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RESEARCH PAPER

HORTICULTURAL STUDIES

Alteration of Antioxidant Activity and Total Phenolic Content during the Eight-Week Fermentation of Apple Cider Vinegar

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Abstract

Apple is one of the delicious fruit consumed by people. Apple cider vinegar was made through the traditional method and the changes occurred during the 8 week fermentation period were determined in this research. Total titratable acidity, pH, total soluble solids (°brix), total phenolic contents, Oxygen Radical Absorbance Capacity (ORAC) and Trolox Equivalent Antioxidant Capacity (TEAC) assays, phenolic contents were determined. Total phenolic substance, ORAC and TEAC values increased significantly weekly and reached the highest level in the 3rd week. Total phenolic substance, ORAC and TEAC values of 3rd week apple vinegar were determined as 1110.63 mg GAE L^{-1}, 10.92 mM and 21.11 µmol TE mL^{-1}, respectively. Apple vinegar samples had gallic acid, catechin, epicatechin, chlorogenic acid, and p-coumaric acid. The major phenolic substances in apple vinegar were gallic acid and chlorogenic acid. While gallic acid value of 3rd and 4th week apple vinegar were detected 11.91 and 23.69 mg L^{-1}, respectively; chlorogenic acid value of 4th and 5th week apple vinegar were found 46.36 and 49.71 mg L^{-1}. Antioxidant activity and phenolic substances values were not significant reduction during the acetic acid fermentation. In this study, the formation process of the functional and sensory properties of apple cider vinegar due to the change in the weekly antioxidant and bioactive component content of apple cider vinegar was emphasized.

1. Introduction

Apple is a fruit commonly consumed by humans. In addition to this consumption as fruit, apple can also be turned into different products (such as jam, puree, apple wine, vinegar). Polyphenolic ingredients in apple composition positively affect human health (Boyer, 2004; Francini and Sebastiani, 2013). There are over 8000 polyphenols which has known as antioxidants in nature. Polyphenols protect our body against damage caused by free radicals (Ganesan and Xu, 2017). Briefly, polyphenolics have been asserted to effective on human health (preventing chronic disease such as cancer, heart attack, hypertension, and diabetes) (Halliwell, 2007). Each of polyphenols may have private health impact (Manach et al., 2004). Apple vinegar comprises of polyphenols such as chlorogenic acid, gallic acid, catechin, epicatechin (Budak et al., 2011). Chlorogenic acid which is abundant in apples has been also indicated to inhibit DNA damage in vitro (Kasai et al., 2000) and displayed a preservative effect against cardiovascular diseases (Laranjinha et al., 1994). Budak et al. (2011) indicated that total phenolic content, chlorogenic acid, antioxidant (ORAC and TEAC) activities values of apple vinegar were the higher determined by surface (traditional) methods with maceration than submersion (industrial) methods with and without maceration. Besides,

different phenolic contents (gallic acid, epicatechin, chlorogenic acid etc.) were detected in apple cider vinegars while chlorogenic acid had been identified as the predominant phenolic content in apple vinegar samples (Budak et al., 2011).

Apple cider vinegar is one of the most commonly known in vinegar types. Although the first known usage of vinegar dates back to a century ago (Johnston and Gaas, 2006; Tan, 2005), vinegar has been widely used in food industry in recent 20 years. There are different kinds of vinegar which are balsamic, cane, champagne, cider, vinegar, distilled, malt, rice wine, sherry, wine (Tan, 2005). Vinegar has a double fermentation processes using different raw materials. These stages are ethanol and acetic acid fermentations. In addition, vinegar is produced by different production methods. While the fermentation in the traditional method (a surface-slow method) occurs on the surface of a barrel following wine or cider; the fermentation in the industrial method (a submersion (quick) method); consists a fermentator in the continuous oxygenation, optimum temperature (Tan, 2005). Acetic acid bacteria are responsible for vinegar production (Ley et al., 1984). Vinegar should contain at least 4% acetic acid (TSE, 2016). The final quality of vinegars depend on the selection of appropriate starter culture, starting material, the production method, maturation and aging (Mas et al., 2014).

