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Comparison of Electrochemical Sensing Platform and Traditional Methods for the Evaluation of Antioxidant Capacity of Apple Cider

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We investigated the antioxidant capabilities of apple cider prepared from six different varieties of apples and three different yeast strains in this study. For the evaluation of antioxidant performance, an unique electrochemical sensing platform was created. Also tested were the DPPH scavenging rate, superoxide anion scavenging rate, hydroxyl radical scavenging rate, and reducing capacity. When traditional assessment methodologies were compared to electrochemical sensing, the results revealed that the electrochemical sensing platform is better for overall antioxidant evaluation of apple cider. Using the electrochemical sensing evaluation platform, we discovered that different yeast strains have a distinct effect on the antioxidant activity of some apple ciders. This low-cost, quick method of evaluating antioxidants has a wide range of applications.

Keywords: Apple cider; Antioxidant capacity; Electrochemical sensor; Scavenging rate; Yeast strains

1. INTRODUCTION

Crushing, pressing, fermentation, maturing, and blending are all used to make apple cider, which is a low-alcohol fermented product manufactured from apples. It is mostly made in England, France, and Germany, and originates in the Normandy region of France. In Europe, the United States, and Australia, it is very popular. Apple cider is made via alcoholic fermentation, which uses yeast to make alcohol from the fermentable carbohydrates in apple juice [1–3]. Apple cider can be categorised in a variety of ways, depending on the brewing method, sugar concentration, and alcohol percentage. According to the brewing method, there are three types [4–8]. (1) Fermented apple cider: Fermented apple cider is created from apple pulp or apple juice that has been fermented. Depending on the qualities of the raw materials

and the needs of the finished product, this type of cider can be fully fermented, semi-fermented, macerated fermented, semi-macerated fermented, and so on. (2) Distilled apple cider: To boost the alcoholic strength of apple juice, it is fermented and then distilled. (3) Champagne-style apple cider: This sort of cider is made from apple juice and fermented in a bottle, and it contains a lot of CO_2.

Polyphenols have gotten a lot of press in recent years because of their significant biological functions. Tannin chemicals and small-molecule phenolic molecules are polyphenols [9–13]. Small-molecule phenols in cider are classified as phenolic acids and their derivatives, as well as flavonoids. Flavan-3-ols, proanthocyanidins, flavonols, dihydrochalcone, and hydroxycinnamic acid and its derivatives are the most abundant polyphenols in cider. Polyphenols are plant secondary metabolites that are often found in plant meals and have a key part in the production of cider [14–17]. Cider's color, taste, flavor, aroma, and stability are all affected. The browning of the fermentation broth contributes to the color of cider. Non-enzymatic browning and enzymatic browning are the two types of browning in cider. Enzymatic browning is linked to the kind and amount of polyphenolic compounds in cider, as well as the activity of polyphenol oxidase [18–21]. Polyphenolic compounds, such as chalcones and glycosylated flavonoids, which release sweetness, and tannins, which release bitterness and astringency, can influence the flavor of cider. The release of tastes is influenced by the degree of structural polymerization of polyphenolic compounds, pH, sugar level, viscosity, and alcohol concentration in the plant [19,22–24].

Polyphenols' implications on human health have gotten a lot of attention. According to studies, the average daily consumption of polyphenols for women is 780 mg and for males is 1058 mg. Dihydrocinnamic acids make up half of them, flavonoids make up 20–50%, and anthocyanins make up roughly 1%. Apple polyphenols are widely employed in the manufacture of food, medicine, and cosmetics because they contain antibacterial, anti-inflammatory, free radical scavenging, metal chelating, coronary heart disease prevention, atherosclerosis prevention, and other biological properties [25–28]. Apple polyphenolic substance composition and content are distributed differently in each apple organ and are regulated by factors such as apple variety, growing region climate, and ripeness. Apple polyphenols enter the fermenter with the apple juice and become components of apple cider through yeast fermentation [29,30], which can have a significant impact on the sensory quality and functional qualities of cider. As a result, the theoretical basis for selecting optimal apple varieties and yeast strains for cider brewing is the influence of different apple varieties and yeast strains on polyphenols and antioxidant capacity in cider under the same process circumstances [31–33].

