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BIOACTIVE CONTENTS AND SOME HORTICULTURAL CHARACTERISTICS OF LOCAL APPLE GENOTYPES FROM TURKEY

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ABSTRACT

Some horticultural characteristics and bioactive contents of some local apple genotypes grown in Bolu province located in western black sea region were investigated. Along with the horticultural characteristics (fruit weight, width etc.), the phenolic spectrum and organic acids as bioactive content were determined. Among major phenolic compounds, catechin, chlorgenic acid, gallic acid, phloridzin and rutine content varied between 10.84- 5.16 mg 100 ml^{-1}; 25.16-7.17 mg 100 ml^{-1}; 7.35- 1.42 mg 100 ml^{-1}; 6.12-1.18 mg 100 ml^{-1} and 5.16-1.01 mg 100 ml^{-1}, respectively. The dominant organic acids were malic acid and fumaric acid among the organic acids. When the apple genotypes were evaluated overall in terms of horticultural properties and bioctive contents; it is suggested that 14BL02, 14BL09, 14BL06 and 14BL01 genotypes displayed superior properties and they may create a significant genetic resource to be use them in breeding activities in apple in future.

Keywords: Apple, organic acids, phenolics, pomology.

INTRODUCTION

The apple has been among the most consumed fruits around the world and of which preferability has been gradually increasing along with its use in the nutrition, food and drug industry (Zhang *et al*., 2010). Apple fruits has also been available on the market year around due to developed storage techniques and marketing strategies. Turkey has very suitable climate and soil conditions that allow to growth many fruit species including pome and stone fruits, nuts, and subtropical fruits (Ercisli, 2009; Erturk *et al*., 2010; Canan *et al*., 2016; Saridas *et al*., 2016; Yazici and Sahin, 2016). By its location and ecological conditions, Turkey has various apple cultivars/genotypes grown in the country for long years and it has been used by the community in various ways in the apple growing regions. Along with the ecological diversity, each region has specific apple cultivars/genotypes.

In the studies, it was revealed that the phenolic compounds which have protective effect in coronary heart diseases and some cancer types demolishes the free radicals and prevent aging due to its antioxidant properties (Rodriguez-Mateos *et al*., 2014). The chemical contents and antioxidant capacities of fruits are affected from various factors. More particularly, the environmental conditions and its genotype structure have significant effects on the formation of these substances (Hegedus *et al*., 2010; Rop *et al*., 2014; Jurikova *et al*., 2014; Lima *et al*., 2014). As a result of these properties, its consumption per capita has been increasing day by day. In accordance with 2017 data of Turkish Statistical Institute, annual apple production in our country was approximately 2.5 million tons in 2015 (TUIK, 2017).

The phenolic compounds determined as secondary metabolism products of plants is a substances group which has a very wide range in the plants and in the recent years, the structure of thousands of phenolic compounds have been determined (Kafkas *et al*., 2006). The phenolic compounds are densely available in the fruits, vegetables, seeds, flowers, leaves, boughs and bodies of plants (Cemeroğlu, 2004; Coşkun, 2006). Phenolic compounds classified into two groups as phenolic acids and flavonoids. The flavonoids which are polyphenolic antioxidants are available in the nature structures of herbal teas, fruits and vegetables. Some part of phenolic compounds is very effective in the formation of taste of fruits and vegetables and principally formation of two significant taste aspects such as bitterness and sourness in the mouth. Other part provides formation of colors in the yellow, yellow-brunet, red-blue tons in the fruits and vegetables. They lead to different problems such as enzymatic browning in processing the fruits and vegetables and making them new products. These cases are very significant in terms of fruits and vegetables and the products which are obtained from them (Cemeroğlu, 2004; Zorenc *et al*., 2016).

The phenolic compounds are also called as ‘‘bioflavonoid’’ due to their positive effects on humans in terms of nutrition physiology. In some studies, they are called as factor P (permeability factor) or vitamin P (Cemeroğlu, 2004; Saldamlı, 2007). The phenolic compounds also gain significance as food component as they lead to enzyme inhibition and they are quality control criterion in different foods (Saldamlı, 2007). The polyphenols were determined as powerful antioxidants and the apples are very rich in terms of flavonoid and phenolic acids and the most significant ones among these phenolic compounds are anthocyanin, catechin, quercetin and chlorogenic acid. The apple is among the fruits which are consumed quite a lot and contains phytochemicals which decrease the risk of diseases such as cancer, cardiovascular diseases, lipid oxidation, immune system damage, asthma and diabetes. In a study, it was determined that the risk of heart attack decreased at the rate of 49% in the men who consumed 100 g apple and above every day compared to the men who consumed less than 18 g apple (Awad *et al*., 2001; Hertog *et al*., 2993). The ratio of total acid content of fruits to the sugar amount is a criterion for ripeness. It is known that the acids are effective on the taste as they decrease the sweetness and increase the sourness. The acidity type and amount is a criterion for the deterioration in the foods (Cemeroğlu and Acar, 1986; Schobinger, 1988; Savran, 1999; Cemeroğlu *et al*., 2004). It was determined that the phenolic compounds inhibit the cancer cells, have antimutagenic activity and reducing effect on blood pressure and decrease the cardiovascular risk (Seeram *et al*., 2006; Bermúdez-Soto *et al*., 2007; Naruszewicz *et al*., 2007; Hellström *et al*., 2010; Jia *et al*., 2012; Ju *et al*., 2012).