The aromatic compounds, polyphenolic compounds and antioxidant activity of vinegar change during the vinegar formation process. Budak et al. (2014) reported that vinegar has high antioxidant and antibacterial activity. Vinegar has been found to be effective in cholesterol metabolism and reducing liver fat. Du et al. (2019) determined that apple pulp obtained by cold pressing technology has significant high antioxidant capacity and bioactive compounds. They reported that vinegar which has high bioactive content can be produced from this pulp. Chlorogenic acid, caffeic acid, phlorizin, gallic acid, coumaric acid, ferulic acid and vanilla acid detected 6.56, 3.03, 1.76, 0.35, 0.33, 0.24, 0.06 mg L^{-1} in apple cider vinegar, respectively (Du et al., 2019). In other study, antioxidant analyzes were performed on the filtered (FAV), clarified (CAV) and packaged (PAV) of apple vinegar samples in the industrial vinegar process (Bakir et al., 2016). They determined that total phenolic content of CAV, FAV, PAV had 383, 357, 459 mg GAE 100 mg^{-1}; TEAC value of CAV, FAV, PAV had 570, 587, 1256 mg TEAC 100 mL^{-1}, respectively. It was observed that apple vinegar contain gallic acid, syringic acid, caffeic acid, p-hydroxybenzoic acid, catechin, and p-coumaric acid (Bakir et al., 2016).

So far, we have not found any previously published studies on determining the weekly antioxidant activity and phenolic components of the apple cider vinegar process. In this study, weekly changes of antioxidant properties and bioactive substances were determined during the apple cider vinegar process.

2. Material and Methods

Apples were harvested in Isparta (in two different regions). Food Engineering laboratory in Suleyman Demirel University was used to convert apples into vinegar and analyzes. Figure 1 shows traditional vinegar production methods. During apple cider and vinegar formation, samples were taken weekly. Apple juice sample was coded as V0. Samples taken during ethanol fermentation and acetic acid fermentation at 1, 2, 3, 4, 5, 6, 7, and 8 weekly samples were coded as V1, V2, V3, V4, V5, V6, V7, and V8, respectively.

Total titratable acidity, pH, total soluble solid (°Brix) of samples were detected according to AOAC (1992) methods. Total titratable acidity of juice, cider, vinegar was expressed as malic acid, lactic acid, acetic acid, respectively. pH meter (WTW, Inolab, USA) and Abbe refractometer (Bellingham Stanley Limit 60/70 Refractometer, England) were used in pH and total soluble solids measurements. Ethanol content of apple cider samples were detected with alcoholometer (Dujardin-Salleron, France).

Folin-Ciocalteu method was used for determination of total phenolic content and "mg GAE L^{-1}" was used to express the values (Singleton et al., 1999).

The hydrophilic ORAC-Fluorescein method were used to detect the Oxygen Radical Absorbance Capacity (ORAC) (Davalos et al., 2005). ORAC values were kinetically calculated in BioTek Instruments (Winooski, Vermont, USA) and indicated as "µmol TE mL^{-1}".

Total antioxidant capacity was made according to the method determined by Seeram et al. (2005). "mM TE" was used in order to express the TEAC assay.

The identification and quantification of phenolic compounds in samples carried out a high-performance liquid chromatography (Shimadzu, Kyoto, Japan) according to Caponio et al. (1999). The system includes a pump (LC-10ADvp), autosampler (SIL-10AD vp), a DAD detector (λmax=278), system controller (an SCL-10Avp), degasser (DGU-14A), column oven (a CTO-10Avp), column (Inertsil ODS-3V C18) (GL Sciences Inc.). Standart chromatogram was shown Figure 2. Phenolic compounds were stated as "mg L^{-1}".

Yeasts were grown on Potato Dextrose Agar (PDA, Merck, Darmstadt, Germany) at 25°C for 5 days. It was added lactic acid (0.14%) (Özdemir et al., 2015). Acetic acid bacteria were counted on Glucose Yeast Extract Agar (GYC, Merck, Darmstadt, Germany) with cycloheximide (100 ppm) at 30°C for 5-7 days (Yetiman, 2012).

