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A Study on The Determination of Total Phenol, DPPH, TSS, Volatile Compounds in Gala Gio Apple Varieties

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Abstract. Apples belong to the Rosaceae family and have been a popular fruit for consumers since ancient times due to their biochemical properties. The Gala series apple varieties are widely grown worldwide and are among the most popular on supermarket shelves. In recent years, the Gala Gio apple variety has been introduced. It is an early-ripening apple with dark red skin, attractive ap-pearance, and high commercial value. As with all fruits, total phenols, antioxidants, and soluble solids are important ripening criteria for apple varieties. In this study, total phenols, antioxidants, and soluble solids were analyzed in the fruits of the Gala Gio apple variety. The study found that the total phenols in Gala Gio were 30.72 mg/100 g GAE, the antioxidants were 35.24%, and the soluble solid contents were 13.8%. Additionally, a total of 26 volatile aroma compounds were identified in the study, including 1 aldehyde, 10 alcohols, 14 esters, and 1 ketone. Among the volatile compounds, alcohols and esters were identified as the predominant compounds. Among the ester group, butanoic acid, 3-methyl-, 3-methylbutyl ester (27.44%), and isoamyl butyrate (25.20%) were the most commonly detected compounds, while within the alcohol group, 1-buta-nol, 3-methyl-, acetate (8.26%) was the most commonly detected compound.

Keywords: apple, aroma, total phenol, antioxidant, soluble solid contents

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1 Introduction

Apple (Malus communis L.) ranks among the top fruit types cultivated worldwide and, in our country, [1]. Apples, which are among the temperate climate fruit species, belong to the Malus genus within the Rosaceae family of the Rosales order. They are a fruit that has been widely preferred by consumers since ancient times due to their biochemical properties. Türkiye is a center of origin for many agricultural products, including fruit cultivation, and possesses a rich variety of fruit species and varieties. The apple has spread to different centers of origin, such as Anatolia, Europe, China, the Himalayas, Japan, Korea, and North America, and 48 varieties are reported to exist. Furthermore, apples contain rich antioxidant components, carbohydrates, essential minerals, and dietary fiber, and are important in terms of taste and nutritional content [2; 1; 3]. However, apples are the second most widely produced pome fruit in the world after bananas. Apples, which are grown in almost every region of Türkiye, can be consumed fresh or processed into various products such as jam, marmalade, fruit juice, wine, and vinegar [4]. Apples are a fruit type that has been cultivated in our country for many years and is one of the leading temperate climate fruits in terms of production and area. According to FAO 2023 data, global apple production totals 97.339.338.8 tons. China ranks first in apple production with 49.601.700

tons, followed by the United States with 5.151.680 tons and Türkiye with 4.602.517 tons.

The provinces that play significant roles incase apple production in Isparta, Karaman, and Niğde. As is well known, both the fresh and dried fruits and leaves of apples are used for many purposes. For example, they are used in vinegar production, jelly production, cakes, pies, cookies, salads, beverages, desserts, main dishes, breakfast foods, and cookie production. Apples are a fruit frequently preferred by conscious consumers due to their delicious taste and the beneficial biochemical content they contain for human health. Apples provide vitamins A and C, are rich in carbohydrates, and are an excellent source of dietary fiber. Consequently, apples also have positive effects on human health due to their anticarcinogenic, antimutagenic, and antimicrobial activity. There is significant evidence that increased apple consumption contributes to improved health by reducing the risk of diseases such as cardiovascular disease and certain types of cancer. As is well known, phenolic compounds are low molecular weight secondary metabolites that are found in high concentrations in fruits and vegetables and exhibit higher antioxidant effects than other bioactive compounds. In addition, many researchers have determined that they have positive effects on human health [5; 6]. It has been reported that phenolic compounds inhibit cancer cells, possess antimutagenic activity and blood pressure-lowering effects, and reduce cardiovascular risk [7; 8; 9]. In one study, the phenolic composition of 67 different apple varieties (new and old varieties) was examined in terms of the concentration of some important phytochemicals and antioxidant activities. For the first time, polyphenolic compounds in Golden Delicious and new varieties derived from it were compared and their correlations evaluated. The total polyphenol content varied between 523.02 and 2723.96 mg per 100 grams of dry matter, depending on the apple variety. The highest correlation was found between total polyphenols and the ABTS method, while the correlation with the FRAP and DPPH methods was lower [10]. This study aimed to determine the soluble solid content, total phenols and antioxidants, and aroma components in Gala Gio apple fruits.

2 Material and Method

2.1 Material

This study used the Gala Gio apple variety as material. The Gala Gio apple variety is characterized by high coloration in fruit color, early ripening, creamy yellow flesh color, firm, juicy, and sweet texture, high sugar content, and medium-sized fruit. Due to its early ripening, it is harvested on average in late July and the first half of August. It is a Gala variety with high commercial value. Fruit samples were obtained during the ripening period from an application and research orchard located in the apple plot of Çukurova University. The trees were grafted onto M9 rootstock, trained in a spindle system, 5 years old, and planted at a distance of 4 x 2.5 m between rows and within rows. The fruit samples were labeled and immediately transported in a cold chain to the Chromatography Laboratory of the Department of Horticulture, Faculty of Agriculture, Çukurova University, for analysis.

