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Some Physical and Chemical Characteristics of Gilaburu (*Viburnum opulus* L.) Fruits in Erzincan Region

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ABSTRACT: This study was conducted in order to identify some physical and chemical characteristics of fruits and seeds in the genotypes of Gilaburu (*Viburnum opulus* L.) naturally grown in Erzincan region. In the study measurements were conducted regarding the weight, height, width, total soluble solid (TSS) content, pH, and titratable acidity (TA) content of the fruits as well as some physical characteristics of the seeds. The contents of organic acids (tartaric acid, malic acid, succinic acid, fumaric acid and acetic acid) and phenolic compounds (gallic acid, catechin, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, protocatechuic acid, vanillic acid, rutin and quercetin) in Gilaburu fruits were analyzed by HPLC. The findings of the study revealed that in the fruits, the content of tartaric acid (1.41 g kg^{-1}-1.24 g kg^{-1}) was higher than those of other organic acids while the content of catechin (284.96 mg kg^{-1}- 352.04 mg kg^{-1}) was higher than those of other phenolic compounds. In addition, mineral elements (K, Ca, Mg, Fe, Mn, Zn and Cu), sugar and vitamin C were determined in fruit samples.

Keywords: Gilâburu (*Viburnum opulus* L.), organic acids, phenolic compounds, Erzincan-Turkey

Erzincan Yöresinde Yetişen Gilaburuların (*Viburnum opulus* L.) Bazı Fiziksel ve Kimyasal Özellikleri

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ÖZET: Bu çalışma Erzincan yöresinde doğal olarak yetişen Gilaburu (*Viburnum opulus* L.) genotiplerinin çekirdek ve meyvelerinin bazı fiziksel ve kimyasal özelliklerini belirlemek amacıyla yapılmıştır.Çalışmada meyvelerin ağırlığı, eni, boyu, pH, toplam kuru madde ve asit içeriklerinin yanı sıra çekirdeklerin bazı fiziksel özellikleri belirlenmiştir. Meyvelerin organik asit (tartarik asit, malik asit, sukkinik asit, fumarik asit and asetik asit) ve fenolik madde (gallik asit, kateşin, caffeic asit, syringik asit, p-kumarik asit, ferulik asit, o-kumarik asit, protokateşik asit, vanillik asit, rutin and kuersetin) içerikleri HPLC ile ölçülmüştür.Meyvelerin tartarik asit içeriği (1.41 g kg^{-1}-1.24 g kg^{-1}) diğer organik asitlerden, kateşin içeriği ise (284.96 mg kg^{-1}- 352.04 mg kg^{-1}) diğer fenolik bileşiklerden daha yüksek bulunmuştur.Ayrıca meyvelerin mineral madde (K, Ca, Mg, Fe, Mn, Zn and Cu), şeker ve vitamin C içerikleri de belirlenmiştir.

Anahtar kelimeler: Gilâburu (*Viburnum opulus* L.), organik asitler, fenolik bileşikler, Erzincan-Türkiye

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INTRODUCTION

Viburnum opulus L. fruit, which is known as "Gilaburu" in Turkey and "Guelder rose", "Cramp bark" and "European Cranberrybush" in Europe belongs to Caprifoliaceae family (Andreeva et al., 2004; Çam and Hışıl, 2007). The genus Viburnum (Caprifoliaceae) is composed of more than 230 species distributed from South America to Southeast Asia (Lobstein et al., 1999). The homeland of Viburnum opulus L. is known as Europe, Northwest Africa, Turkistan and Canada (Davis, 1972; Richard and Pierre, 1992). This fruit species is widely grown particularly in Kayseri and Erzincan regions in Turkey and serves to a variety of purposes (Soylak et al., 2002; Çam and Hışıl, 2007). In Central Anatolia region, the traditional drink gilaburu is obtained from Viburnum opulus fruits. Gilaburu is a fast growing, bushy shrub, to 2-4 meters, the fruits of which are utilized as dried fruits, pickle and jam. Gilaburu fruits are traditionally used in the treatment of kidney problems and kidney stones. Additionally, it has sedative effects, acts as a vasodilator and an effective antispasmodic that helps to relieve muscle cramps and spasms (Anonymous, 2010).

