawberry accessions tested, the total phenolics ranged from 138 mg to 228 mg gallic acid equivalent per 100 g fresh fruit. The total monomeric anthocyanin content was the highest in wild accession FV-2 (53.51 mg/100 g) while the lowest in FV-6 as 25.11 mg per 100 g fresh weight. The total ellagic acid content was between 15.18 and 26.36 mg per 100 g. All wild strawberries exhibited higher antioxidant activity than cv. Camarosa. Thus, it can be concluded that wild strawberry is a good source of polyphenols, ellagic acid and antioxidants.

Introduction

The garden strawberry (*Fragaria × ananassa* Duch.) is a cultivated hybrid species of the genus *Fragaria* (collectively known as the strawberries) and it is appealing to the human senses of sight and taste due to its bright red color, juicy texture, sweetness and distinct flavor. The wild aromatic diploid species *F. vesca* L. (also known as woodland strawberry) is genetically not related to the octoploid *F. x ananassa* because *F. vesca* L. is not an ancestor of *F. x ananassa* (Chandler et al., 2012). *F. x ananassa* is cultivated both open field and in greenhouses throughout the world. Although commercial strawberry (*F. × ananassa* Duch.) cultivation only started around the end of 1970 in Turkey, the country is currently one of the biggest strawberry producers in the world after USA with an annual production quantity of 353,000 tons (FAO, 2012). In Turkey, there are nine different agro-climatic regions and strawberries are grown in each of those with Mediterranean and subtropical climate regions being the most important producers. The diversity in climate across the country, together with the cultivation of short-day and day-neutral cultivars, and implementation of various planting practices, allow consumers to enjoy this fruit almost all year round (Ozdemir et al., 2013; Torun et al., 2014).

Fragaria vesca L., the wild strawberry, is the most extensively distributed species in the 200-2450 m a.s.l., particularly in the highlands of the Black Sea region of Turkey and the species is characterized by considerable geographical and ecological adaptation capacity. The highly aromatic and sweet fruits were used predominantly for fresh consumption or preservation (Ercisli, 2004). Since this fruit is not cultivated, wild strawberry is a valuable product in Turkey (Ozsen and Erge, 2013). Fruits of wild strawberries are generally ovate, bright red, soft and aromatic. Since the Middle Ages, wild-type strawberries were praised for their intense flavor (Darrow, 2005). The aroma of wild strawberry is perceived as very aromatic and more herbaceous than those of *F.×ananassa* cultivars (Hirvi and Honkanen, 1982). Therefore, *F. vesca* serves as a comparison with regard to flavor. Zabetakis and Holden (1997) hypothesis that the aroma of *F. vesca* may be more 'strawberry-like' than those of *F. x ananassa*. Ulrich et al. (2007) and Ulrich and Olbricht (2013) indicated diversity of aroma patterns in wild and cultivated *Fragaria* accessions

In recent years some introduced short-day and day-neutral cultivars such as 'Fern', 'Camarosa', 'Festival', 'Fortuna', 'Rubygem' have been widely produced in Turkey, 'Camarosa' being most common due to its high market segment (Esitken et al., 2010; Ozdemir et al., 2013). Wild strawberries with unique aroma can suffer from poor climatic adaptation and hence they might have inadequate quality and productivity (Ercisli, 2004).

More recently, there is an increasing interest in colorful berry fruits including strawberry, raspberry, blackberry, mulberry, blueberry, elderberry etc. These fruits are popularly consumed not only in fresh and frozen forms but also as processed and derived products including dried and canned fruits, yogurts, beverages, jams, and jellies (Seeram et al., 2006).

Berries provide significant health benefits because of their high levels of polyphenols, antioxidants, vitamins, minerals, and fibers (Halvorsen et al., 2002; Anttonen and Karjalainen, 2005). It has been demonstrated that a wide diversity of phytochemical levels and antioxidant capacities exist within and across berries (Moyer et al., 2002; Hegedus et al., 2008). Furthermore, accumulating evidence suggests that genotype has a profound influence on concentrations of bioactive compounds in berries (Halvorsen et al., 2002; Anttonen and Karjalainen, 2005).

Some berries, such as strawberries, have been identified as sources of phenolic compounds like gallic and ellagic acids, which have potential cancer chemo preventive activity (Xue et al., 2001). These different bioactive phenolic compounds, including flavonoids, tannins, and phenolic acids, have received considerable interest in bearing possible relations to human health.