Vinegar productions was done in duplicate and two in parallel and all experiments were repeated

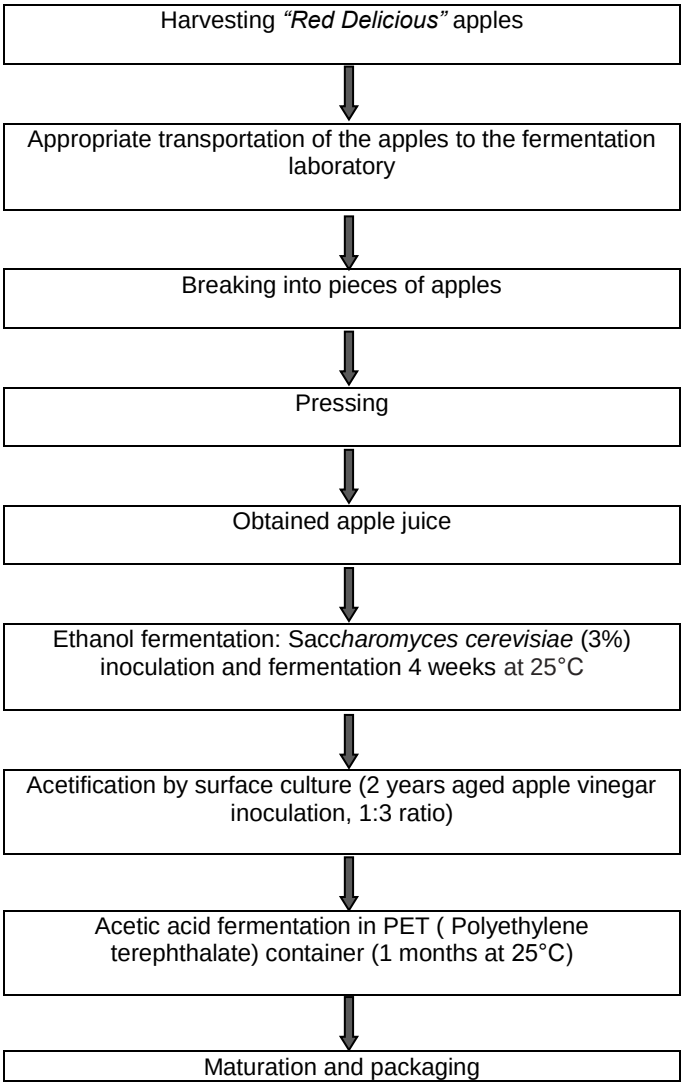


Figure 1. Flow chart of apple cider vinegar

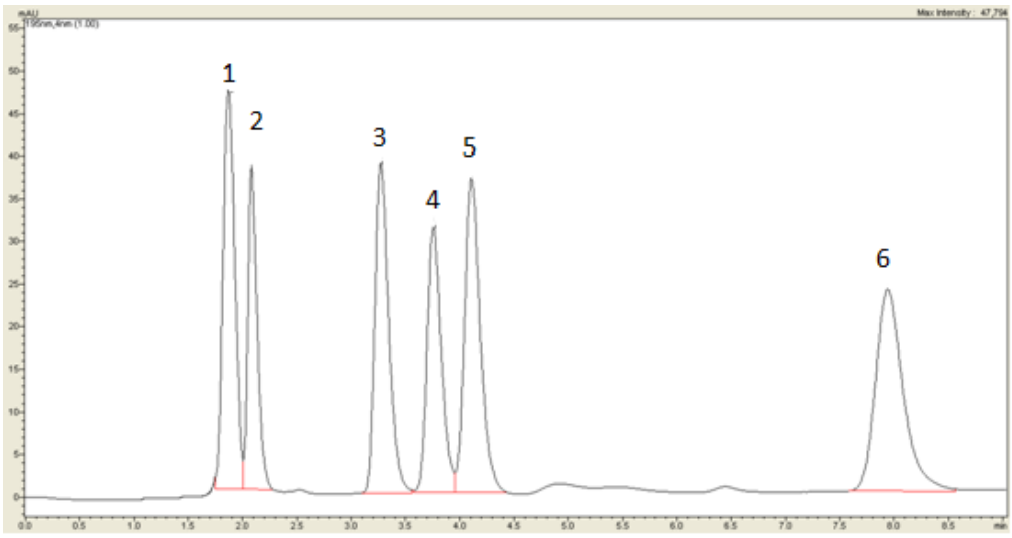


Figure 2. A chromatogram of standard (1: gallic acid, 2: chlorogenic acid, 3: catechin, 4: caffeic acid, 5: epicatechin, and 6: rutin)