In this century with a rapidly increasing population, different fruit species need to be grown and their uses need to be promoted. In order to serve this purpose, studies have been conducted on this fruit species in our country. In the studies, the contents of L-malic acid, L-ascorbic acid and oxalic acid in fresh gilaburu juice were determined respectively as 863.73 mg 100 ml^{-1}, 35.20 mg 100 ml^{-1} and 57.55 mg 100 ml^{-1} (Çam and Hışıl, 2007). Sönmez et al. (2007) have identified some physical characteristics of the seeds of gilaburu fruit. In the study, the height of the seeds was determined as 6.56-8.44 mm, their width as 6.24- 8.57 mm, thickness as 1.62-2.52 mm and geometric mean as 4.19- 5.31 mm. Organic acids are the compounds in plants generated by the metabolic processes such as tricarboxylic acid and shikimic acid metabolisms and stored in vacuoles. While fruits generally contain malic acid and citric acid, grapes contain tartaric acid. The primary acid found in many fruits is either citric or malic acid. Secondary acids are phenolic acids. Besides, there are also few amounts of other organic acids in fruits such as succinic acid, oxalic acid and salicylic acid (Cemeroğlu et al., 2001). Phenolic compounds are the secondary metabolites formed in subsidiary compounds that are synthesized during aromatic amino acid metabolism.

The consumption and growth of gilaburu has recently been increasing along with a growing awareness about its beneficial effects on human health. There are only a limited number of studies about this fruit species. On the basis of this lack of studies, this study aimed at the identification of some physical and chemical characteristics of gilaburu genotypes naturally grown in Erzincan region. The genotypes with superior characteristics were identified via selection and the physical and chemical characteristics of the associated fruits were analyzed. The organic acids and phenolic compounds identified in the fruits in this study are important substances in both human health and fruit juice processing industry. Hence, with this study, the physical and chemical characteristics of gilaburu were identified and an effort has been made to classify this fruit among the other fruit species as well as to determine its nutrients.

MATERIAL AND METHODS

This study was conducted in four districts of Erzincan region with high gilaburu potential. The study areas are Konakbaşı and Kılıçkaya villages. The fruits of these local gilaburu genotypes were firstly cleaned and sampled. Subsequently, the fruit samples were stored at -80 °C until the analyses.

Identification of Physical Characteristics of the Fruits: Four gilaburu genotypes were tested and examined in the study. In order to identify pomological characteristics of these genotypes, 10 fruits were randomly selected from each genotype and fruit weight, seed weight (with a scale reading to 0.1 g), fruit height, fruit width, shell thickness, seed height, number of fruits per bunch, fruit color, fruit juice color, seed width, seed thickness (with a compass reading to 0.01 mm), content of TSS (with a hand refractometer) and TA (by titration method) were identified. Fruit flesh and skin colors were identified by observation and comparison. Subsequently, pH was determined in fruit juice (by pH meter).

Identification of Organic Acids: The standards (tartaric acid, malic acid, succinic acid, fumaric acid and acetic acid) used in organic acid analyses were obtained from Sigma company (St. Louis, MO, USA) and H2SO4 with chromatic purity was obtained from Merck company (Darmstadt, Germany). Milli-Q pure water (Bedford, MA, USA) was used in the preparation of standards and samples. About 50 g samples were smashed from gilaburu samples and 7 g from each sample was weighed into centrifuge tubes. Organic acids were extracted according to a modification of the method of Bevilacqua and Califano (1989). 25 ml of 0.009 N H_2SO_4 was added into the samples and samples were homogenized. Subsequently, they were blended in mixer for 1 h and centrifuged at 7000 g for 5 min. Af-

ter separated from the solid part via centrifugation, the liquid part was first filtrated through a raw filter paper and then filtrated twice through a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA).

Organic acids were subjected to analysis by HPLC equipment (Agilent HPLC 1100 series G 1322 A, Germany) according to the method of Bevilacqua and Califano (1989). Aminex HPX - 87 H, 300 mm x 7.8 mm column (Bio-Rad Laboratories, Richmond, CA, ABD), was used in HPLC system and the equipment was controlled by Agilent software running on a personal computer. The detectors were adjusted at the wavelengths of 214 and 280 nm. 0.009 N H_2SO_4 filtrated through 0.45 µm membrane filter was used as the mobile phase.

Analysis of Phenolic Compounds: The phenolic compounds were separated by HPLC according to the method of Rodriguez-Delgado et al. (2001). Chromatographic separation was performed by Agilent 1100 (Agilent, USA) HPLC system using a DAD detector (Agilent, USA) and 250x4.6 mm, 4µm ODS column (HiChrom, UK). Solvent A Methanol-acetic acid-water (10:2:88) and solvent B Methanol-acetic acid-water (90:2:8) were used as mobile phase and gradient elution program presented in Table 1 was employed. Separation was performed at 254 and 280 nm and flow rate and injection volume were determined respectively as 1 mL min^{-1} and 20 mL min^{-1}.

Analysis of Sugars: The samples were prepared according to the method described by Melgarejo et al. (2000) with minor modifications; briefly, the sample of 5 g fruit was centrifuged at 12.000 rpm for 2 minutes at 4 ºC. Then the supernatant was filtrated with SEP-PAK C18 cartridges and transferred into a vial to used for analyze. Analysis of sugars was performed by HPLC with µbondapak-NH_2 column and refractive index (RI) detector using 85% acetonitril as a mobile phase. The calculation of concentrations was based on standards prepared in the laboratory.