Especially in the recent reports, the determination of phenolic compounds and organic acids has revealed the higher content in of local cultivars/genotypes as they have a great importance in terms of human health and nutrition and due to their antioxidant properties. In this study, some local apple genotypes grown in Bolu were investigated in terms of bioactive content and some horticulture characteristics.

MATERIALS AND METHODS

Plant material: In this study, ten local apple genotypes (14BL01, 14BL02, 14BL03, 14BL04, 14BL05, 14BL06, 14BL07, 14BL08, 14BL09, 14BL10) were assessed. They were harvested in the periods when the fruits belonging to genotypes have grown in full maturity. The fruit samples picked homogenously were stored at -80 °C until their laboratory analyses were conducted after the pomological measurements were determined. In the research, local apple varieties were determined and examinations were made on some pomological properties. In the determination of pomological properties of local varieties determine; average fruit weight, seed weight (with a balance sensitive to 0.01 g (Radvag PS 4500/C/1, Poland)), fruit length, fruit width, fruit stem length, fruit stem thickness, stem cavity width, stem cavity depth, fruit skin thickness, core width, core length, seed length, seed width, seed thickness (with a caliper sensitive to 0.01 mm (Model No. CD-6CSX, Mitutoyo, Japan)), pulp hardness (with hand penetrometer by lifting a thin layer on fruit surface (FT 327; McCormick Fruit Tech, Yakima, Washington)), soluble solids content (SSC) (with hand refractometer (model PAL-1, McCormick Fruit Tech., Yakima, Washington)) and titratable acidity (TA) (with titration method) were determined in 10 fruits which were randomly taken from each genotype (Richard, 1991). A sample of juice was also taken from each fruit. The taste and juiciness states of fruits were determined with sensory observations. The pulp color and fruit shell color were determined by observation and comparison. The shape index was determined by dividing fruit length by fruit width. The fruit volume was calculated by putting the fruits into 500 ml measuring cylinder of which specific part is filled with water. The fruit density was determined by dividing the fruit weight by fruit volume (Richard, 1991).

Analysis of phenolic compounds: In the research, the gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, phloridzin, protocatechuic, vanillic acid, rutin and quercetin phenolic compounds were determined. In the separation of phenolic acids with HPLC (Agillent 1100 series HPLC G 1322 A, Germany) the method developed by Rodriguez-Delgado *et al*. (2001) was modified and used. The samples collected were distilled with distilled water at the ratio of 1:1 and after they were centrifuged at 15000 rpm for 15 min., the supernatant was filtered with 0.45µm millipore filters and then injected to HPLC. The chromatographic separation was conducted by using DAD detector (Agilent. USA) and 250*4.6 mm, 4µm ODS colon (HiChrom, USA) in Agilent 1100 (Agilent) HPLC system. Solvent A Methanol-acidic acid-water (10:2:88), Solvent B Methanol-acidic acid-water (90:2:8) were used as the mobile phase. The separation was conducted at 254 and 280 nm and the flow rate was determined as 1 mL/min. and the injection volume was determined as 20 µL.

Analysis of organic acids: The samples collected were kept at deepfreeze (-20 ºC) until the time of analysis. In the research, the citric acid, tartaric acid, malic acid, succinic acid, oxalic acid and fumaric acid contents were determined among the organic acids. In the extraction of organic acids, the method developed by Bevilacqua and Califano (1989) was modified and used. 5 g was taken from the fruit samples obtained and transferred to centrifuge tubes. These samples were homogenized by adding 20 ml 0.009 N H_2SO_4 (Heidolph Silent Crusher M, Germany). Then, it was mixed on the agitator (Heidolph Unimax 1010, Germany) for 1 hour and centrifuged at 15000 rpm for 15minutes. The aqueous part which was separated at centrifuge was filtered from first coarse filter paper, then 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) for two times and finally SEP-PAK C_{18} cartridge.