Method

2.2 Measurement of The Amount of Soluble Solid Content.

It was calculated as (%) in the fruit juice collected from 3 randomly selected fruits from the fruit samples using a hand Refractometer.

2.3 Determination of Total Phenol.

Following homogenization and weighing of the samples, the total phenolic content of the fruit juice was quantified by a colorimetric method based on the Folin–Ciocalteu reagent, as described by Spanos [11]. Absorbance was measured at 760 nm using a Multiskan™ GO spectrophotometer, and concentrations were calculated from a standard calibration curve generated with gallic acid. The results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight (mg GAE/100 g).

2.4 Determination of Total Antioxidant Capacity.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity was evaluated according to the method of Brand-Williams [12] with minor adjustments. A 0.06 µM DPPH solution was freshly prepared in ethanol prior to analysis. For the assay, 50 µL of the sample was mixed with 1950 µL of the DPPH solution. The mixture was vortexed for 1 min and then allowed to stand in the dark at ambient temperature for 30 min. Absorbance was measured at 515 nm against a reagent blank. The percentage of DPPH radical inhibition was calculated using the following formula:

$$\%\text{Inhibition} = 100 \times [(\text{Abs blank } (t = 30)) - (\text{Abs sample})]/[(\text{Abs blank } (t = 30))]$$

where Abs sample is the absorbance of the reaction in the presence of the sample (sample dilution + DPPH solution), Abs blank is the absorbance of the blank for each sample dilution (sample dilution + DPPH solvent), and Abs control is the absorbance of the control reaction (sample solvent + DPPH solution).

2.5 Volatile compounds were extracted by solid-phase microextraction (SPME).

Volatile constituents were isolated from apple pulp, with approximately three randomly selected fruits used per replicate. From each homogenized sample, 1 g was transferred into a headspace vial, followed by the immediate addition of 1 mL $CaCl_2$ solution. The vial was incubated at 40 °C for 30 min to allow equilibration. Volatile compounds were then extracted using a grey SPME fiber (DVB/CAR/PDMS). The absorbed aroma compounds were subsequently desorbed and identified by gas chromatography–mass spectrometry using a Shimadzu GC-2010 Plus system [13].

3 Results and Discussion

In this study, according to the analyses performed on Gala Gio variety fruits, total phenols were determined to be 30.72 ± 0.76 mg/GAE 100 g, DPPH % 35.24 ± 2.00, and soluble solid content % 13.83 ± 0.06. In a study conducted by Bozbuğa and Pırlak [14] under Niğde ecological conditions, the soluble solid content was found to be lowest in the Granny Smith variety at 12.2% and highest in the Fuji variety at 16.5%. In a study conducted in the Battalgazi district of Malatya province to determine the fruit quality performance of certain apple varieties under plain conditions, the SÇKM value of apple varieties was found to be 12.60% (Granny Smith) - 16.83% (Fuji), the pH value was 3.41 (Fuji) - 4.48 (Pink Lady), total phenolic content 338 mg 100 g GAE^{-1} (Pink Lady) - 854 mg 100g GAE^{-1} (Golden Delicious), and total antioxidant content %46 (Golden Delicious) - %67 (Granny Smith). Özden and Özden [15], in their study on different fruit types, determined the total phenolic content in apples to be 698.67 mg/GAE/kg in the Granny Smith variety and 810.48 mg/GAE/kg in the Gala variety; and antioxidant activity in the same varieties at 45.66% and 61.79%, respectively. In a study conducted under Ardahan's ecological conditions, the phenolic compound content and antioxidant activity in the fruit peel of apple varieties were 209.7–578. 9 mg/100 g and 30.5–73.4%, while in the fruit flesh, they were determined to be 46.9–112.2 mg/100 g and 21.7–57.8% [9]. Yıldırım et al. [16] determined the total phenolic content and antioxidant activity in the fruit flesh to be 411.46 mg/100 GAE (G. Delicious), 471. 8 mg/100 g GAE (Pink Lady), 417.7 mg/100 g GAE (Granny Smith), and 47.06% (Golden Delicious), 32.52% (Pink Lady), and 49.54% (Granny Smith), respectively. The results obtained in this study differed from those reported in the literature. DPPH and total phenol contents were found to be lower than those reported in the literature. However, the amount of soluble solid content was found to be consistent with the literature data. The difference in total phenol and DPPH values is thought to be significantly influenced by variety, ecological and cultivation conditions, rootstock, pruning, and training systems. The study identified a total of 26 volatile aroma compounds in the Gala Gio variety, including 1 aldehyde, 10 alcohols, 14 esters, and 1 ketone (Table 1).