In recent years, electrochemical detection methodology for antioxidant evaluation has become a well-established evaluation tool. It provides the benefits of low cost and speed. We brewed apple cider with various apple kinds as raw materials and different yeasts as fermentation strains in this study. To assess the changes in polyphenol composition and antioxidant activity of apple cider during and after fermentation, we used an electrochemical sensor platform and traditional methodologies. We looked at how different apple cultivars and yeast strains affected fermentation polyphenol content and antioxidant activity.

2. EXPERIMENTAL

2.1. Regents

Apple varieties include Red Fuji, Gala, Sansa, Red Jade, Snake Fruit and Golden Crown. Yeast strains include brewer's yeast (#1), Delb's spore-round yeast (#2) and heat-resistant Kluyver yeast (#3). Protocatechuic acid, catechin, procyanidin B2, epicatechin, rhizosphingoside, ellagic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, hypericin, quercetin, sodium hydroxide, zinc acetate, hydrochloric acid, ethyl acetate, chromatographic methanol, phosphate buffer (0.2 mol/L, pH 6.6), potassium ferricyanide, trichloroacetic acid, DPPH, o-triophenol, H_2O_2, ferrous sulfate, salicylic acid, and ethanol were all analytically grade and used without purification. The cider was fermented at a low temperature with a long maceration time of 16-18°C. The fermentation time was extended to about 15 d, and the alcoholic fermentation was stopped by decanting the tank when the alcoholic content reached about 6 degrees.

2.2. Electrochemical platform fabrication

The fabrication of the electrochemical platform followed prior findings [34,35]. A specified amount of zinc acetate solution (15 mM) was typically added to a 1 mL 1 wt% chitosan solution (in 1% acetic acid). After 1 min of shaking, 0.1 M NaOH was added to start the gelation process. The pH of the final chitosan-Zn hydrogel ranged between 6.2 and 6.5. Depolymerization was achieved by introducing H_2O_2 and apple cider vinegar into a chitosan-zinc ions hydrogel. GCE, Ag/AgCl, and Pt electrodes were placed into the hydrogel after depolymerization for electrochemical measurements. From -1.2V to -0.7V, differential pulse voltammetry (DPV) was recorded.

2.3. HPLC determination of polyphenols

Sample preparation: aspirate 10 mL of cidar sample and adjust pH to around 7 with 1 M NaOH. To mix the supernatant, extract with 20 mL of ethyl acetate three times. Adjust the pH of the remaining fraction to around 2 with 2 M HCl. Extract three times with 20 mL ethyl acetate, mix the supernatant, and evaporate to dryness at 40°C by rotation. HPLC was used to determine the residue, which was dissolved in 10 mL of chromatographic methanol.

Chromatographic conditions: mobile phase A: 1% acetic acid, mobile phase B: methanol, flow rate 1 mL/min, injection volume 20 μL, column temperature: 19 ℃. Gradient elution: mobile phase B increased from 5% to 30% at 0~10 min, from 30% to 50% at 10~25 min, from 50% to 60% at 25~30 min, and from 60% to 70% at 30~35 min.

Quantitative analysis: weigh 1 mg of each standard accurately in methanol, dissolve in 10 mL volumetric flasks, then dilute to different concentration gradients. Under the above chromatographic conditions, the standard curve was drawn with the concentration X as the horizontal coordinate and the peak area Y as the vertical coordinate. The samples were identified using the same chromatographic conditions, and the corresponding contents were calculated using the peak regions as a standard curve.