The organic acids were analyzed in HPLC device (Agilent HPLC 1100 series G 1322 A, Germany) by using the method developed by Bevilacqua and Califano (1989). In HPLC system, Aminex HPX - 87 H, 300 mm x 7.8 mm colon (Bio-Rad Laboratories, Richmond, CA, USA) was used and the device was controlled with the computers including Agilent package program. DAD detector in the system (Agilent, USA) was set to 214 and 280 nm wavelengths. In the study, 0.009 N H_2SO_4 filtered at 0.45 µm membrane filter was used as mobile phase.

Statistical analysis: The study was planned as three repetitions and 20 fruits per repetition. The introductory statistics belonging to analysis and measurement results was offered as average ± standard deviation. In the statistical evaluations, Windows SPSS 20 was used and the differences between the means was evaluated by subjecting to ANOVA variance analysis and determined with Duncan multiple comparison test ($p<0.005$).

RESULTS AND DISCUSSION

Pomological properties: In the study, it was determined that the fruit weight varied between 32.44 g (14BL08) and 127.32 g (14BL02). It was determined that in the fruits belonging to genotypes investigated, 14BL02 had maximum fruit width as 72.38 mm and 14BL08 variety had minimum fruit width as 40.28 mm. It was determined that the shape index of genotypes varied between 0.96 (14BL03-14BL09) and 0.69 (14BL01). In terms of soluble solids content of genotypes investigated in the study, the highest value was determined at 14BL04 as 16.13% and the lowest soluble solids content ratio was determined at 14BL08 as 9.18%. When average fruit lengths of 10 genotypes were investigated in this paper, it was determined that the shortest one was 14BL08 as 33.70 mm while the longest one was 14BL09 variety as 66.30 mm. In their study conducted with the aim of determining the morphological and pomological properties of 10 apples grown in districts and villages of Catak (Van) and Tatvan (Bitlis), Özrenk *et al*., (2011) stated that average fruit weights varied between 20.9-139.3 g. While Kaya and Balta (2007) determined in a study conducted in territory of Gevaş (Van) that average fruit weights varied between 32.29 g and 138.25 g in local apple varieties, Serdar *et al*. (2007) determined in a study conducted on local apple varieties grown in Camili territory of Artvin that average fruit weights varied between 54.33-206.0 g. In this research, it was seen that some parameters showed differences as well as it may be said that the fruit weight showed similarities with other studies conducted. It is thought that these differences on fruit size may vary depending on the genetic factors, ecological conditions and cultural practices. In their study, Coşkun and Aşkın (2016) reported that Gelin Elması variety had the maximum average fruit width (76.56 mm) and Kızıl Ahmedi variety had the minimum average fruit width (64.86 mm). In a study conducted in territory of Gevaş, the shape index of fruits was determined varying between 0.80-0.90 (Kaya and Balta, 2007). It was noted that the shape index values determined in the research showed similarity with the values in our study. In accordance with these values, it is thought that the fruit shape was smooth and carried one of preferability criteria on the market. As a result of the studies conducted on the fruits, Coşkun and Aşkın (2016) determined the average fruit length varying between variety 24 (65.82 mm) and Kızıl Ahmedi variety (53.93 mm). In a study, Goffreda *et al*. (1995) determined that water soluble dry matter ratio in NJ55 apple varied between 13% and 14.8%. In their study conducted on summer apple varieties, Özrenk *et al*., (2011) determined that water soluble dry matter ratios in the study varied between 10.0% and 15.4%. In a study conducted on Erzurum conditions, Karlıdağ and Eşitken (2006) determined that SSC ratios varied between 9.10-13.80% in the apple varieties. In a research conducted on Pink Lady, Golden Delicious, Lady Williams apples, the titratable acidity contents were determined varying between 0.90%, 0.32% and 0.83% (Cripps and Richards, 1993). In the study, the pomological parameters of apple genotype were investigated and it was seen that the values obtained showed similarity with the findings of other researchers (Özrenk *et al*., 2011; Coşkun and Aşkın, 2016) to a large extent (Table 1-4).

Table 1. Some physical properties of fruit and seed in apple genotypes.

Varieties	Fruit weight (g)	Fruit width (mm)	Fruit length (mm)	Fruit volume (ml)	Fruit shape index	Fruit density (g ml^{-1})	Seed weight (g)
14BL01	41.92 b*	58.62 b	40.86 de	40.00 de	0.69 f	1.05 cd	0.06 a
14BL02	127.32 a	72.38 a	61.88 ab	86.68 a	0.85 c	1.47 a	0.04 b
14BL03	52.4 b	59.80 b	57.70 abc	53.32 c	0.96 a	0.98 d	0.04 b
14BL04	48.44 b	60.90 ab	50.88 bcd	41.00 de	0.83 c	1.18 c	0.04 b
14BL05	51.56 b	59.84 b	56.54 abc	46.00 d	0.94 ab	1.12 c	0.03 bc
14BL06	64.24 b	68.08 ab	54.40 bc	73.32 bc	0.79 d	0.88 e	0.04 b
14BL07	44.36 b	61.08 ab	47.70 cd	40.00d e	0.78 de	1.11 c	0.02 c

14BL08	32.44 b	40.28 c	33.70 e	30.00 f	0.83 c	1.08 d	0.04 b
14BL09	72.88 b	69.06 ab	66.30 a	76.30 b	0.96 a	0.96 d	0.04 b
14BL10	48.12 b	60.58 b	50.86 bcd	38.33 de	0.83 c	1.26 b	0.04 b

*:There are significant differences ($p<0.05$) among the genotypes having different letters in same column.

