Ecological Life Sciences
ISSN: 1308 7258 (NWSAELS)
ID: 2018.13.3.5A0101

Status : Original Study
Received: January 2018
Accepted: July 2018

Erinç Koçak
Ege University, erinckocak@gmail.com, İzmir-Turkey
Evren Demircan
Beraat Özçelik
İstanbul Teknik University, İstanbul-Turkey
evrendemircan@itu.edu.tr; ozcelik@itu.edu.tr

DOI	http://dx.doi.org/10.12739/NWSA.2018.13.3.5A0101	
ORCID ID	0000-0001-9054-4703	0000-0001-8533-6037
	0000-0002-1810-8154	
CORRESPONDING AUTHOR	Evren Demircan	

ANTIOXIDANT CAPACITIES AND PHENOLIC PROFILES OF OTTOMAN STRAWBERRY FRUIT AND OTTOMAN STRAWBERRY JAM

ABSTRACT

The objective of this study was to quantify the antioxidant capacity of four wild strawberry cultivars (Ottoman) harvested in Elazig, Alkaya, Kargucak and Pences as different species of Fragaria vesca and two Ottoman strawberry jams by various spectrophotometric methods (TPC, CUPRAC, TFC, DPPH, ABTS, FRAP) and to evaluate their phenolic profile by HPLC-DAD. The highest antioxidant capacity was determined in the extracts of Pences cultivar with the concentrations of 60.14, 153.20, 45.08 mg TROLOX/g of dry weight in FRAP, CUPRAC and DPPH assays respectively. Quercetin, (+) catechin, and gallic acid were detected by HPLC in all strawberry samples in substantial amounts.

Keywords: Wild Strawberry, Fragaria Vesca, Flavonoids, Spectrophotometric Analysis, HPLC

1. INTRODUCTION

The rapidly rising consumer demand for the health promoters rich in antioxidants leads the scientists to conduct studies on the species whose commercial valorisation has not been accomplished so far. Berries play an important role in a healthy diet. Among berries, strawberries draw a particular attention since they contain considerable amounts of vitamin C and anthocyanins. In Karadeniz Ereğli on the western coast of the Black Sea a local strawberry cultivar, 'Ottoman', is used for jam processing. 'Ottoman' is male sterile and rich in aromatic substances [1]. This is the Fragaria vesca species well known with its fragrance and special pink colour, cultivated in many other regions in Turkey such as Elazig located in the eastern part of Anatolia [2], in Bolu Province, at the east of Marmara [1 and 3]. The fruits of Ottoman Strawberry (OS) have either an oblate conical form or a round shape. The flesh of the fruit has a white colour with a soft skin. The fruits are considerably small and its ripening period is comparatively short in contrast to other strawberry types (harvested within a month, usually in May or June). The structure of the mesocarp is free from voids and has high moisture content [1]. Scientific evidence suggests that strawberries have some health benefits likewise reduced risk of cancer, improved neurofunction, vision and memory, prevention from weight gain due to the bioactive phytochemicals they include (phenolic acids, flavonoids, proanthocyanidins, ellagitannins, triterpenoids, lignans). The

How to Cite:
Koçak, E., Demircan, E., and Özçelik, B., (2018). Antioxidant Capacities and Phenolic Profiles of Ottoman Strawberry Fruit and Ottoman Strawberry Jam, **Ecological Life Sciences (NWSAELS)**, 13(3): 119-130. DOI: 10.12739/NWSA.2018.13.3.5A0101.

anticancerogenic property and the anti-inflammatory functions of strawberries depend strongly on their antioxidant capacity [4]. In literature, several studies related to the antioxidant capacity of wild strawberries have been conducted so far. Mahmood, Anwar [5], studied the effect of ripeness on the total phenolic content (TPC) and total flavonoid content (TFC) of four different strawberry cultivars (Korona, Tufts, M.Lavegiata, M.Macroura) and observed an increase in the percentage of TPC, TFC, flavonoid and phenolic acids as the maturity progresses from unripened to fully ripened stage. The highest level of TPC among three South American Fragaria chiloensis, Fragaria vesca and Fragaria ananassa species was reported in Fragaria vesca species by Cheel, Theoduloz [6]. Häkkinen [7] reported that strawberry jam-cooking caused only a small loss of flavonols and ellagic acid.

To our knowledge, no research has been conducted until now which investigated the antioxidant capacities of OS with different origins and OS jam by more than two antioxidant assays. In addition, no results with respect to the TFC of OS and OS jam have been reported in literature. This research aims to investigate the effects of cultivation in different areas on the antioxidant capacity and phenolic profiles of OS and OS jam by various spectrometric methods and HPLC.

2. RESEARCH SIGNIFICANCE

Strawberries play an important role in our diet since these fruits are considered as potential sources of several antioxidants and phenolic substances. In the past, a number of scientific researches revealed this evidence by conducting different antioxidant assays with cultivated strawberries. On the other hand, the number of studies focusing on the antioxidant capacity of wild strawberries is limited. This article aims to clarify the antioxidant capacity of Ottoman species known as one of the wild strawberry species by several assays and discuss the effect of cultivar and location on the phenolic profile and antioxidant capacity of strawberries.