The aims of this work were to evaluate and compare some biochemical and bioactive contents in wild and cultivated strawberry fruits as well as to assess the genotypic influence on investigated characteristics in strawberry fruits.

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Material and methods

Plant material

The research was conducted in 2012 on harvested fruits from fifteen wild strawberry (*Fragaria vesca*) accessions and 'Camarosa' cultivar (*Fragaria x ananassa*) grown in the Senkaya district in Northeastern Turkey at 40°33'43'N 42°20'47'E and 1800 m altitude. The mountainous growing area was covered by Scotch pine (*Pinus sylvestris*) forests. The climate of the sampling region has typical Black Sea climate (warm and humid) with an average annual humidity of 67 % and precipitation of 47 kg/m^3. Near the sampling region for wild strawberries, there were some commercial non-modern strawberry plantations of the 'Camarosa' cultivar. Thus there were similarities between wild and cultivated strawberries in terms of growing conditions in the study area. Neither chemical treatment nor extra fertilizer was applied during experiment. Irrigation was only applied to avoid damage by drought. Because of high altitude, no fungicide against grey mold (*Botrytis cinerea* Pers.) was used. Wild fruits were harvested per plant (per accession) and cultivated strawberry fruits were harvested from 30 plants at fully mature stage.

Sample preparation and determination of chemical contents

About 100 g of fruit samples for each accession were frozen at -20 °C. At the time of analysis, fruits were thawed and homogenized in a standard food blender. Slurries were used to determine chemical (TSS, titratable acidity and pH) and bioactive content (vitamin C, total phenolic, antioxidant activity, ellagic acid, total anthocyanins). Total soluble solid (TSS) contents was determined by refractometer (Model RA-250HE, Kyoto Electronics Manufacturing Co. Ltd., Japan). Levels of titratable acidity (TA), pH and vitamin C were determined using standard methodology (AOAC, 2005).

Total phenolic content

For the extraction and determination of total phenolic content, a single extraction procedure was designed to assay phenols (Bartolome et al., 1995). For each replicate, a 3 g aliquot of slurry was transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, water, and acetic acid (70:29.5:0.5, v/v) for 2 h. After filtration, acetone was removed by rotary evaporation, after which the concentrated samples were brought to a final volume of 20 mL with deionised water. Next, Folin-Ciocalteu's phenol reagent and water were incubated for 8 min, followed by the addition of 7 % sodium carbonate. After 2 h, the absorbance was measured by an automated UV-VIS spectro-photometer at 750 nm. Gallic acid was used as standard. The results were expressed as mg gallic acid equivalents on 100 g fresh weight basis (mg GAE/100 g FW).

Determination of antioxidant activity

Total antioxidant activity was estimated by two standard procedures: FRAP and DPPH assays. The FRAP assay (Benzie and Strain, 1996) was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1, v/v/v) to form the FRAP reagent just prior to analysis. For each assay laboratory duplicate, 2.97 mL of FRAP reagent and 30 µL of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm using a spectrophotometer. The results were expressed as µmol trolox equivalent on g fresh weight basis.

Free radical scavenging activity was determined according to the method of Suja et al. (2005) with slight modifications. Fruit extract (1 mL) was added to 2 mL DPPH solution (2 mL of 0.02 g/L DPPH) in ethanol. The reduction of DPPH was measured at 517 nm against a blank assay for 30 min. The percentage of the remaining radical in medium is calculated as the absorbance of the sample divided by that of DPPH control at the same time multiplied by 100. The amount of sample needed to decrease the initial DPPH concentration by 50 %, EC_{50}, was calculated graphically. The results were expressed as µmol on g fresh weight basis.

Determination of total anthocyanins

Total monomeric anthocyanins (TMA) were determined by the pH differential method (Giusti and Wrolstad, 2001), using a UV-VIS spectrophotometer. Absorbance was measured at 533 nm and 700 nm in buffers at pH 1.0 and 4.5 using A = (A533 - A700) pH 1.0 - (A533 - A700) pH 4.5 with a molar extinction coefficient of 29,600. Total anthocyanin content was expressed as mg pelargonidin-3-glucoside equivalent in 100 g FW.