Table 2. Continue of Table 1.

Varieties	Fruit stalk length (mm)	Fruit stalk thickness (mm)	Fruit stalk cavity width (mm)	Fruit stalk cavity depth (mm)	Fruit peel thickness (mm)	Core cavity width (mm)	Core cavity length (mm)
14BL01	6.48 e*	1.25 c	8.93 c	3.09 b	0.33 b	11.42 d	9.93 f
14BL02	9.01 bc	2.20 a	14.10 ab	4.58 ab	0.45 b	18.24 bc	17.42 a
14BL03	9.18 abc	1.15 c	13.23 ab	6.26 a	1.57 a	17.79 bc	15.02 bcd
14BL04	10.44 a	1.06 c	13.06 ab	3.86 ab	0.16 b	18.02 bc	13.66 cde
14BL05	6.38 e	1.24 c	12.26 abc	4.46 ab	0.27 b	17.55 bc	16.39 ab
14BL06	6.89 de	1.52 b	12.19 abc	3.98 ab	0.27 b	22.63 a	15.65 abc
14BL07	9.57 ab	1.27 c	15.37 a	3.95 ab	0.14 b	15.59 cd	11.65 ef
14BL08	8.32 bcd	1.22 c	10.57 bc	3.23 b	0.43 b	12.36 d	11.70 ef
14BL09	8.30 bcd	1.19 c	14.14 ab	4.36 ab	0.25 b	19.46 abc	16.83 ab
14BL10	7.71 cde	1.20 c	13.77 ab	3.62 b	0.30 b	20.80 ab	13.32 de

*:There are significant differences ($p<0.05$) among the genotypes having different letters in same column.

Table 3. Continue of Table 1.

Varieties	Seed width (mm)	Seed length (mm)	Seed thickness (mm)	SSC (%)	pH	TA (%)
14BL01	3.70 c*	5.74 b	1.98 ab	12.09 d	4.14 a	1.13 g
14BL02	3.79 bc	6.12 b	2.12 ab	11.10 fg	3.14 e	2.13 d
14BL03	4.24 abc	7.43 a	1.41 b	12.15 c	3.66 d	2.65 b
14BL04	3.83 abc	6.55 ab	2.31 ab	16.13 a	3.87 c	2.20 c
14BL05	4.31 abc	6.47 ab	2.55 a	11.07 g	3.66 d	3.06 a
14BL06	4.05 abc	6.49 ab	2.07 ab	15.16 b	3.63 d	2.16 cd
14BL07	4.58 ab	5.66 b	2.29 ab	11.05 g	4.04 b	1.07 h
14BL08	4.51 abc	6.14 ab	2.20 ab	9.18 h	4.12 a	1.33 f
14BL09	4.67 a	7.42 a	2.25 ab	11.14 ef	3.66 d	2.67 b
14BL10	4.42 abc	6.52 ab	2.40 a	11.16 e	3.18 e	1.67 e

*:There are significant differences ($p<0.05$) among the genotypes having different letters in same column.

Table 4. Sensory analysis on the fruits of apple genotypes.

Varieties	Fruit taste	Juiciness	Fruit pulp color	Fruit peel color
14BL01	Sour	Well	Light yellow	Pink
14BL02	Sweet	Little	Light yellow	Light yellow
14BL03	Tart	Little	Light yellow	Green
14BL04	Sour	Little	Light yellow	Yellow
14BL05	Sour	Little	Light yellow	Pink
14BL06	Sour	Little	Light yellow	Pink
14BL07	Tart	Well	Light yellow	Yellow
14BL08	Sour	Well	Light yellow	Pink
14BL09	Tart	Little	Light yellow	Yellow
14BL10	Sweet	Little	Light yellow	Green