2.4. Determination of reduction capacity

Mix 2.5 mL of phosphate buffer with 0.2 mL of sample in 1 mL of water (0.2M, pH 6.6). In a water bath at 50°C for 20 minutes, add 2.5 mL of 1 percent potassium ferricyanide, shake well, and then add 1 mL of 10% trichloroacetic acid. Centrifugation at 3000 r/min for l0 minutes was used to separate the solution. The absorbance was measured at 700 nm after 2.5 mL of supernatant, 2.5 mL of distilled water, and 0.5 mL of 0.1 percent $FeCl_3$ were added.

2.5. Determination of DPPH scavenging rate

2 mL of 0.1 mM DPPH radical alcohol solution was added to 2 mL of aqueous solution containing 0.2 mL of the sample. At room temperature and sheltered from light, the absorbance was measured at 517 nm for 30 min. The clearance was calculated according to the following formula: Clearance (%)=$(1-A_l/A_0)\times100\%$ where A_0 and A_1 are the blank and sample absorbance values at 517 nm, respectively.

2.6. Determination of superoxide anion scavenging rate

The reaction was stopped by adding 1 mL of 8 mM HCl and measuring the absorbance at 299 nm. The catechol solution in the sample solution background group was replaced with the same volume of distilled water, and the sample in the blank control group was replaced with the same volume of distilled water. The following formula was used to calculate the % clearance of superoxide anion. Clearance rate (percentage) = $[A_0-(A_x-A_{x0})]/A_0 \times 100\%$ The absorbance of the blank control solution and the absorbance of the sample solution backdrop, respectively, are A0 and Ax0.

2.7. Determination of hydroxyl radical scavenging rate

9 mM $FeSO_4$, 9 mM salicylic acid/ethanol solution, and 1 mL sample solution were added to the test tube, with the blank control group receiving the same volume of distilled water as the sample. Using distilled water as a reference, the absorbance of each concentration was measured at 510 nm. The following formula was used to calculate the hydroxyl radical scavenging rate: Scavenging rate (percentage) = $[A_0-(A_x-A_{x0})]/A_0\ 100 \times \%$, where A_0, A_x, and A_{x0} are the absorbance of the blank control solution, sample solution absorbance, and sample solution background absorbance, respectively.

3. RESULTS AND DISCUSSION

The effect of apple cultivars on variations in antioxidant activity in cider fermentation broth was first examined. Figure 1 shows that the DPPH scavenging rate of each sample decreased slightly during the process of manufacturing cider from several apple, however the values varied greatly. The

absorbance of Red Fuji1#, for example, ranged from 0.86 to 0.94, while Gala 1#'s ranged from 0.44 to 0.61. Each sample (except Gala1#) had a declining and subsequently increasing superoxide anion scavenging rate, with the lowest trough on the fourth day, but at varying rates of drop [36]. On the fourth day, the rate of superoxide anion scavenging in Gala1# cider rose, but then decreased. The hydroxyl radical scavenging rate in Gala1#, Sansa1#, and Red Jade1# ciders showed a falling trend followed by an ascending trend, but the troughs occurred at various times. The hydroxyl radical scavenging rate tended to increase and then decline in cider made from Snake Fruit1#, Golden Crown1#, and Red Fuji1#, and the time of the largest peak was different. During cider brewing with several apple, the reduction capacity of all samples (except Red Fuji1#) showed a declining and then increasing pattern, with the lowest peak occurring at day 7. During fermentation, however, the reduction capacity values differed greatly among the samples. On day 2, the lowering capacity of Red Fuji1# as a raw material for cider fermentation peaked, then declined.