Determination of total ellagic acid

Extraction and hydrolysis of ellagitannins methods were used (Pineli et al., 2011). Frozen strawberries (75 g) were lyophilized for 4 days in darkness. The moisture of fresh strawberries and the residual moisture of lyophilized strawberries were determined by gravimetry, after drying at 105 °C until constant weight. The results were used to convert data from dry basis to fresh basis. Samples of lyophilized strawberry powder (0.5 g, for each replicate) were extracted three times in 80 % methanol (50 mL the first time, 25 mL the next two times) at 15,000 rpm for 1 min while cooled in ice bath, and vacuum filtered through Whatman 1 filter paper. An aliquot of 2 mL of the combined extracts was dried under nitrogen stream and 2 mL of 2N trifluoroacetic acid (TFA) were added. Hydrolysis was performed in a glycerin bath at 120 °C for 60 min. Hydrolized extracts were rota-evaporated at 95 °C for 2 min, re-suspended at 1 mL of HPLC grade methanol, filtered in 0.22 µm PTFE filters, and analyzed by HPLC. Identification and quantification of total ellagic acid was achieved using analytical reversed-phase HPLC in a Varian Pro Star system with auto sampler and ternary pump and UV-VIS detector. Some extracts were also analyzed in a HPLC coupled with DAD detector (Shimadzu, SPD-M10A DAD, Germany), in order to confirm the structures previously identified by time retention with the UV-VIS detector. The column used was 250 mm x 4.6 mm, i.d., 5 µm, Prodigy ODS3 reversed-phase silica and elution solvents were A, water: tetrahydrofuran: TFA (98:2:0.1) and B, acetonitrile. Solvent gradient was: 17 % B for 2 min, increasing to 25 % B after 5 min, to 35 % B after a further 8 min and to 50 % B after 5 min. Samples were injected in duplicate. Calibration was performed by injecting the external standard ellagic acid (Sigma Chemical Co., USA) three times at five different concentrations, in the range of 0.1-10 µg. Results were expressed as mg/100 g FW basis.

Statistical analysis

All data were analyzed using SPSS software and procedures. Analysis of variance tables were constructed using the Least Significant Difference (LSD) method at $p<0.01$.

Results and discussion

Biochemical characteristics

Results related to some biochemical characteristics (TSS, acidity, pH) and vitamin C of fifteen wild strawberries and cv. Camarosa are given in Tab. 1. Remarkable significant differences among wild strawberry accessions and between the group of wild strawberries

Tab. 1: Selected biochemical characteristics of fruits of wild and cultivated strawberries.

Accessions	SSC (%)	pH	Acidity (%)	Vitamin C (mg/100 g fresh fruit)
FV-1	6.91ab	3.31^{NS}	1.47^{NS}	51.10c
FV-2	6.60b	3.27	1.11	54.07b
FV-3	6.67ab	3.15	1.18	43.69ef
FV-4	6.78ab	3.08	1.24	50.67cd
FV-5	6.86ab	3.10	1.37	49.11cd
FV-6	6.70ab	3.09	0.95	45.18de
FV-7	7.13ab	3.30	1.11	53.74bc
FV-8	6.84ab	3.18	1.06	57.37a
FV-9	7.32ab	3.21	1.01	51.06c
FV-10	7.51a	3.24	1.03	45.51de
FV-11	6.67ab	3.13	1.24	38.55f
FV-12	7.10ab	3.06	1.20	47.44d
FV-13	6.96ab	3.11	1.21	44.18e
FV-14	7.34ab	3.20	1.18	41.71ef
FV-15	7.42ab	3.20	1.31	44.16e
'Camarosa'	6.90ab	3.16	1.17	38.66f

Means within a column followed by the same letter are not significantly different at $p<0.01$
NS: Non significant

and cv. Camarosa were found for most of the biochemical characteristics (Tab. 1). TSS and Vitamin C values in the fruit of strawberry accessions were found to be different from each other at $p<0.01$, while pH and acidity of accessions were found not to be different from each other at $p<0.01$ (Tab. 1).

TSS and Vitamin C of the wild and cultivated accessions are shown in Tab. 1. TSS and Vitamin C contents varied from 6.61 % (FV-2) to 7.51 % (FV-10) and 38.55 mg/100 g (FV-11) to 57.37 mg/100 g (FV-8) among accessions, respectively. The pH and acidity varied from 3.06 ('FV-12') to 3.31 ('FV-1') and 0.95 % ('FV-11') to 1.47 % ('FV-1') among accessions, respectively (Tab. 1).