3. EXPERIMENTAL METHOD

3.1. Raw Material

In this research, a single sample cultivated in the province Elazig (OE) and three different samples cultivated in the villages of Karadeniz Ereğli (Alkaya, Kargucak and Pences) in the Black Sea region of Turkey were collected during the harvesting period 2013-2014. The strawberries were milled with liquid nitrogen using a grinder (IKA, Germany) and then stored at -80°C until the analysis. The OS jams were purchased from Azim Konserve San.Tic.A.S., a local producer located in Karadeniz Ereğli.

3.2. Chemicals

Chemicals used in research were analytical grade and purchased from Sigma Aldrich (Germany), Fluka (Germany) and Merck (Germany).

3.3. Extraction of OS Samples

In order to obtain more accurate results in the further analysis, there must be sufficient antioxidants in the sample extract which makes the choice of a solvent critical. Therefore, three types of solvent were tested in the extraction:

- 80% acetone-distilled water (v/v),
- 60% ethanol-distilled water mixture (v/v),
- Methanol-water-formic acid mixture (80:19.9:0.1 v/v respectively).

Having performed all of the experiments for the determination of the antioxidant capacity, it was observed that the total antioxidant capacities of the acetone-water extracts was 3-4 times higher than those of the other extracts for all samples. Hence, 80% acetone-water mixture was chosen as the solvent for further analysis. 0.1g of each freeze dried sample was weighed and treated with 5mL of the solvent. The mixture was vortexed for 2 minutes and shaken for 10 minutes in an ultrasound bath (VWR-D218, Germany) filled with ice. Then, each sample was centrifuged at 5000rpm at 4°C for 10 minutes using Sigma2-16PK centrifuge (Germany). The supernatant was evaporated at 40°C and 500rpm using Buchi-diagonal rotary evaporator (Switzerland). After the evaporation, the residues were freeze dried for 16 h in the lyophilizator (Christ-USA). The freeze dried extracts were treated with methanol of 10mL.

3.4. Preparation of OS Jam Extracts

The dry matter of the jams was determined using Abbe refractometer (China). The Brix of the first sample (J1) and the second sample (J2) were measured 69.5% and 70.5% respectively. Due to the high level of sugar, it was difficult to mill the jams. They were stored at -80°C without being milled until the extraction.

3.5. Determination of the Total Phenolic Content (TPC)

TPC was measured using Folin-Ciocalteu colorimetric method described by Velioglu, Mazza [8]. Briefly, the appropriate dilutions (0.2mL) of the samples were oxidized with 1.5mL of 10% Folin-Ciocalteu reagent at room temperature. Then the reaction was neutralized with 1.2mL sodium carbonate (7.5%m/v). The absorbance was measured at 765nm with a spectrophotometer (Synergy HT, USA) after the incubation of 1.5h at room temperature in the dark. Quantification was done on the basis of a calibration curve of gallic acid in methanol (10, 20, 40, 80, 100, 200mg/L). Results were expressed as gallic acid equivalent (GAE), i.e., mg gallic acid/g DW.

3.6. Determination of the Total Flavonoid Content (TFC)

TFC was measured using a colorimetric method [9]. A 0.25-mL sample of freeze dried extract was mixed with 1.25mL distilled water and 0.075mL 0.05g/mL $NaNO_2$. After 6 min, 0.15mL of 0.1g/mL $AlCl3 \cdot 6H_2O$ solution was added. After 5 min 0.5mL of 1 mol/L NaOH was added to the mixture. The mixed solution was allowed to stand for 15 min before the measurement of the absorbance at 510nm. Quantification was done on the basis of a calibration curve of Rutin in methanol (4, 8, 10, 20, 40, 80, 100, 200, 400mg/L). The TFC was calculated and expressed as mg Rutin/g DW.

3.7. Determination of the Copper (II) Reducing Antioxidant Capacity (CUPRAC)

CUPRAC was measured using a colorimetric method [10]. The appropriate dilutions of 0.1mL samples were mixed with 1mL of 0.01mM $CuCl_2 \cdot 2H_2O$, 1mL of 7.5x 10-3mM Neocuproine and 1mL of 1M ammonium acetate buffer (pH=7) and 1mL of H_2O (total volume:4.1mL). The samples were allowed to stay in the dark at room temperature for 30 minutes. Next, the absorbance was measured at 450nm. Quantification was done on the basis of a calibration curve of TROLOX in methanol (10, 20, 40, 80, 100, 200, 400, 800mg/L). Results were expressed as mg TROLOX/g DW.