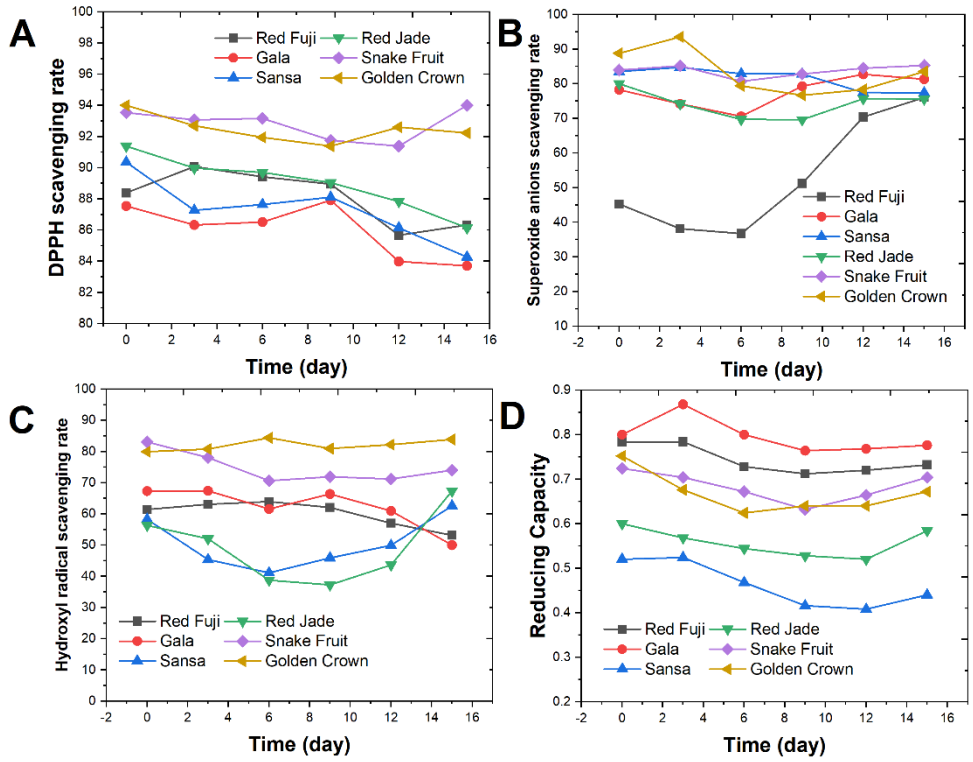

Figure 1. Changes of (A) DPPH·scavenging rate, (B) superoxide anions scavenging rate, (C) hydroxyl radical scavenging rate and (D) reducing capacity of fermentation ciders made from different apple varieties along with the fermentation time.

Figure 2 demonstrates how the electrochemical sensor platform detected the antioxidant characteristics of different apple varieties during the fermentation process. The antioxidant properties of Red Fuji1# and Red Jade1# decreased and then increased with the fermentation process, while those of Gala1# and Sansa1# decreased gradually with the fermentation process. The antioxidant activity of Snake Fruit1# and Golden Crown1#, on the other hand, did not change appreciably as a result of the

fermenting procedure. It can be demonstrated that standard antioxidant activity evaluations can only evaluate distinct active clusters one at a time, making a comprehensive index difficult to establish [37]. However, the electrochemical sensing platform can give an evaluation of the overall antioxidant effect of the samples.