Our results for the strawberry fruit samples were within the previously published ranges of fruit TSS, acidity, vitamin C and pH (Voca et al., 2008; Pineli et al., 2011; Ozdemir et al., 2013; Silva et al., 2013), which varied from 6.01-11.13 % for TSS, 3.01-3.81 for pH, 31-85 mg per 100 g for Vitamin C and 0.7-1.3 % for acidity, respectively. Cordenunsi et al. (2005) reported values of vitamin C ranging from 47 to 80 mg per 100 g of fresh weight, depending on the strawberry cultivars. Similar values were reported for vitamin C with 52.25 to 53.21 mg (Hansawasdi et al., 2006), and with 38.4 to 72.1 mg per 100 g of fresh mass, depending on the cultivar (Laugale and Bite, 2006). In modern strawberry breeding, new cultivars carry high or medium sugar but low acid contents and lead to high sugar-acid ratios. The *F. vesca* accessions in this study are characterized by very diverse brix-acid ratios. Additionally, high acid contents involve also astringency especially in combination with low sugar values.

Vitamin C, including ascorbic acid and dehydroascorbic acid, is one of the most important nutritional quality factors in many horticultural crops and has many biological activities in the human body. The content of vitamin C in fruits can be influenced by various factors such as genotypic differences and cultural practices etc. (Lee and Kader, 2000). In our study, the wild and cultivated strawberry plants found were cultivated under the same growing conditions and thus the genotypic effect seems more dominant.

According to Voca et al. (2008), the quantity of total soluble solids in the investigated strawberry cultivars ranged from 6.00 to 10.01 %, while Laugale and Bite (2006) reported values for total soluble solids from 8.4 to 11.6 %, depending on the strawberry cultivar. The soluble solid levels are used as indicator of maturity and also to determine fruit quality of strawberry.

Bioactive content

In modern strawberry breeding, the direction recently changed and today's strawberry cultivars are characterized by rather different patterns of secondary metabolites. Bioactive compounds in strawberries and other horticultural crops can be defined as secondary plant metabolites (Milivojevic et al., 2011) Bioactive contents of the analyzed strawberry accessions are presented in Tab. 2. We found significant differences in the level of total phenolic, total monomeric anthocyanin, total ellagic acid and antioxidant activity (characterized by using two different methods) among the assayed genotypes ($p<0.01$) (Tab. 2).

The total phenolic content of strawberry accessions ranged from 138 mg (cv. Camarosa) to 228 mg GAE per 100 g (FV-2) (Tab. 2). Results showed a genotype-dependent total phenolic accumulation in strawberry fruits and also indicated that all wild accessions had higher total phenolic contents than cv. Camarosa (Tab. 2). The total phenolic content of strawberry fruits grown in different countries were reported to be between 96 and 223 mg GAE per 100 g fruits (Voca et al., 2008; Pineli et al., 2011), which is comparable to our findings. Torronen and Maatta (2002) determined values of total phenols in strawberry cultivars ranging from 96 mg/100 g to 133 mg/100 g of fresh weight. All these results indicate that besides other berries and small fruits (Jurikova et al., 20011; Jurikova et al., 2012a; Jurikova et al., 2012b), strawberry fruits might also be a good source of total phenolics. The various factors such as genotype, agronomic practices, maturity level at harvest, postharvest storage, climatic and geographical locations affect the total phenolic content of horticultural plants (Milivojevic et al., 2011; Milivojevic et al., 2012). As indicated before, in this study genotypic effect was more dominant.

The observed total monomeric anthocyanin contents greatly differed among the strawberry accessions and varied from 25.11 mg (FV-6) to 53.51 mg (FV-2) pelargonidin-3-glycoside equivalent in 100 g FW (Tab. 2). This indicates that within polyphenolics strawberry fruit is also a good source for anthocyanin. A wide variation (12-44 mg per 100 g FW) in anthocyanin content was reported in strawberry cultivars previously (Castro et al., 2002; Voca et al., 2008; Oszmianski and Wojdylo, 2009; Pineli et al., 2011). Meyers et al. (2003) reported an average anthocyanin content of 41.4 mg/100 g in strawberry cultivars in the USA. They found great differences among the analyzed cultivars.

Total ellagic acid contents also varied among the accessions. When cultivars were compared, the highest total ellagic acid content was observed for FV-4 berries as 26.36 mg per 100 g and lowest in FV-7 berries as 15.18 mg per 100 g (Tab. 2). Pinto et al. (2008) and Pineli et al. (2011) reported a significant variation among full ripe strawberry cultivars for total ellagic acid content. The values determined for ripe strawberry cultivars were between 16.6 and 19.4 mg/100 g. Ellagic acid is considered the most important phenolic compound in strawberries (Hakkinen et al., 1999). There is a particular interest in ellagic acid due to its potential chemoprotective, anti-inflammatory and antibacterial effects properties (Vattem and Shetty, 2005).