three times. A one-way analysis of variance (ANOVA) was applied using SPSS 18.0 (SPSS, 2010). The mean ± SEM was used to express the results.

3. Results and Discussion

pH values, total acidity (%) and total soluble solids (°Brix) of weekly samples were shown in Table 1. While pH had steadily decreased, total acidity had gradually increased during fermentation. pH, total acidity, brix values have significantly changed in the first week. Chemical transformation in yeast fermentation has also significantly affected these values (P<0.05). pH changes in the 1st, 2nd, 3rd in weeks were not found to be statistically significant (P>0.05). Besides, after the 3rd week, the pH tended to decrease significantly (P<0.05) and the pH was observed at close values until the 8th week (P>0.05). In the 8th week, the pH value showed a decrease and the pH value of V8 had 3.38 and it was observed that the vinegar formation was completed. Total acidity of 2nd, 3rd, 4th weeks had increased (P>0.05) but total acidity of 7th and 8th weeks had significantly increased (P<0.05). While the total acidity values of the wine and the juice were close to each other, the value of the vinegar was higher than them (P<0.05). It was considered that the increase of the total acidity value in these stage of fermentation, might be caused by the production of mainly acetic acid and other organic acids, also the stability of the pH value might be caused by a weak acid property of the organic acid. Because, while the pH value was expressed as a negative logarithm of the concentration of dissociated hydrogenions, titratable acidity deals with measurement of the total acid concentration contained within a food, regardless of the effectiveness of the acid, that is, whether it is weak or strong. While the pH values of the wine and the juice were close to each other, the value of the vinegar was lower than them (P<0.05). This situation has also observed in vinegar productions using different fruits (Sadler and Murphy, 2010). Budak (2010) reported that total acidity of apple vinegar samples was 57.2 g L^{-1}. Moreover, total soluble solid of apple juice was 14 (°Brix), this value decreased step by step in the ethanol fermentation (P<0.05). Especially, there was a significant decrease in brix value in the first week (P<0.05). Because, sugar has turned into ethanol by alcohol fermentation (Treck and Teuber, 2002). Total soluble solid of V8 had shown 2.15 °Brix in the end of fermentation. Alcohol value reached its highest value at 3rd week, and this value remained the same in the 4th week. Acetic acid fermentation was started in the 4th week. That's why alcohol value decreased with the initiation of acetic acid fermentation (P<0.05). Since, acetic acid bacteria operates under oxygen, alcohol, suitable temperature conditions for acetic acid fermentation (Guillamon and Mas, 2011). Yeast counted during ethanol fermentation. Acetic acid bacteria counted during acetic acid fermentation (Table 1). In the post-inoculation yeast count was the highest observed at the end of the 1st week. Yeast count and decreased in sugar consumption is balanced with each other. The yeast value entered the stationary phase in the 3rd and 4th weeks. Finally, yeast entered the death phase at 5th week and counting could not be made. Acetic acid bacteria count was determined between 4.55 and 5.80 log kob mL^{-1}.