3.8. Determination of Ferric (III) Reducing Ability of Plasma (FRAP)

FRAP was measured using a modified version of the method [11]. Reagents included 300mmol/TROLOX, Acetate buffer, pH3.6 (3.1g Sodiumacetate+16 mL acetic acid in 1 L of solution), 0.156g of TPTZ (2,4,6-tripyridyl-s-triazine) in 50mL of ethanol (Sigma, Switzerland), 0.5404g of $FeCl_3 \cdot 6H_2O$+2mL HCl (37% m/m) in 100mL of solution (prepared daily). FRAP reagent was prepared by mixing 80mL of (1), 8mL of (2), and 8mL of (3). A volume with 0.1mL of sample extract was mixed with 0.9mL of FRAP reagent. The mixture was vortexed. After 4 minutes, the absorbance was measured at 593nm. Quantification was done on the basis of a calibration curve of TROLOX in methanol (1, 2, 4, 8, 10, 20, 40 and 80mg/L). Results were expressed as mg TROLOX/g of DW.

3.9. Determination of ABTS Cation Radical Scavenging Activity

ABTS(2,2azinobis-3ethylbenzothiazoline-6sulfonicacid-diammoniumsalt) cation radical scavenging activity was measured using the method [12]. 0.22g of ABTS reagent was dissolved in 200mL distilled water and 0.038g of K2S2O8 was dissolved in 2mL of distilled water. Both solutions were mixed and stored overnight to complete the radicalization. This ABTS solution is diluted with 0.05M KPi buffer (Mixture of 0.05M potassiumdihydrogenphosphate and 0.05M dipotassiumhydrogenphosphate, pH=8) until its absorbance reaches 0.9±0.2. The pH of the mixture was adjusted to 7.4 at the end. 0.1mL of sample extract was mixed with 1mL of ABTS solution. It was shaken for 10 seconds. The absorbance was measured at 734nm after 30 seconds. The absorbance values were subtracted from the blank values of pure methanol. Quantification was done on the basis of a calibration curve of TROLOX in methanol (4, 8, 10, 20, 40, 80 and 100mg/L). Results were expressed asmg TROLOX/g of DW.

3.10. Determination of DPPH Radical Scavenging Activity

This experiment was based on the method of Kumaran and Karunakaran [13] and Tezcan, Gultekin-Ozguven [14]. 0.1mL of sample extract was mixed with 2mL of 0.1 mM DPPH (1,1diphenyl-2picyrlhydrazyl) in methanol solution and shaken for 10 seconds. The mixture was stored in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm against methanol. The measured absorbance of the mixture was subtracted from the blank values. Quantification was done on the basis of a calibration curve of TROLOX in methanol (10, 15, 20, 30, 40, 60 and 80mg/L). The radical scavenging activity was calculated using the formula:

〖(A〗_blank-A_sample)/A_blank x100 (1)

or the mg TROLOX equivalent values were calculated with respect to the standard for 1g DW.

3.11. Determination of the Phenolic Profile of OS Cultivars and OS Jam

Major phenolics were determined following the method of Capanoglu, Beekwilder [15]. Extracts were filtered through a 0.45µm membrane. Two milliliters of sample extracts were analyzed by HPLC (Waters 2487) equipped with photodiodearray detector (Waters 2687) with a supelco SUPERCOSIL LC-18 column (250x4.6mm, particle size 5 µm.). Themobile phase was the water-trifluoroacetic acid (0.1%) (solvent A) and acetonitrile-trifluoroacetic acid (0.1%) (solvent B) at a flow rate of 1ml/min. A linear gradient was used as follows: at 0 min 95% solvent A and 5% solvent B; at 45min 65% solvent A and 35% solvent B; at 47 min 25% solvent A and 75% solvent B; at 54 min

returns to initial conditions. The detection was achieved at 260, 280, 315, 360 and 510nm. All the analyses were run in duplicate and results were expressed in terms of mg/100g DW.

4. STATISTICAL ANALYSIS

The extraction of all samples was carried out in duplicate. Results are shown as mean and standard deviations. In order to evaluate the relationship among dependent variables, correlation tests were carrried out. Pearson correlation coefficients for each pair of antioxidant assays were determined and other statistical analysis to compare means of the results was performed by SPSS software version 20.0. (SPSS, Inc. Chicago, Illinois). One way ANOVA test was performed in order to reveal the significant differences among the mean values of the antioxidant assays for each variety. The main factor studied was the influence of variety on the antioxidant activity and total phenolic content. If a statistical significant effect was found, means were compared using Tukey's honestly significant difference (HSD) multiple comparison test. All statistical tests were performed at a 5% significance level.

5. FINDINGS AND DISCUSSIONS

The total antioxidant capacity (TAC) of the extracts was determined using four different in vitro assays since antioxidant activity occurs by different mechanisms which means employing a method depending on one mechanism may not reflect the true antioxidant capacity [16]. The results of the sample extracts obtained for OS cultivated in Elazig (OE), Alkaya (OA), Kargucak (OK) and Pences (OP); OS jam with a Brix of 69.5% (J1); OS jam with a Brix of 70.5% (J2) in Total Phenolic content (TPC), Total Flavonoid content (TFC) assays and the results of the antioxidant analysis in addition to the one way ANOVA-Tukey HSD test results are summarized in Table 1. Among the examined strawberry varieties, OP had the highest TPC; whereas OE showed the lowest TPC. There was a 3.33 fold difference between the highest and lowest values of TPC for strawberries. The OP extracts had the highest and the OE extracts had the lowest value among all strawberries in TFC assay. Among the strawberry samples, OP had the highest values in CUPRAC, DPPH and FRAP assays while OA cultivar yielded the highest score in ABTS assay. It was figured out that there was approximately 2.31 fold difference in CUPRAC and 1.88 fold difference in ABTS, 3.16 fold difference in DPPH and 1.36 fold difference in FRAP assays between the highest and lowest values. On the other hand, OS jam had the lowest antioxidant capacity.