The total antioxidant activities of the sixteen strawberry fruit extracts determined by DPPH and FRAP methods are shown in Tab. 2. The antioxidant capacity differed greatly among strawberry accessions for both antioxidant-determining assays (Tab. 2).

Tab. 2: Bioactive content and antioxidant activity in fruits of wild and cultivated strawberry accessions.

Genotypes	Total phenolics (mg GAE per 100 g FW)	Total anthocyanin (mg per 100 g FW)	Ellagic acid (mg per 100 g FW)	Antioxidant activity DPPH (µmol per g FW)	Antioxidant activity FRAP (µmol per g FW)
FV-1	193bc	33.01bc	25.08ab	8.18bc	37.05fg
FV-2	228a	53.51a	22.87ab	7.91bc	49.11a
FV-3	170c	28.62cd	20.10bc	10.27ab	30.82jk
FV-4	210ab	47.66ab	26.36a	7.96c	47.18b
FV-5	201b	43.07b	17.95bc	8.11bc	43.14d
FV-6	190bc	25.11d	17.05bc	9.31b	39.05ef
FV-7	177bc	31.64c	15.18c	9.96ab	38.10efg
FV-8	214ab	51.78ab	24.48ab	8.00bc	47.62b
FV-9	207ab	39.64bc	18.91bc	8.07bc	44.09cd
FV-10	187bc	29.66cd	23.36ab	11.38a	33.15i
FV-11	191bc	38.41bc	18.55bc	9.67ab	37.70g
FV-12	217ab	49.07ab	20.47b	7.94c	46.10b
FV-13	211ab	45.18ab	19.62bc	7.98c	45.01c
FV-14	195abc	45.64ab	18.15bc	8.40bc	43.18d
FV-15	171c	29.37cd	21.18ab	10.84ab	31.05j
Camarosa	138d	36.15bc	18.56bc	11.20ab	27.10k

Means within a column followed by the same letter are not significantly different at $p<0.01$
FW: Fresh weight

In FRAP assay, we found significant differences among the accessions ($P<0.01$). The genotype FV-2 showed the highest total antioxidant capacity (49.11 µmol per g) in FRAP assay. This genotype was followed by FV-8, FV-4, FV-12 and FV-13 genotypes, which had 47.62, 47.18, 46.10 and 45.01 µmol per g FRAP. Overall, the lowest antioxidant activity was observed in 'Camarosa' as 27.10 µmol per g (Tab. 2). These results are similar to the data reported by PINELI et al. (2011) who determined 24-27 µmol per g FRAP values. HALVORSEN (2002) reported FRAP values in wild *Fragaria vesca* fruits between 66 and 70 µmol per g and in *Fragaria × ananassa* fruits between 19-24 µmol per g FRAP value.

In the DPPH assay, antioxidant activity in strawberry samples varied from 7.94 µmol per g ('FV-12') to 11.38 µmol per g ('FV-10'), which is consistent with previous findings in strawberry. PINELI et al. (2011) reported DPPH values between 10.10 and 12.83 µmol per g fruit. Lower DPPH value indicates higher antioxidant activity. The antioxidant activity of strawberry fruit extracts is primarily correlated with total phenolic contents of the fruit rather than any individual phenolic compound or vitamin C content (ROUSSOS et al., 2009). Antioxidants from fruits and vegetables, especially with an intense coloration, are considered an important protection factor against oxidative stress and its deleterious consequences to human health (PINELI et al., 2011). The hypothesized health benefits related to strawberry consumption include their role in the prevention of inflammation, oxidative stress and cardiovascular disease, certain types of cancers, type 2 diabetes, obesity, and neurodegenerative disorders (GIAMPIERI et al., 2012).

Conclusion

This study showed marked differences in the selected biochemical and bioactive values among wild (less common) and commercial strawberry fruits. All wild material in general had higher levels of TSS, vitamin C, total phenolic and total ellagic acids than cv. Camarosa. Therefore, *Fragaria vesca* genotypes are one of the most valuable source with respect to examined polyphenolic compounds. It is clear that the same amount of *F. vesca* fruits gives a higher intake of phenolic compounds etc. than *F x ananassa*. Considering that all accessions were grown under identical conditions and in the same location, one can clearly see the variability between genotypes, which is quite typical for strawberries. Due to our findings, one can assume a high potential value of wild strawberries in terms of bioactive content and an importance for nutraceutical manufacturers. Nevertheless, wild accessions should be grown in controlled conditions in successive years to ascertain their real potential and stability of the characters.

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