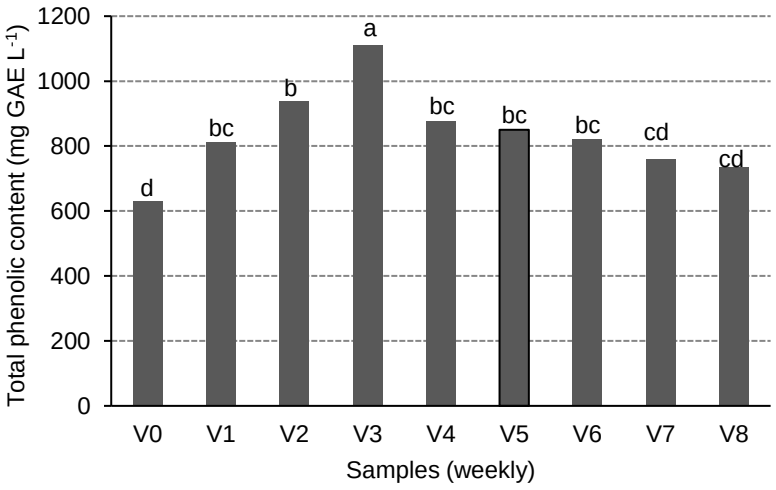
Total phenolic contents (TPC) of samples were presented in Figure 3. Total phenolic substance value increased significantly weekly (P<0.05) and reached the highest level in the 3rd week. Total phenolic substance was 1110.63 mg GAE L^{-1} in V3

Table 1. Chemical properties and phenolic compounds of samples (8 weeks)

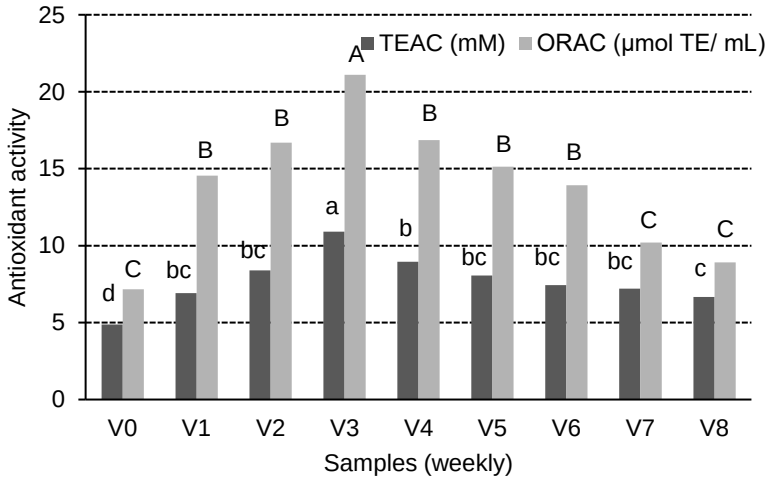
S	pH	TA (%)	TSS	A	Y	AAB	GA	CA	C	E	p-CA
V0	4.38 ±0.04^{a}	1.64 ±0.09^{c}	14.00 ±0.46^{a}		5.56 ±0.08^{b}		7.94 ±1.17^{b}	12.16 ±1.41^{b}	1.24 ±0.33^{a}	0.41 ±0.05^{b}	0.06 ±0.01^{b}
V1	3.91 ±0.08^{b}	2.32 ±0.01^{bc}	6.25 ±0.89^{b}	7.30 ±0.24^{b}	7.14 ±0.09^{a}		9.33 ±1.02^{b}	33.52 ±0.51^{b}	1.69 ±0.11^{a}	1.28 ±0.10^{b}	0.07 ±0.01^{b}
V2	3.90 ±0.07^{b}	2.31 ±0.02^{bc}	4.75 ±0.26^{bc}	8.35 ±0.14^{a}	5.81 ±0.10^{b}		10.36 ±0.76^{b}	35.38 ±0.91^{ab}	2.05 ±0.16^{a}	3.24 ±0.08^{a}	0.12 ±0.01^{ab}
V3	3.67 ±0.09^{bc}	2.33 ±0.01^{bc}	3.45 ±0.20^{cd}	8.59 ±0.15^{a}	4.38 ±0.20^{b}		11.91 ±2.20^{b}	41.05 ±1.05^{a}	2.09 ±0.49^{a}	3.65 ±0.20^{a}	0.16 ±0.04^{ab}
V4	3.53 ±0.05^{cd}	2.23 ±0.03^{bc}	3.60 ±0.12^{cd}	8.55 ±0.15^{a}	4.18 ±0.11^{b}	5.24 ±0.02^{a}	23.69 ±1.35^{a}	46.36 ±2.78^{a}	1.92 ±0.15^{a}	3.55 ±0.18^{a}	0.19 ±0.01^{a}
V5	3.43 ±0.04^{cd}	3.47 ±0.11^{b}	3.25 ±0.43^{cd}	3.45 ±0.16^{c}		5.56 ±0.09^{a}	25.58 ±1.48^{a}	49.71 ±2.93^{a}	1.75 ±0.42^{a}	2.67 ±0.14^{a}	0.17 ±0.03^{ab}
V6	3.47 ±0.05^{cd}	3.87 ±0.03^{b}	2.95 ±0.32^{d}			5.80 ±0.03^{a}	27.22 ±0.99^{a}	45.64 ±1.56^{a}	1.50 ±0.48^{a}	2.79 ±0.31^{a}	0.13 ±0.02^{ab}
V7	3.51 ±0.04^{cd}	4.54 ±0.07^{a}	2.35 ±0.08^{d}			5.10 ±0.05^{a}	24.83 ±2.93^{a}	43.45 ±2.01^{a}	1.59 ±0.42^{a}	2.80 ±0.27^{a}	0.11 ±0.04^{ab}
V8	3.38 ±0.05^{d}	5.19 ±0.09^{a}	2.15 ±0.08^{d}			4.55 ±0,03^{a}	25.68 ±3.39^{a}	45.07 ±2.48^{a}	1.54 ±0.47^{a}	2.66 ±0.48^{a}	0.08 ±0.01^{ab}