Table 1. Total phenolic content and Total Antioxidant capacities of the examined freeze dried strawberry sample extracts

Extract	TPC[1]	TFC[2]	CUPRAC[3]	ABTS[4]	DPPH[5]	FRAP[6]
OE	17.85±1.03^{a}	20.75±0.16^{a}	66.42±9.79^{a}	61.17±2.52^{a}	20.24±2.32^{a}	44.00±3.86^{a}
OA	36.81± 3.48^{b}	31.17±3.59^{b}	91.66±6.22^{ab}	84.46±6.67^{b}	33.76±6.50^{b}	53.28±1.89 ^{ab}
OK	53.65± 4.45^{c}	34.75±1.88^{bc}	136.84±3.61^{bc}	44.91±4.54^{c}	60.49±9.46^{c}	44.39±3.47^{a}
OP	59.60± 4.71^{c}	45.08±3.53^{ab}	153.2±2.87^{c}	60.94±1.37^{a}	64.03±3.78^{c}	60.14±3.03 ^{ab}
J1	1.86±0.08	1.6±0.21	3.47±0.07	0.657±0.002	0.984±0.035	3.90±0.29
J2	1.84±0.19	1.41±0.04	3.47±0.04	0.66±0.001	0.992±0.037	3.22±0.17

[1]Total phenolic content (TPC) expressed in mg GAE/g DW
[2]Total flavonoid content (TFC) expressed in mg Rutin/g DW
[3]CUPRAC: Copper (II) reducing antioxidant capacity expressed in mg TROLOX/g DW;
[4]ABTS: Cation radical scavenging activity expressed in mg TROLOX/g DW;

[5]DPPH: Radical scavenging activity expressed in mg TROLOX/g DW
[6]FRAP: Ferric (III) reducing ability of plasma expressed in mg TROLOX/g DW;
OE, OA, OK, OP: Ottoman strawberry cultivated in Elazig, Alkaya, Kargucak, Pences respectively.
J1: Ottoman strawberry jam
Brix:69.5%
J2: Ottoman strawberry jam
Brix:70.5%.
[*]All analysis was run in duplicate. Three measurements were made for each replicate. The values are given as the mean of duplicate runs with its standard deviations. Columns belonging to the same data set with different letters are significantly different ($p<0.05$ n=6 analysis)

When the means of each strawberry cultivar were compared according to the antioxidant assay, it was observed that OP and OK varieties slightly differ from the other genotypes in CUPRAC, DPPH and TPC assays. The differences in the antioxidant capacities of OS might issue that geographical location and climate conditions play an important role in the phenolic content and antioxidant activity. One way ANOVA test results revealed that there was a significant difference between OE and all other OS in TPC, TFC and DPPH assays. In contrast, the distinction among the varieties cultivated in Karadeniz Ereğli is not as clear as in the case of OE variety since OK and OP varieties gave close results in all assays except for ABTS. Nevertheless, within the group of Karadeniz Ereğli cultivars, one may claim that there is a significant difference between OA and OP cultivars in all assays except for FRAP. This may be an important issue to evaluate the differences among the cultivars harvested in the same region and in the same year showing that geographical location might have a moderate level of impact on the antioxidant activities of strawberries even though the locations where the samples were collected are not so far from each other. When it comes to the relationship between the antioxidant assays and the mean value of each cultivar in each assay, Pearson correlation coefficients of the sample extracts are given in Table 2. A significant correlation was observed between TPC and CUPRAC assay ($r=0.952$, $p\leq0.01$); TPC and DPPH assay ($r=0.943$, $p\leq0.01$). In addition, the results of CUPRAC and DPPH assays were closely similar ($r=0.93$; $p\leq0.01$), suggesting that the two assays are almost interchangeable in the case of strawberry. Besides, there was a significantly strong correlation between ABTS and FRAP assays ($r=0,867$; $p\leq0.01$) in agreement with the results of the previous study [17].