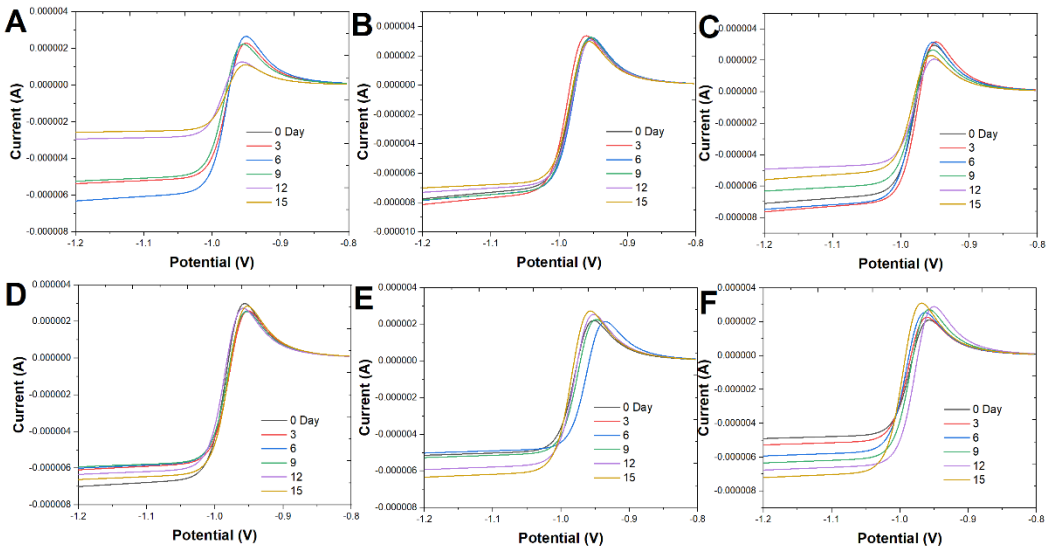

Figure 2. Electrochemical based antioxidant evaluation of fermentation ciders made from (A) Red Fuji, (B) Gala, (C) Red Jade, (D) Sansa, (E) Snake Fruit and (F) Golden Crown. Pulse amplitude = 50 mV; step amplitude = 4 mV; pulse time = 50 ms; scan rate = 8 mV/s.

During the fermentation of cider by different yeasts employing Red Fuji, the DPPH scavenging rate, superoxide anion scavenging rate, hydroxyl radical scavenging rate, and reducing capacity trends are shown in Figure 3. The three yeast strains did not demonstrate evident variability, but did varied in the time of peak values, which could be due to the different growth and fermentation rates of different yeast strains [38]. The rate of superoxide anion scavenging in cider fermented by the three yeasts initially reduced, then gradually increased, possibly due to dissolved oxygen in the apple fermentation broth. On the second day of fermentation, the reduction ability of the cider fermented by the three yeasts was greater than that of the fresh apple juice and the fermentation broth at other stages. This could be because the cider was made with leached pomace fermentation, which meant that the polyphenols in the apple pomace were continually dissolved into the fermentation broth during the early stages of fermentation due to leaching [39]. Because the reduction capacity of apple fermentation cider could only be tested in clear cider during fermentation, the reduction capacity of apple fermentation cider increased during the pre-fermentation stage before gradually decreasing with the oxidation of polyphenols.