S:Samples, TA: Total acidity (%), TSS:Total soluble solids (°Brix), A: Alcohol, Y: Yeast (log kob mL^{-1}), AAB: Acetic acid bacteria (log kob mL^{-1}), GA: Gallic acid (mg L^{-1}), CA:Chlorogenic acid (mg L^{-1}), C: Catechin (mg L^{-1}), E: Epicatechin (mg L^{-1}), p-CA: p-Coumaric acid (mg L^{-1})

Data expressed as mean ± standard error (SEM). a, b, c, d: There is a statistically significant difference between groups in the same column without common letters (P<0.05).



a



b

Figure 3. Total phenolic contents (a) and antioxidant activity (b) of samples (Data expressed as mean ± standard error (SEM). a, b, c, d: There is a statistically significant difference between samples without common letters (P<0.05). Capital letters and lower case letters are evaluated among themselves (P<0.05).

sample. TPC of the V4 sample decreased to 876.25 mg GAE L^{-1} with the start of acetic acid fermentation (P<0.05). After the 4th week, TPC values started to decrease, but no significant decreases were observed (P>0.05). TPC values of V5, V6, V7, and V8 had 850.15, 820.03, 758.56, 734.55 mg GAE L^{-1}, respectively. The weekly analyzed results show that the total phenolic matter values increased during ethanol fermentation (P<0.05) and did not change throughout acetic acid fermentation (P>0.05). This increase could be associated with the release of the phenolic acids bound to sugar or/and organic acid molecules in the juice in the alcohol fermentation (Crozier et al., 2009).

In the literature research conducted so far, although there is no weekly follow-up in apple vinegar process, total phenolic substance results in apple juice, wine and vinegar samples have been determined. It was indicated that the total phenolic content were 3392 (Rababah et al., 2005), 2110-3470 (Wu et al., 2004), 1100-3570 (Podsedek et al., 2000; Liu et al., 2001), 977 mg GAE L^{-1} (Wolfe et al., 2003) in apple juice samples; 730-1343 mg GAE L^{-1} in apple cider produced with different techniques (Budak et al., 2015); 908, 568, 757, 416 mg GAE L^{-1} of apple vinegars produced with different techniques (Budak et al., 2011), 33-284 mg GAE L^{-1} apple vinegars (Du et al., 2019), 357-459 mg GAE L^{-1} (Bakir et al., 2016), 43-495 mg GAE L^{-1} in eleven apple vinegars purchased from local markets in China (Liu et al., 2019).