Analysis of Vitamin C: Ascorbic acid content was determined following the modified HPLC (Agilent 1100 series HPLC G 1322 A, Germany) analytical procedure outlined by Cemeroğlu et al. (2007). The 5 g of sample was transferred to a 50 mL volumetric flask including 10 mL 6% (W/V) metaphosphoric acid (Sigma, M6285, %33.5). The sample was then homogenized at 24 000 rpm for 15 second, and centrifuged at 14 000 rpm for 10 min at 1ºC. 5 ml of the supernatant was filtered through 0.45µm PTFE syringe filters (Phenomenex, UK) and placed in an amber colored vial (AIM, Screw vial, SV-15A). Quantification of ascorbic acid was made by an external standard method using an L-ascorbic acid Standard (Sigma A5960). Samples were separated on a Luna C18 column (250 x 4:60 mm, 5 µ from Phenomenex) at 25 ºC by an HPLC. The mobile phase was 25 mM KH2PO4 (adjusted to pH 2.2 with phosphoric acid) with a flow rate of 1 ml min^{-1}. L-ascorbic acid was detected at 254 nm.

Analysis of Mineral Elements: Fruit samples were dried at 65 °C until the constant weight after washing with distilled water. The samples have been prepared for analysis by grinding. Total Phosphorus content was measured using spectrophotometer. Total mineral contents (K, Ca, Mg, Fe, Mn, Zn and Cu) of the samples were also determined using Atomic Absorption Spectrophotometer (Kacar, 1984).

RESULTS AND DISCUSSION

The following findings were obtained in the study conducted in Konakbaşı and Kılıçkaya villages: The number of fruits per bunch ranged between 29 and 71; fruit weight ranged between 0.765 g and 0.768 g, fruit width ranged between 1.02 mm and 1.03 mm; fruit height ranged between 1.04 mm and 1.08 mm; shell thickness ranged between 0.013 mm and 0.014 mm; TSS content ranged between 12% and 13.4%; pH ranged between 3.47 and 3.50; seed weight ranged between 0.104 g and 0.112 g; seed height ranged between 0.83 mm and 0.91 mm; seed width ranged between 0.71 mm and 0.82 mm; seed thickness ranged between 0.21 mm and 0.23; TA content ranged between 12.2% and 13.1%. Besides, colors of fruit flesh and skin varied dark red (Table 2).

Table 1. HPLC program

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
15	85	15
25	50	50
35	15	85
45	0	100

Table 2. Pomological traits of gilaburu fruits

Genotypes	NFPB (groove)	FW (g)	FWI (mm)	FH (mm)	CFF	CFS	ST (mm)	TSS (%)	pH	SW (g)	SH (mm)	SWI (mm)	ST (mm)	TA (%)
KNB 02	71	0.768	1.03	1.04	Dark red	Dark red	0.013	12.0	3.47	0.112	0.91	0.82	0.21	12.2
KLÇ 02	29	0.765	1.02	1.08	Dark red	Dark red	0.014	13.4	3.50	0.104	0.83	0.71	0.23	13.1

NFPB: number of fruits per bunch, FW: fruit weight, FWI: fruit width, FH:fruit height, CFF: Color of fruit, CFS: Color of fruit skin, ST: shell thickness, SW: seed weight, SH: seed height, SWI: seed width, ST: seed thickness, TA: titratable acidity.

Table 3. Organic acids content of gilaburu fruits

Genotypes	Tartaric acid (g kg^{-1})	Malic acid (g kg^{-1})	Succinic acid (g kg^{-1})	Fumaric acid (mg kg^{-1})	Acetic acid (g kg^{-1})
KNB 02	1.41	1.37	0.052	0.15	0.026
KLÇ 02	1.24	1.21	0.046	0.16	0.032

Table 4. Phenolic compounds content of gilaburu fruits (mg kg^{-1})

Genotypes	Gallic acid	Catechin	Chlorogenic acid	Caffeic acid	Syringic acid	P-coumaric acid	Ferulic acid	O-coumaric acid	Protocatechuic acid	Vanilic acid	Rutin	Quercetin
KNB 02	108.29	284.96	29.51	26.26	30.29	0.104	55.90	13.91	20.93	22.49	17.81	6.11
KLÇ 02	118.17	352.04	44.33	38.35	24.70	0.117	44.98	17.16	36.27	22.10	20.02	8.32

Table 5. Nutrient content of gilaburu fruits

Genotypes	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)
KNB 02	1663.62	2970	1856	1340	2.9	1.6	1.7	0.6
KLÇ 02	1300.14	2680	1752	1190	2.1	1.7	1.5	0.5