Phenolic compounds: In this paper, it was seen that the apple genotypes investigated had statistically significant differences in terms of gallic acid, catechin, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, phloridzin, protocatechuic, vanillic acid, rutin and quercetin contents ($p \leq 0.05$) (Table 5). In the research, it was seen that the catechin, chlorogenic acid, gallic acid, phloridzin and rutin contents were higher than other phenolic and they were the phenolic compounds dominant on the apple. In terms of gallic acid content, the highest value was determined at 14BL01 genotype as 7.35 mg 100 ml^{-1} and the lowest value was determined at 14BL05 genotype as 1.42 mg 100 ml^{-1}. In terms of catechin content, the lowest value was determined at 14BL08 variety as 5.16 mg 100 ml^{-1} while the highest value was determined at 14BL03 genotype as 10.84 mg 100 ml^{-1}. The highest chlorogenic acid content was determined at 14BL01 genotype as 25.16 mg 100 ml^{-1} and it was seen that it had the highest value among the phenolic acids. In terms of the caffeic acid content of genotypes investigated, it was determined that similar results were obtained at two genotypes. In this regard, the highest value was determined at 14BL01 and 14BL03 genotypes as 1.12 mg l^{-1}. In terms of syringic acid, p-coumaric acid and ferulic acid contents, it was seen that the lowest and highest values varied between 0.12-1.09 mg 100 ml^{-1}, 0.07-0.25 mg 100 ml^{-1} and 0.16-1.50 mg 100 ml^{-1}, respectively (Table 5). In terms of the o-coumaric acid, phloridzin, protocatechuic acid, vanillic acid, rutin and quercetin contents which have antifungal and antimutagen properties, it was seen that the changes varied in the range of 0.34-2.57 mg 100 ml^{-1}, 1.18-6.12 mg 100 ml^{-1}, 0.04-0.11 mg 100 ml^{-1}, 0.04-0.24 mg 100 ml^{-1}, 1.01-5.16 mg 100 ml^{-1} and 0.05-0.16 mg 100 ml^{-1} (Table 6). In a study, Coşkun and Aşkın (2016) stated that the highest caffeic acid content of Kızıl Ahmedi variety was determined as 8.18 mg kg^{-1} and Starking Delicious variety followed this with the value of 6.82 mg kg^{-1}. In terms of local varieties and standart cultivars, it may be said that local varieties had lower caffeic acid content. In terms of local varieties, Uzun Yumra variety had the highest caffeic acid content as 6.71 mg kg^{-1} and Batum variety as 5.91 mg kg^{-1}, Variety 24 as 4.09 mg kg^{-1} and Gelin cultivar as 0.37 mg kg^{-1} followed this, respectively. It was stated that the lowest caffeic acid content was determined Yayla Pınarı cultivar as 2.89 mg/kg (Coşkun and Aşkın, 2016). In their study, Karadeniz and Eksi (2001) reported that chlorogenic acid and epicatechin contents of Amasya cultivar were averagely 258.2 mg l^{-1} and 126.8 mg l^{-1}, the contents of Starking Delicious cultivar were averagely 152.1 mg l^{-1} and 90.8 mg l^{-1}, the contents of Golden Delicious cultivar were averagely 132.4 mg l^{-1} and 40.9 mg l^{-1}, respectively as a result of the distribution of phenolic substances in the apple juice by variety. When the results obtained in the research conducted by Coşkun and Aşkın (2016) were compared with the results of this study, it was seen that both chlorogenic acid and epicatechin contents of Starking Delicious cultivar were low. In a study conducted on seven different apple varieties, it was noted that total phenolic contents of Golden Delicious and Granny Smith apple cultivars were 8.0 and 9.0 mg GAE g^{-1}, respectively (Drogoudi *et al*. 2008). In the researches conducted on the phenolic compound contents of apple fruits, it was seen that there were similar and different results (Ju *et al*. 1996; Escarpa and Gonzalez, 1998; Wolfe and Liu, 2002; Tsao *et al*., 2003; Veberic *et al*., 2005; D'Abrosca *et al*., 2007; Vieira *et al*. 2009; Zhang *et al*., 2010). It is thought that majority of findings obtained in this research showed similarity with the results of these researchers and the differences may be resulted from the genetic factors, climate conditions and cultural practices.

Table 5. Gallic acid, catechin, chlorogenic, caffeic, syringic, *p*-coumaric and ferulic contents of apple genotypes (mg 100 ml^{-1}).