Table 2. Pearson's correlation coefficients for quantitative determinations in strawberry samples

	TPC	TFC	CUPRAC	ABTS	DPPH	FRAP
TPC		0.910	0.952	0.835	0.943	0.894
TFC			0.925	0.903	0.857	0.883
CUPRAC				0.862	0.930	0.907
ABTS					0.724	0.867
DPPH						0.877
FRAP						

[a]99% confidence interval, significant at $p\leq0.01$

Although there is sufficient knowledge about the antioxidative features of cultivated strawberries in literature, there is a limited number of investigations reported in the past and related to the antioxidant activity of wild strawberries. On a dry weight basis, average levels of TPC's reported here for OE extracts (17.85mg/g of

DW) are consistent with the previously reported values of 1600-2410 mg/100g of DW [18] and 2200mg/100g of DW [19]. In addition, the results of OE variety for TPC were in accordance with the results of the study [20] which are in the range of 2000-2800mg GAE/100g of DW). It was reported by Mahmood, Anwar [5] and Rekika, Khanizadeh [21] that in strawberry, TPC ranged from 491–1884mg GAE /100g of DW and 0.426-0.937mg GAE/g of FW respectively which disagree with the results of this study (1.78-5.9mg GAE/g of FW). One might state that the difference in the results might be influenced by cultivar and climate conditions. Rekika, Khanizadeh [21] studied the antioxidant capacity of 18 selected strawberry varieties (Fragaria ananassa Duch.) by ABTS assay and found out between the range of 0.198 and 0.272mmol TROLOX/g of DW which is fairly in agreement with the results of this study (0.179-0.337mmol TROLOX/gof DW). In another scientific research, the antioxidant activity of strawberry according to ABTS, FRAP and DPPH was determined as 11.5, 24.9; 15.9µmol TEAC/g of FW respectively within a reaction time of 120 min [22]. The results of FRAP assay in our study belonging to OP cultivar (24.03µmol TEAC/g of FW) and OA cultivar (21.28µmol TEAC/g of FW) and the values of OA cultivar in DPPH assay (13.48µmol TEAC/g of FW) confirm the previous values. However, our results of ABTS assay are higher (17.94-33.74µmol TEAC/g FW) compared to the values in the referring study. Each of these methods provides an estimate depending on the reaction time which is shorter in our study (4 min in FRAP, 30 min in DPPH, 30 seconds in ABTS), the complexity of the reaction kinetics and the polymerization potential of the phenolics present in the sample [22]. Therefore, it might be concluded that the difference between the results might have been caused by these factors. The results of ABTS assay are consistent with the previously reported values 33.1 µmol TEAC/g of FW [23] which indicates a close result to the findings in this research (24.44µmol TROLOX/g of FW in OE extracts). However, there is some evidence in the literature contradicting the results of this study. Aaby, Skrede [20] determined 4.3mmol TEAC/100g of FW (0.364mmol TE/g of DW) content in freeze dried strawberries extracted with 40mL of a mixture containing acetone, water, acetic acid (70:29.5:0.5 v/v). Our results of OS samples are at considerably lower levels (0.176 and 0.24mmol/g of DW).

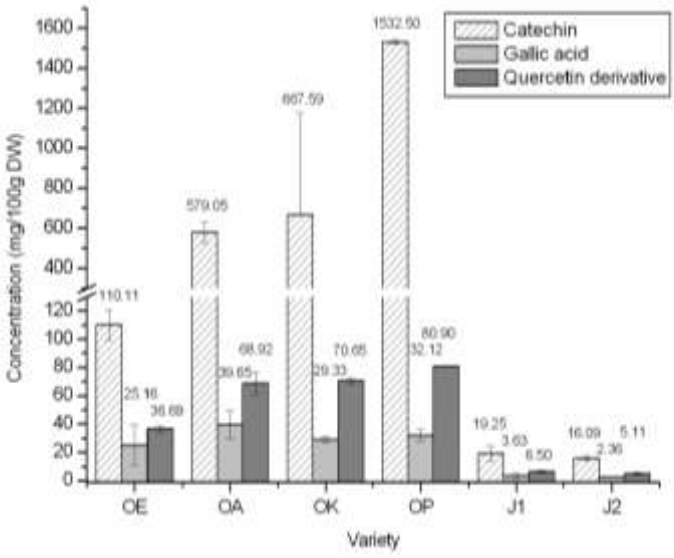

Figure 1. The main phenolics (+catechin, gallic acid and quercetin derivatives) most abundant in Ottoman strawberry samples collected from Elazig, Alkaya, Kargucak, Pences and analyzed by HPLC

In another research, seven cultivars of strawberry (Diamante, Elsanta, Honeoye, Madeleine, Marmolada, Miranda and Miss as Fragaria ananassa species) were analyzed for TPC, TFC and the antioxidant capacity before and after low sugar jam production to evaluate their changes after thermal processing [24]. Fresh fruits had TPC ranging from 251.97 to 713.06mg GAE/100g of DW. The quantities of the antioxidant capacity in DPPH for strawberry jam were determined between the range of 0.2-0.62mmol TE/kg of FW and between 212.78-383.19mg GAE/100g of DW in TPC. The results of TPC were higher than our findings (185 mg GAE/100g of DW) whereas the values of DPPH assay were lower than our results (2.76mmol TE/kg of FW). The reason for the disagreement in results might be based on the different jam recipe, storage conditions, processing procedure and the level of anthocyanin degradation during cooking depending on the variety of strawberries.