Table 6. Sugar and vitamin C ccontent of gilaburu fruits

Genotypes	Glikoz (g $100g^{-1}$)	Fruktoz (g $100g^{-1}$)	Sakaroz (g $100g^{-1}$)	Vitamin C (mg $100g^{-1}$)
KNB 02	2.346	1.675	0.064	33.432
KLÇ 02	2.421	1.597	0.069	32.761

In the studies of researchers on the determination of pomological characteristics of gilaburu fruits, the number of fruits per bunch was identified as around 75.25; fruit weight as 0.7-0.86 g; fruit width as 8.0-11.45 mm; fruit height as 11.83 mm; pH as 3.24-3.9 and content of TSS as 7.81-14.37 % (Karadeniz et al., 2003; Bolat and Özcan, 1995; Kollmann and Grubb, 2002).

In this study conducted in Erzincan region, after the identification of physical characteristics of the two superior locally grown gilaburu genotypes, the most superior two genotypes (KNB 02 and KLÇ 02) were further selected among these best genotypes and their contents of organic acids and phenolic compounds were identified. In the study, the content of organic acids namely, tartaric acid, malic acid, succinic acid, fumaric acid and acetic acid as well as the content of phenolic compounds namely, gallic acid, catechin, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, o-coumaric acid, protocatechuic acid, vanillic acid, rutin and quercetin in Gilaburu fruits were identified.

Tartaric acid content was identified to range between 1.24-1.41 g kg^{-1}; malic acid content to range between 1.21-1.37 g kg^{-1}; succinic acid content to range between 0.046-0.052 g kg^{-1}; fumaric acid content to range between 0.15-0.16 mg $kg^{-1}$and acetic acid content to range between 0.026-0.032 g kg^{-1} (Table 3). Considering the content of organic acids in fruits, the most abundant component was malic acid while acetic acid was the least abundant. In the study of Çam and Hışıl (2007) on gilaburu juices, L-ascorbic acid content was identified as 35.20 mg 100 ml^{-1}; L-malic acid content as 863.73 mg 100 $ml^{-1}$and oxalic acid content as 57.55 mg 100 ml^{-1}. In a similar study by Altun and Yilmaz (2007), salicylic acid content in Viburnum opulus fruits was identified as 1.26% and chlorogenic acid content was identified as 1.24%.

The findings of our study conducted in Erzincan region are in agreement with the findings of similar studies on this topic. Regarding the content of phenolic compounds in gilaburu fruits, gallic acid was identified to range between 108.29- 118.17 mg kg^{-1}; catechin content to range between 284.96-352.04 mg kg^{-1}; chlorogenic acid content to range between 29.51-44.33 mg kg^{-1}; caffeic acid content to range between 26.26-38.35 mg kg^{-1}; syringic acid content to range between 30.29-24.70 mg kg^{-1}; p-coumaric acid content to range between 0.104-0.117 mg kg^{-1}; ferulic acid content to range between 44.98-55.90 mg kg^{-1}; o-coumaric acid content to range between 13.91-17.16 mg kg^{-1}; protocatechuic acid content to range between 20.93-36.27 mg kg^{-1}; vanillic acid content to range between 22.10-22.49 mg kg^{-1}; rutin content to range between 17.81-20.02 mg kg-1and quercetin content to range between 6.11-8.32 mg kg^{-1}. Catechin and p-coumaric acid were the most and least abundant components among all phenolic compounds, respectively (Table 4). In the Ph.D. study of Çam (2005) regarding the identification of organic acid and phenolic compound contents of gilaburu juices in Kayseri region, L-malic acid content was calculated as 9.422 g L^{-1}, oxalic acid content as 0.573 g L^{-1}, tartaric acid content as 0.095 g L^{-1} and L-ascorbic acid content as 0.736 g L^{-1}. With respect to phenolic compounds, the same researcher calculated chlorogenic acid content as 798.81 mg L^{-1}, caffeic acid content as 26.22 mg L^{-1} and p-coumaric acid content as 3.38 mg L^{-1}. In addition, mineral elements (Table 5), sugar and vitamin C (Table 6) in samples of fruit were determined and found to be compatible with previous studies (Bolat and Özcan, 1995; Çam and Hışıl, 2007).

Considering the chemical compounds of fruits belonging to gilaburu genotypes in Erzincan region, it is concluded that the fruits have rather abundant amounts of organic acids and phenolic compounds. The cultivation and widespread growth of these genotypes may enable the consumption of gilaburu as an alternative fruit species and its utilization as a considerable raw material in fruit juice processing industry. This will contribute to the local economy as well as to the appraisal of the significance of the fruit in human nutrition.

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