Varieties	Gallic	Catechin	Chlorogenic	Caffeic	Syringic	*p*-Coumaric	Ferulic
$14^{BL}01$	7.35 ± 0.005 ^{a*}	10.26 ± 0.009 ^{b}	25.16 ± 0.010 ^{a}	1.12 ± 0.004 ^{a}	1.09 ± 0.006 ^{a}	0.16 ± 0.003 ^{b}	1.42 ± 0.007 ^{b}
$14^{BL}02$	5.16 ± 0.005 ^{d}	9.83 ± 0.008 ^{c}	19.88 ± 0.012 ^{b}	1.01 ± 0.007 ^{b}	0.98 ± 0.005 ^{c}	0.25 ± 0.008 ^{a}	1.50 ± 0.003 ^{a}
$14^{BL}03$	3.43 ± 0.005 ^{g}	10.84 ± 0.009 ^{a}	15.14 ± 0.008 ^{c}	1.12 ± 0.006 ^{a}	0.97 ± 0.002 ^{c}	0.14 ± 0.006 ^{b}	1.01 ± 0.005 ^{d}
$14^{BL}04$	6.01 ± 0.011 ^{c}	7.14 ± 0.004 ^{h}	9.17 ± 0.004 ^{i}	0.98 ± 0.005 ^{c}	0.45 ± 0.004 ^{f}	0.09 ± 0.004 ^{d}	0.46 ± 0.005 ^{h}
$14^{BL}05$	1.42 ± 0.008 ^{j}	7.56 ± 0.010 ^{g}	12.01 ± 0.010 ^{e}	0.46 ± 0.006 ^{g}	0.12 ± 0.003 ^{i}	0.07 ± 0.004 ^{e}	0.56 ± 0.006 ^{g}
$14^{BL}06$	4.51 ± 0.010 ^{f}	7.99 ± 0.010 ^{f}	10.11 ± 0.010 ^{g}	0.68 ± 0.006 ^{f}	0.19 ± 0.004 ^{h}	0.09 ± 0.006 ^{d}	0.16 ± 0.003 ^{i}
$14^{BL}07$	3.11 ± 0.008 ^{h}	8.15 ± 0.006 ^{e}	11.01 ± 0.008 ^{f}	0.95 ± 0.009 ^{d}	0.26 ± 0.004 ^{g}	0.12 ± 0.004 ^{c}	0.46 ± 0.004 ^{h}

14^{BL}08	2.15 ± 0.005 ^{i}	5.16 ± 0.008 ^{j}	13.02 ± 0.011 ^{d}	1.01 ± 0.010 ^{b}	0.53 ± 0.004 ^{e}	0.10 ± 0.002 ^{d}	0.66 ± 0.005 ^{f}
14^{BL}09	6.16 ± 0.011 ^{b}	9.12 ± 0.009 ^{d}	10.01 ± 0.008 ^{h}	0.76 ± 0.006 ^{e}	1.01 ± 0.008 ^{b}	0.07 ± 0.004 ^{e}	1.12 ± 0.004 ^{c}
14^{BL}10	5.12 ± 0.011 ^{e}	6.17 ± 0.012 ^{i}	7.17 ± 0.009 ^{j}	0.46 ± 0.010 ^{g}	0.67 ± 0.004 ^{d}	0.09 ± 0.005 ^{d}	0.75 ± 0.009 ^{e}

*:There are significant differences ($p<0.05$) among the genotypes having different letters in same column.

Table 6. *o*-Coumaric, phloridizin, protocatechuic, vanillic, rutin and quercetin contents of apple genotypes (mg 100 ml^{-1}).

Varieties	*o*-Coumaric	Phloridizin	Protocatechuic	Vanillic	Rutin	Quercetin
14^{BL}01	2.57 ± 0.006 ^{a}*	5.74 ± 0.006 ^{b}	0.10 ± 0.002 ^{b}	0.24 ± 0.005 ^{a}	4.13 ± 0.006 ^{c}	0.05 ± 0.001 ^{f}
14^{BL}02	1.87 ± 0.004 ^{c}	6.12 ± 0.008 ^{a}	0.04 ± 0.001 ^{f}	0.16 ± 0.001 ^{c}	5.16 ± 0.009 ^{a}	0.13 ± 0.005 ^{b}
14^{BL}03	1.15 ± 0.008 ^{d}	5.17 ± 0.004 ^{c}	0.06 ± 0.003 ^{e}	0.21 ± 0.010 ^{b}	4.16 ± 0.005 ^{b}	0.09 ± 0.004 ^{d}
14^{BL}04	2.11 ± 0.001 ^{b}	3.11 ± 0.010 ^{e}	0.07 ± 0.002 ^{d}	0.09 ± 0.004 ^{d}	1.16 ± 0.005 ^{h}	0.13 ± 0.002 ^{b}
14^{BL}05	0.97 ± 0.006 ^{g}	4.12 ± 0.008 ^{d}	0.11 ± 0.004 ^{a}	0.05 ± 0.001 ^{g}	2.55 ± 0.006 ^{e}	0.07 ± 0.001 ^{e}
14^{BL}06	1.08 ± 0.006 ^{e}	5.13 ± 0.007 ^{c}	0.06 ± 0.004 ^{e}	0.10 ± 0.004 ^{d}	1.01 ± 0.008 ^{j}	0.09 ± 0.002 ^{d}
14^{BL}07	0.99 ± 0.008 ^{f}	4.16 ± 0.006 ^{d}	0.09 ± 0.003 ^{b}	0.06 ± 0.004 ^{f}	2.11 ± 0.006 ^{g}	0.16 ± 0.006 ^{a}
14^{BL}08	0.34 ± 0.004 ^{j}	2.32 ± 0.219 ^{f}	0.06 ± 0.004 ^{e}	0.07 ± 0.003 ^{e}	3.14 ± 0.010 ^{d}	0.05 ± 0.002 ^{g}
14^{BL}09	0.57 ± 0.007 ^{i}	3.12 ± 0.009 ^{e}	0.08 ± 0.005 ^{d}	0.04 ± 0.002 ^{h}	1.12 ± 0.006 ^{i}	0.11 ± 0.004 ^{c}
14^{BL}10	0.68 ± 0.006 ^{h}	1.18 ± 0.004 ^{g}	0.09 ± 0.004 ^{c}	0.07 ± 0.003 ^{e}	2.17 ± 0.007 ^{f}	0.09 ± 0.004 ^{d}