5.1. The determination of the phenolic profile of the samples

Analysis of the sample extracts by HPLC revealed a great diversity of compounds. Gallic acid, (+) catechin and quercetin could be detected in all samples (Figure 1) and other minor phenolic acids such as caffeic acid, p-coumaric acid, neochlorogenic acid were identified and quantified on the basis of their retention time and absorbtion spectra in comparison with their standards (Figure 2).

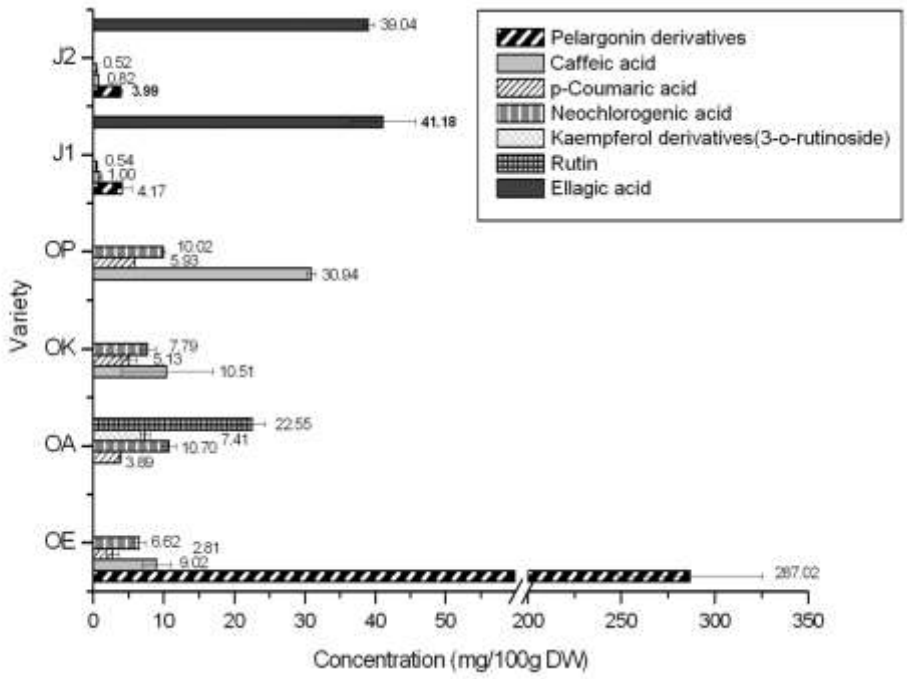

Figure 2. Other minor phenolics abundant in the examined Ottoman strawberry samples collected from Elazig, Alkaya, Kargucak, Pences and analyzed by HPLC

OE: ottoman strawberry-Elazığ
OA: ottoman strawberry-Alkaya
OK: ottoman strawberry-Karucak
OP: ottoman strawberry
Jam-Brix: 69.5%
J2: ottoman strawberry Jam
Brix: 70%. Data is expressed in terms of mg/100gDW

(+) Catechin detected at 280 nm was found to be the most common phenolic in all samples. Its quantity was detected as 1532.5mg/100g of DW in OP extracts as the most abundant compound whereas it was identified in much lower values in other OS extracts differing from 110.1 to 667.59 mg/100g of DW. The research in the past relevant to the phenolic profile of OS reveals the presence of (+) catechin (6.88mg/100g of FW, [25]), quercetin (3.80mg/100g of FW, [25]) and kaempferol derivatives (1.22mg/100g of FW, [25]). On the other hand, Kahkonen, Hopia [18] reported (+) catechin as 8.1-10mg/100g of DW in three different Finnish origin wild berries (Fragaria ananassa Bounty, Jonsok, Senga sengana). The strawberry cultivars inspected in this study contain considerably higher amounts of (+) catechin. When it comes to the contribution to the TAC, (+) catechin gave strongpositive correlations withall antioxidant assays except for ABTS, reaching the highest values R2=0.771 in CUPRAC and R2=0.812 in TPC assays. The other flavanol (quercetin) resulted in a surprisingly higher positive correlation in regard with the contribution to the TAC (R2=0.948 in TPC; and R2=0.947 in CUPRAC) although its concentration was detected in all samples at much lower levels than that of (+) catechin. This may be linked to the superior antioxidant capability of quercetin due to its structure (having o-dihydroxy group in B-ring, presence of 3-5 –OH groups and 2-3 double bond in conjugation with 4-oxo function, [26]). p-Coumaric acid was detected in all samples at 312 nm at various concentrations. In contrast to previous study [17], the antioxidant contribution to TPC and CUPRAC analysis was evident where it showed the strongest correlations (R2=0.978; R2=0.997, respectively) among the other phenolics to antioxidant assays. Another research determined p-coumaric acid level in OS within the range of 0.42 and 0.64mg/100g frozen fruits and ellagic acid was found at 0.52-2.09mg/100g frozen fruit collected at different maturation stages [27]. The results of our research do not confirm these findings which may depend on the different sample preparation and method of extraction.