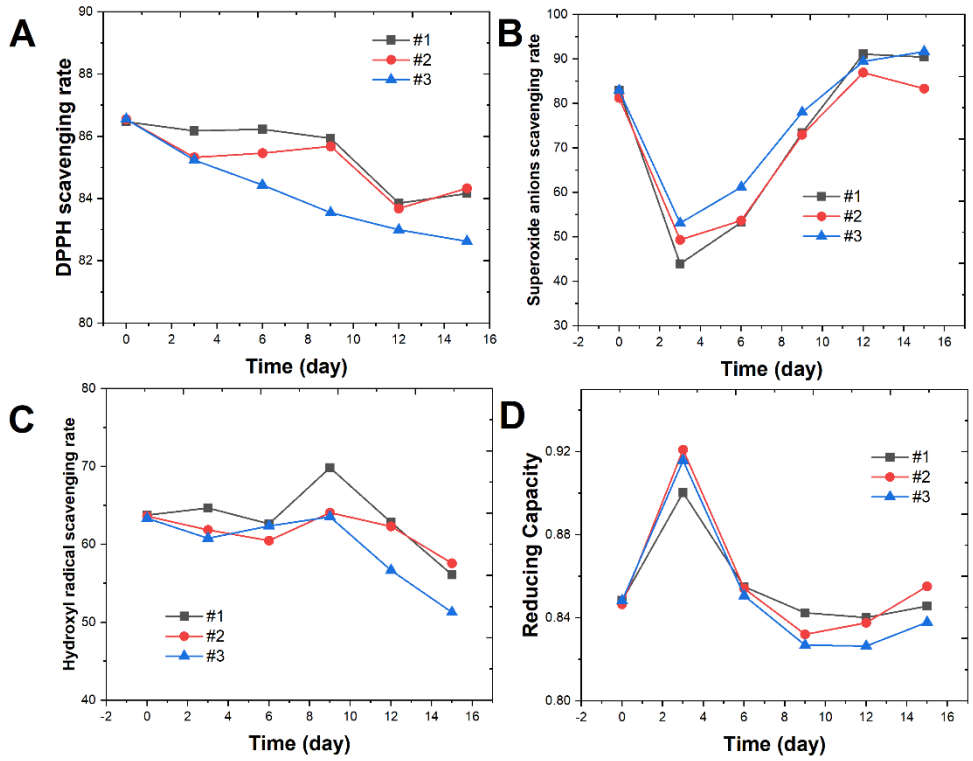

Figure 3. Changes of (A) DPPH· scavenging rate, (B) superoxide anions scavenging rate, (C) hydroxyl radical scavenging rate and (D) reducing capacity of fermentation ciders made from Red Fuji with three different yeasts.

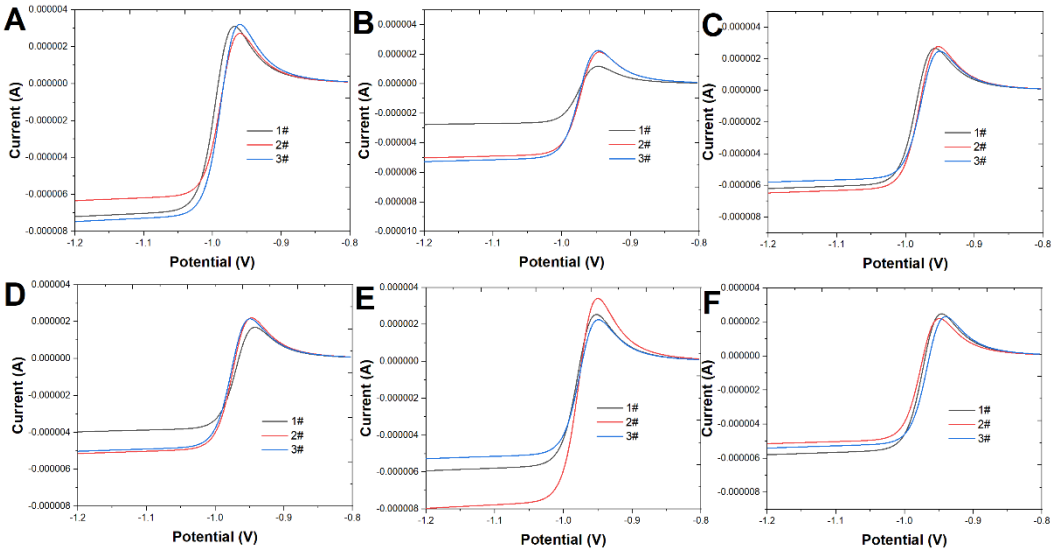

Figure 4. Electrochemical based antioxidant evaluation of fermentation ciders made from (A) Red Fuji, and., (B) Gala, (C) Red Jade, (D) Sansa, (E) Snake Fruit and (F) Golden Crown using 1#, 2# and 3#. Pulse amplitude = 50 mV; step amplitude = 4 mV; pulse time = 50 ms; scan rate = 8 mV/s.

Figure 4 illustrates the results of testing the antioxidant activity of the electrochemical sensing platform on three distinct yeasts with Red Fuji, Gala, Sansa, Red Jade, Snake Fruit, and Golden Crown

[40]. As can be observed from the graph, there is no discernible difference between the three yeasts used in Red Fuji's apple cider. Red Jade, Snake Fruit, and Golden Crown are all examples of this. In Gala and Sansa, however, 1# produced significantly higher antioxidant activity. In the cider made from Snake Fruit, on the other hand, 2# had the lowest antioxidant activity.