*:There are significant differences ($p<0.05$) among the genotypes having different letters in same column.

Organic acids: The organic acids are the chemicals which have vital importance in the protection of human health as well as that they flavor the fruits. In some studies, it has been understood that the organic acids, especially malic acid, citric acid and tartaric acid, make significant contributions to the human in various aspects such as strengthening the immune system, preventing the renal calculi, eliminating the oral diseases, decreasing the poisoning risks caused by the toxic metals, beautifying and strengthening the skin and decreasing the fibromyalgia symptoms (Abraham and Flechas, 1992; Penniston *et al*., 2007). In this research, it was determined that there were statistically significant differences among the genotypes in terms of organic acid contents ($p<0.05$). In terms of organic acid contents of fruits belonging to apple genotypes investigated, it was seen that the major organic acid was malic acid. Thus, malic acid is known as apple acid. In the relevant research, it was determined that the citric acid and succinic acid following the malic acid were higher compared to other acids. In terms of organic acid contents of apple genotypes, the highest citric acid content was determined at 14BL06 variety as 0.57 mg 100 ml^{-1} and lowest content was determined at 14BL10 variety as 0.15 mg 100 ml^{-1}. The highest tartaric acid content was determined at 14BL07 as (0.37 mg 100 ml^{-1}) and the lowest content was determined at 14BL03 (0.04 mg 100 ml^{-1}). The higher malic acid content which was major in the apple was determined at 14BL01 as 4.62 mg 100 ml^{-1} and the lowest content was determined at 14BL08 as 2.06 mg 100 ml^{-1}. In terms of organic acid contents, it was determined that the succinic acid, oxalic acid and fumaric acid contents varied between 0.17-0.51 mg 100 ml^{-1}, 0.16-0.33 mg 100 ml^{-1}, 0.15-1.12 mg 100 ml^{-1}, respectively (Table 7). In the research, Abacı and Sevindik (2014) determined that the highest malic acid content was determined at Yayla Pınarı apple cultivar (7106.05 mg kg^{-1}) and the lowest content was determined at Kızıl Ahmedi cultivar (1916.50 mg kg^{-1}) in terms of organic acids. Same researchers stated that the oxalic acid content was determined at Kızıl Ahmedi cultivar as 7.95 mg kg^{-1} and at Uzun Yumra variety as 4.70 mg kg^{-1}. In the same study, the highest citric acid content was determined at Yayla Pınarı (55.55 mg kg^{-1}) and lowest content was determined at Starking Delicious (24.10 mg kg^{-1}). It was determined that the tartaric acid content varied between 382.55 mg kg^{-1} (Variety 24) and 84.00 mg kg^{-1} (Yayla Pınarı) (Abacı and Sevindik, 2016). In the studies conducted on apple fruits, malic, malonic and citric acids were determined as a result of organic acid analysis. The malic acid was determined as the organic acid which had the highest content among the organic acids. Most of researchers stated that the organic acid having the highest content in the apple fruit was determined as malic acid (Loue, 1968; Lindsay and Norvell, 1978; Kwang *et al*., 1996; Shyi and Sun 1999; Zhao *et al*., 1995; Lee *et al*., 2000). The malic acid content in Starking variety was determined to be higher than the content in Golden variety. The malonic acid followed the malic acid and it was notified that lowest amount of organic acid was the citric acid (Mordoğan and Ergun, 2001). In the research conducted by Wu *et al*. (2007), it was determined that highest organic acid was malic acid and highest total organic acid content was determined at Granny Smith apple and Ralls apple variety followed this variety. It was stated that similar results were obtained in the studies conducted by different researchers and the malic acid was the dominant organic acid (Hulme and Wooltorton, 1957; Ulrich, 1970; Beruter, 2004; Hecke *et al*., 2006; Petkovsek *et al*., 2007). In this research, it was determined that malic acid had the highest content and fumaric acid, citric acid, succinic acid and tartaric acid followed this, respectively when organic acid contents of fruits belonging to apple varieties investigated. Along with that, it was determined that oxalic acid was the organic acid which had the lowest content. It was determined that the results of organic acid analysis conducted by researchers showed parallelism with majority of findings obtained in this study and there were some differences at some values even a little. It is thought that these differences may be resulted from the differences on genetic structures of apple varieties, ecological factors and differences on analysis technique.