Caffeic acid was detected as the second most abundant phenolic acid in OP, OK and OE extracts whose quantity was determined higher than that of the previous study [25]. On the other hand, the caffeic acid content of OE extracts was found only a bit higher (13.92% more) than that in Kelebek and Selli [25]’s report. The similarity between the results of OE extracts and those in literature [25] could be associated with harvesting locations which were not so far from each other and possess similar climate conditions. In contrast to what was previously observed [17] no relevant peaks indicating the presence of ellagic acid were detectable in the examined samples except for OS jams. High ellagic acid content of OS jam (39.04-41.18mg/100g of DW) could be associated with processing conditions. That is, cooking may have risen the ellagic acid content of the jams. On the other hand, it seems that heat treatment did not have a significant effect on the anthocyanin content since certain amount of pelargonin derivatives were detected (3.99-4.16mg/100g of DW) in OS jams. Anthocyanins were also analyzed by HPLC at 510 nm and expressed as pelargonin chloride which was detected only in the OE cultivar and OS jams. In a recent study [25] pelargonin 3-glucoside was found as the most abundant anthocyanin at lower levels (9.11 mg/100g of FW) than our results. The pelargonin content of OE extracts (28.7mg/100g of FW) was in agreement with the previous research of Buendia, Gil [28] reported as pelargonin 3-glucoside, in 15 different Fragaria ananassa species from Spain between 20.2 and 35.5mg/100g of FW.

6. CONCLUSION AND RECOMMENDATIONS

The strawberries examined in this study exhibited potent antioxidant activity. The results showed that OP extracts had the strongest antioxidant capacity in TPC, CUPRAC, DPPH and FRAP assays, whereas the OS jams had the lowest antioxidant capacity in all assays. The phenolic profile revealed a great deal of diversity among all samples having a wide range of concentration. However, three main phenolics identified as (+) catechin, quercetin and gallic acid were detected in all strawberry samples in substantial amounts. In addition, p-coumaric acid and caffeic acid were identified as the most abundant phenolic acids in all examined samples respectively. The ellagic acid content was detected in OS jams in considerably higher concentrations than the other phenolics.

REFERENCES

1. Serce, S.G.K., Ozdemir, E., Bakan, M., Paydas, S., and Kaska, N., (2005). Genetic Resources of Turkish Strawberry Varieties (in Turkish). GIDA-TARIM Cine Tarim A.S., Vol: Nisan-Mayis 68, pp:60-64.
2. Turhan, E. and Kargi, S.P., (2007). Strawberry Production in Turkey. J Chronica Horticulturae, Vol:47, Number:2, pp:18-20.
3. Kaska, N., (1996). Strawberry Growing in Turkey. III International Strawberry Symposium 439.
4. Crecente-Campo, J., Nunes-Damaceno, M., Romero-Rodriguez, M.A., and Vazquez-Oderiz, M.L., (2012). Color, Anthocyanin Pigment, Ascorbic Acid and Total Phenolic Compound Determination in Organic Versus Conventional Strawberries (Fragaria X Ananassa Duch, Cv Selva). Journal of Food Composition and Analysis, Vol:28, Number:1, pp:23-30. WOS:000311179700004.
5. Mahmood, T., Anwar, F., Abbas, M., and Saari, N., (2012). Effect of Maturity on Phenolics (Phenolic Acids and Flavonoids) Profile of Strawberry Cultivars and Mulberry Species from Pakistan. International Journal of Molecular Sciences, Vol:13, Number:4, pp:4591-607. WOS:000303454700037.
6. Cheel, J., Theoduloz, C., Rodriguez, J.A., Caligari, P.D.S., and Schmeda-Hirschmann, G., (2007). Free Radical Scavenging Activity and Phenolic Content in Achenes and Thalamus from Fragaria Chiloensis Ssp Chiloensis, F Vesca and F. X Ananassa Cv. Chandler. Food Chemistry, Vol:102, Number:1, pp:36-44. WOS:000243541900006.
7. Häkkinen, S., (2000). Flavonols and Phenolic Acids in Berries and Berry Products. University of Kuopio.
8. Velioglu, Y.S., Mazza, G., Gao, L., and Oomah, B.D., (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. Journal of Agricultural and Food Chemistry, Vol:46, Number:10, pp:4113-17. WOS:000076655800039.
9. Dewanto, V., Wu, X.Z., Adom, K.K., and Liu, R.H., (2002). Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. Journal of Agricultural and Food Chemistry, Vol:50, Number:10, pp:3010-14. WOS:000175355200045.
10. Apak, R., Güçlü, K., Ozyurek, M., and Karademir, S.E., (2004). Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: Cuprac Method. Journal of Agricultural and Food Chemistry, Vol:52, Number:26, pp:7970-81. WOS:000225985700037.