The differences in the total phenolic content of apple juice can vary according to apple varieties and growing conditions, and this change is reflected in the products produced from apple (apple wine, apple cider vinegar). TEAC ($ABTS^{\cdot}$) and ORAC values of apple samples were presented in Figure 3. ORAC and TEAC values were similar tendency in weekly measurements of apple samples. ORAC and TEAC values increased weekly until the 3rd week (P<0.05). ORAC and TEAC values reached the highest antioxidant value in the 3rd week.

TEAC and ORAC value of V3 (apple cider) had 10.92 mM and 21.11 µmol TE mL^{-1}, respectively. While the decrease in the ORAC value in acetic acid fermentation was significant ($P<0.05$), the decrease in the TEAC value was not significant ($P>0.05$). It has been stated that yeast use and fermentation conditions affect phenolic compounds during ethanol fermentation (Brandolini et al., 2007). Because, phenolic compounds related to sugar are released when yeast uses sugar, and antioxidant activity increases during fermentation (Crozier et al., 2009). Ubeda et al. (2011) reported that ORAC value of balsamic vinegar, apple vinegar, sherry vinegar, persimmon vinegar, red wine vinegar had 40049, 8986, 7879, 1857, and 1462 µmol TE kg^{-1}, respectively. Budak et al. (2011) determined that ORAC values between 3.00 and 5.89 µmol mL^{-1} in apple vinegar samples, while TEAC values between 5.4 and 13.5 mmol L^{-1}. In our study, ORAC values of apple vinegar (V8) had 8.90 µmol TE mL^{-1}.

Gallic acid, catechin, epicatechin, chlorogenic acid, and p-coumaric acid were detected in all samples (Table 1). Contents of catechin, epicatechin and p-coumaric acid were lower than gallic acid and chlorogenic acid content in all samples. Gallic acid content of samples increased weekly until the 4rd week ($P>0.05$). Gallic acid value of V3 and V4 had 11.91 and 23.69 mg L^{-1}, respectively ($P<0.05$). This increases could be associated with the release of the phenolic acids bound to sugar or/and organic acid molecules in the juice, in the alcoholic mediums (Crozier et al., 2009). Differences in gallic acid value were not significant in acetic acid fermentation ($P>0.05$). Chlorogenic acid was the dominant phenolic substance in apple cider and apple cider vinegar samples; especially, V4 and V5 samples had the highest content of chlorogenic acid. Budak et al. (2011) reported that chlorogenic acid of apple cider vinegar sample had 18.67 mg L^{-1} and chlorogenic acid was the dominant phenolic substance in apple vinegar. Catechin, epicatechin, p-coumaric acid content of V8 had 1.54, 2.66 and 0.08 mg L^{-1}, respectively. It has been shown that epicatechin significantly changed 2nd week while coumaric acid significantly changed 4th week ($P<0.05$). The leading polyphenols in apple cider vinegar were chlorogenic acid, caffeic acid, phlorizin, vanilla acid, gallic acid, coumaric acid and ferulic acid (Du et al., 2019). Phenolic compounds in apples changes induced by apple cultivar, breeding approaches, fruit postharvest and transformation into juice. Total and individual polyphenols in apple cultivars and cultivation may have been shown to vary (Volz and McGhie, 2011). As a result of weekly analyzes, we was observed that gallic acid and chlorogenic acid content was the dominant phenolic component in apple cider vinegar.

4. **Conclusion**

This study is the first detailed report determining the weekly change in antioxidant properties and bioactive substances during fermentation of apple cider vinegar. These values reached the highest value as a result of ethanol fermentation and no significant change was observed during acetic acid fermentation. Significant biochemical changes were observed especially until the 4th week of fermentation. As a result, it was observed that the antioxidant and phenolic component values increased with the release of phenolic compounds bound to sugar as a result of using the sugar in the fruit by yeast. It is important for human health to increase the usage area and consumability of apple cider vinegar and to benefit from its functional properties. In addition, being preferred for its sensory properties, apple wine and vinegar is one of the important functional products for health. Determining the weekly change of apple cider vinegar made from apple fruit in terms of antioxidant and phenolic components (especially gallic acid and chlorogenic acid) is important in terms of detecting the change in fermentation steps. This study will shed light on the emergence of new studies especially in fermentation stages.

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