Table 7. Organic acid contents of apple genotypes (mg 100 ml^{-1}).

Varieties	Citric	Tartaric	Malic	Succinic	Oxalic	Fumaric
14BL01	0.44 ± 0.004 c*	0.12 ± 0.002 d	4.62 ± 0.014 a	0.51 ± 0.005 a	0.29 ± 0.004 b	1.12 ± 0.001 a
14BL02	0.52 ± 0.004 b	0.09 ± 0.005 e	3.69 ± 0.005 b	0.43 ± 0.005 b	0.22 ± 0.005 c	0.76 ± 0.006 b
14BL03	0.33 ± 0.006 f	0.04 ± 0.006 g	3.65 ± 0.012 bc	0.36 ± 0.008 c	0.16 ± 0.006 d	0.45 ± 0.008 c
14BL04	0.17 ± 0.004 i	0.11 ± 0.004 d	2.98 ± 0.006 d	0.43 ± 0.008 b	0.21 ± 0.010 c	0.35 ± 0.008 d
14BL05	0.42 ± 0.011 d	0.12 ± 0.006 d	2.56 ± 0.008 f	0.22 ± 0.004 e	0.16 ± 0.005 d	0.15 ± 0.006 g
14BL06	0.57 ± 0.008 a	0.34 ± 0.009 b	3.61 ± 0.064 c	0.18 ± 0.004 g	0.21 ± 0.004 c	0.35 ± 0.010 d
14BL07	0.25 ± 0.008 h	0.37 ± 0.000 a	2.59 ± 0.002 f	0.21 ± 0.006 f	0.33 ± 0.005 a	0.17 ± 0.010 f
14BL08	0.36 ± 0.006 e	0.28 ± 0.008 c	2.06 ± 0.012 g	0.28 ± 0.005 d	0.16 ± 0.008 d	0.22 ± 0.013 e
14BL09	0.30 ± 0.011 g	0.06 ± 0.001 f	3.66 ± 0.013 bc	0.17 ± 0.004 g	0.21 ± 0.006 c	0.17 ± 0.003 f
14BL10	0.15 ± 0.005 j	0.09 ± 0.004 e	2.76 ± 0.008 e	0.22 ± 0.004 e	0.17 ± 0.005 d	0.22 ± 0.006 e

*:There are significant differences ($p<0.05$) among the genotypes having different letters in same column.

Conclusion: In this research, the horticultural and bioactive content of fruits belonging to apple genotypes grown in the province of Bolu were investigated. This study is the first research to date on these apple genotypes. The territory of Bolu has a climate structure at which various fruits may be grown conveniently. It has affected having a different climate structure that the province of Bolu is located at the transition route between Black Sea and Marmara Region. In terms of fruit growing, this difference may be used in the dissemination of alternative fruit cultivation with various product patterns. The apple among the fruit types with soft seed has grown in this territory densely and it has a significant position in the agricultural production of region. Along with that, these lead to loss of value on the market that this fruit is not quoted over a good price in the harvesting period and there are lack of knowledge and opportunity about different evaluation areas. Therefore, the chemical compositions of local apple genotypes grown in the territory were revealed with this research and the compounds which are important in terms of evaluating the relevant cultivars in different areas were determined. The phenolic compounds taking place among these compounds are in short supply in the fruits and vegetables but they are very significant as they lead to various problems in processing these products (especially in the fruit juice industry). They are effective in flavoring the products and especially in the formation of a sour taste in the mouth. The organic acids are effective on the taste depending on the balance of acid-sugar. In terms of the pomological properties of apple varieties investigated in this research; it was determined that 14BL02, 14BL06 and 14BL009 genotypes are hopeful in terms of fruit weight and sizes especially which affect the attractiveness of fruit. In terms of phenolic compounds, it was seen that 14BL01, 14BL02, 14BL03 and 14BL07 genotypes come to forefront. In terms of organic acid contents of apple genotypes investigated, it was determined that 14BL01 and 14BL06 genotypes are hopeful. In the light of findings obtained in this research, it is thought that relevant hopeful varieties have the potential to be candidate for developing new industrial cultivars for future studies. Conducting studies which will reveal the relation of phenolic compounds and organic acids with genes and developing new cultivars are significant in terms of protecting gene resources of our country and we hope that this research will create resource for these kinds of studies.

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