11. Benzie, I.F.F. and Strain, J.J., (1996). The Ferric Reducing Ability of Plasma (Frap) as a Measure of ''Antioxidant Power'': The Frap Assay. Analytical Biochemistry, Vol:239, Number:1, pp:70-76. WOS:A1996UY64800010.
12. Miller, N.J. and RiceEvans, C.A., (1997). Factors Influencing the Antioxidant Activity Determined by the Abts (Center Dot+) Radical Cation Assay. Free Radical Research, Vol:26, Number:3, pp:195-99. WOS:A1997WT96200002.
13. Kumaran, A. and Karunakaran, R. J., (2006). Antioxidant and Free Radical Scavenging Activity of an Aqueous Extract of Coleus Aromaticus. Food Chemistry, Vol:97, Number:1, pp:109-14. WOS:000235723300015.
14. Tezcan, F., Gultekin-Ozguven, M., Diken, T., Ozcelik, B., and Erim, F.B., (2009). Antioxidant Activity and Total Phenolic, Organic Acid and Sugar Content in Commercial Pomegranate Juices. Food Chemistry, Vol:115, Number:3, pp:873-77. WOS:000265348100015.
15. Capanoglu, E., Beekwilder, J., Boyacioglu, D., Hall, R., and De Vos, R., (2008). Changes in Antioxidant and Metabolite Profiles During Production of Tomato Paste. Journal of Agricultural and Food Chemistry, Vol:56, Number:3, pp:964-73. WOS:000252969100049.
16. Karadag, A., Ozcelik, B., and Saner, S., (2009). Review of Methods to Determine Antioxidant Capacities. Food Analytical Methods, Vol:2, Number:1, pp:41-60. WOS:000263075700005.
17. Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., De Vos, C.H.R., Capanoglu, E., Bovy, A., and Battino, M., (2008). Antioxidants, Phenolic Compounds, and Nutritional Quality of Different Strawberry Genotypes. Journal of Agricultural and Food Chemistry, Vol:56, Number:3, pp:696-704. WOS:000252969100012.
18. Kahkonen, M.P., Hopia, A.I., and Heinonen, M., (2001). Berry Phenolics and Their Antioxidant Activity. Journal of Agricultural and Food Chemistry, Vol:49, Number:8, pp:4076-82. WOS:000170598600094.
19. Asami, D.K., Hong, Y.J., Barrett, D M., and Mitchell, A.E., (2003). Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. Journal of Agricultural and Food Chemistry, Vol:51, Number:5, pp:1237-41. WOS:000181087100020.
20. Aaby, K., Skrede, G., and Wrolstad, R.E., (2005). Phenolic Composition and Antioxidant Activities in Flesh and Achenes of Strawberries (Fragaria Ananassa). Journal of Agricultural and Food Chemistry, Vol:53, Number:10, pp:4032-40. WOS:000229049000043.
21. Rekika, D., Khanizadeh, S., Deschenes, M., Levasseur, A., Charles, M.T., Tsao, R., and Yang, R., (2005). Antioxidant Capacity and Phenolic Content of Selected Strawberry Genotypes. Hortscience, Vol:40, Number:6, pp:1777-81. WOS:000231867500048.
22. Ozgen, M., Reese, R.N., Tulio, A.Z., Scheerens, J.C., and Miller, A.R., (2006). Modified 2,2-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (Abts) Method to Measure Antioxidant Capacity of Selected Small Fruits and Comparison to Ferric Reducing Antioxidant Power (Frap) and 2,2 '-Diphenyl-1-Picrylhydrazyl (Dpph) Methods. Journal of Agricultural and Food Chemistry, Vol:54, Number:4, pp:1151-57. WOS:000235627800024.

23. Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., and Battino, M., (2005). Plant Genotype Affects Total Antioxidant Capacity and Phenolic Contents in Fruit. Nutrition, Vol:21, Number:2, pp:207-13. WOS:000227517500015.
24. Levaj, B., Bursać Kovačević, D., Bituh, M., and Dragović-Uzelac, V., (2012). Influence of Jam Processing Upon the Contents of Phenolics and Antioxidant Capacity in Strawberry Fruit (Fragaria Ananassa× Duch.). Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam, Vol:7, Number: SPECIAL ISSUE-7th, pp:18-22.
25. Kelebek, H. and Selli, S., (2011). Characterization of Phenolic Compounds in Strawberry Fruits by Rp-Hplc-Dad and Investigation of Their Antioxidant Capacity. Journal of Liquid Chromatography & Related Technologies, Vol:34, Number:20, pp:2495-504. WOS:000299376300002.
26. Apak, R., Guclu, K., Demirata, B., Ozyurek, M., Celik, S.E., Bektasoglu, B., Berker, K.I., and Ozyurt, D., (2007). Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the Cuprac Assay. Molecules, Vol:12, Number:7, pp:1496-547. WOS:000250488100024.
27. Kosar, M., Kafkas, E., Paydas, S., and Baser, K.H.C., (2004). Phenolic Composition of Strawberry Genotypes at Different Maturation Stages. Journal of Agricultural and Food Chemistry, Vol:52, Number:6, pp:1586-89. WOS:000220285600028.
28. Buendia, B., Gil, M.I., Tudela, J.A., Gady, A.L., Medina, J.J., Soria, C., Lopez, J.M., and Tomas-Barberan, F.A., (2010). Hplc-Ms Analysis of Proanthocyanidin Oligomers and Other Phenolics in 15 Strawberry Cultivars. Journal of Agricultural and Food Chemistry, Vol:58, Number:7, pp:3916-26. WOS:000276232700006.