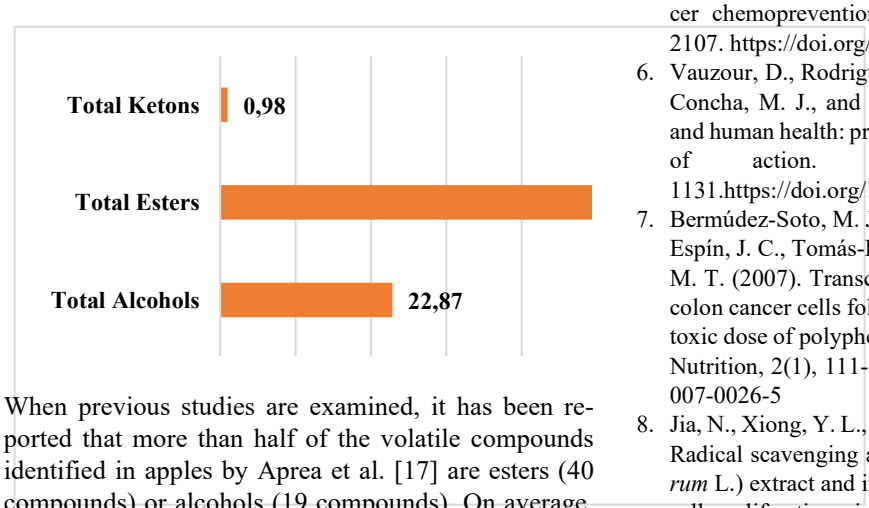
Table 1. Volatile Compounds of apple with SPME grey fiber (DVB/CAR/PDMS)

Retention time	Compound name	%
	Aldehydes	
Kas.19	(E)-2-Hexenal	0.56
	Total aldehydes	0.56
	Alcohols	
7.025	1-Butanol 3-methyl-	Nis.32
11.282	1-Butanol 3-methyl-acetate	Ağu.26
11.513	1-Hexanol	0.76
19.117	3-Pentanol, 2,4-dimethyl	0.45
10.516	2-Pentanol, acetate	Oca.38
11.042	1-Hexanol	Oca.84
15.488	2-Heptanol, acetate	2.Haz
15.985	Oct-3(Z)-enol	0.42
18.89	4,4-Dimethyl-3-hexanol	Oca.26
21.801	Phenol, 2-methoxy-4-(2-propenyl)	Oca.58
	Total alcohols	22.87
	Esters	
8.269	Acetic acid, butyl ester	Oca.28
13.59	Butanoic acid, 2-methylpropyl ester	4.May
15.138	Butanoic acid, 1-methylbutyl ester	0.61
16.812	Butanoic acid, 3-methyl-, 3-methylbutyl ester	27.44
18.783	(Z)-Butanoic acid, 4-hexenyl ester	0.56
18.957	Butanoic acid, 1-methylhexyl ester	0.43
19.667	Octanoic acid, 3-methylbutyl ester	Oca.25
19.735	Butanoic acid, 4-hexen-1-yl ester	Mar.39
22.089	Butanoic acid, 3-methylbutyl ester	Oca.23
14.746	Isoamyl Isobutyrate	May.82
14.881	Isobutyrate <isoamyl->	Oca.64
15.73	Isoamylbutyrate	25.Şub
16.715	Isoamyl-2-Methyl Butyrate	Oca.56
19.511	Butanoate hexyl-, 3-methyl-	Oca.13
	Total esters	75.59

	Ketons	
12.085	2-Heptanone	0.98
	Total ketons	0.98

Among these compounds, the alcohol and ester groups were particularly dominant. The distribution of volatile compounds was determined as follows: aldehydes 0.56%, alcohols 22.87%, esters 75.59%, and ketones 0.98%. Among the esters, butanoic acid, 3-methyl-, 3-methylbutyl ester (27.44%), isoamyl butyrate (25.20%), and isoamyl isobutyrate (5.82%) were detected at the highest levels. Within the alcohol group, the compounds 1-Butanol, 3-methyl-, acetate (8.26%), 1-Butanol, 3-methyl (4.32%), and 2-Heptanol, acetate (2.60%) were determined (Figure 1).

Fig. 1. Total volatile compounds (%) of apple with SPME grey fiber (DVB/CAR/PDMS)



When previous studies are examined, it has been reported that more than half of the volatile compounds identified in apples by Aprea et al. [17] are esters (40 compounds) or alcohols (19 compounds). On average, the most abundant compounds were identified as hexyl acetate (24.4%), hexanol (23.0%), butyl acetate (15.4%), 3-methyl butyl acetate (13.5%), butyl alcohol (4.3%), and 2-methyl-1-butanol (3.4%).

4 Conclusion

The Gala Gio apple variety is ecologically suitable for apple cultivation in the Adana Çukurova region, particularly on M9 dwarf rootstocks using the spindle training system, due to the appealing color of the fruit, its early ripening, and the high amount of soluble solid content, aroma, and the favorable market demand. It has been determined that this apple variety is profitable and preferable for producers when grown in ecologically suitable regions for apple cultivation in Adana Çukurova, particularly when cultivated on M9 dwarf rootstocks using the spindle training system. This study will shed light on more comprehensive studies involving other Gala apple series and other apple varieties.

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