The influence of apple varietals on catechin content variation during cider fermentation was next examined. Figure 5A demonstrates that the catechin concentration of Red Fuji, Gala, Snake Fruit, and Golden Crown ciders remained low during fermentation. The catechin content of cider prepared from Sansa and Red Jade, on the other hand, varied greatly and peaked at the end of alcoholic fermentation. When the particular data is combined, it can be observed that, while the catechin content varied during the brewing of cider from different apple varieties, the pattern was for the catechin content to increase first, then drop.

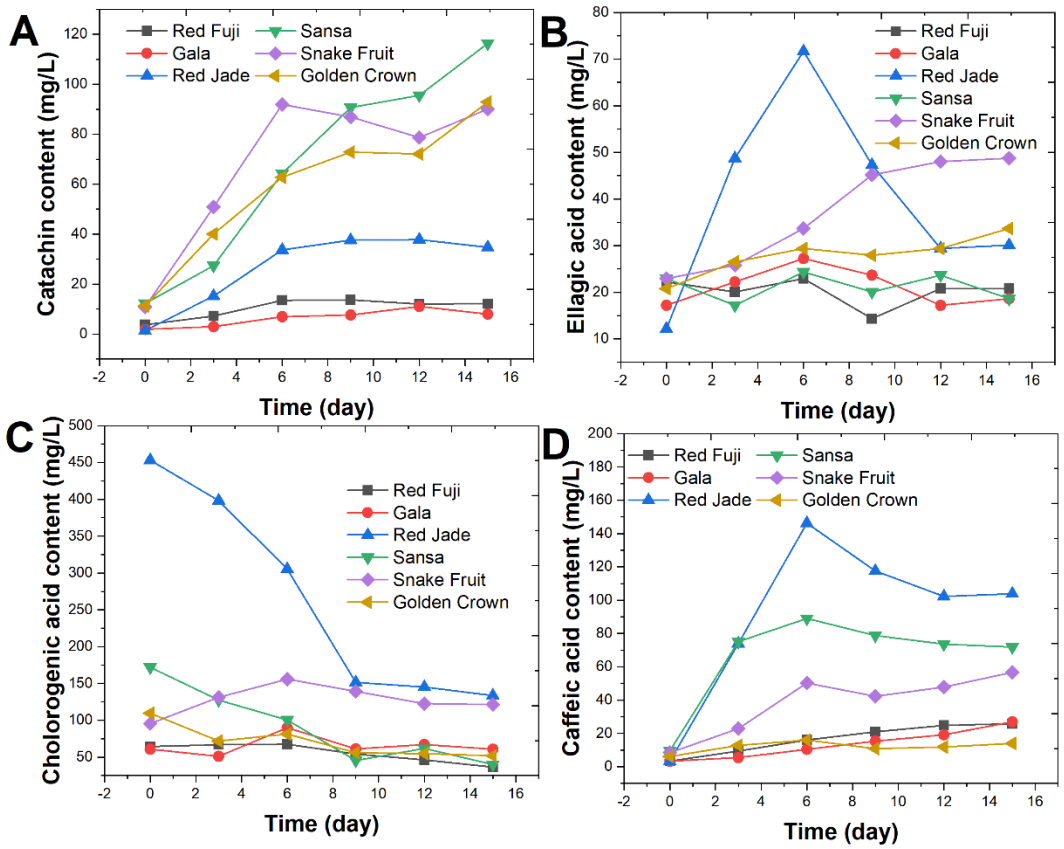

Figure 5. Changes of (A) catechin, (B) ellagic acid, (C) chlorogenic acid and (D) caffeic acid content of fermentation ciders made from different apple varieties along with the fermentation time.

With Red Fuji, Gala, Sansa, Red Jade, and Golden Crown, ellagic acid levels changed within a limited range during the cider manufacturing process, as shown in Figure 5B. The ellagic acid content in Snake Fruit cider, on the other hand, surged abruptly on the second day before dropping to a lower level. This could be owing to the high levels of ellagic acid in the Snake fruit rind, which was immersed in the fermentation broth during the pre-fermentation period.

The chlorogenic acid level of Gala, Sansa, and Red Jade ciders decreased over time, as seen in Figure 5C. During the brewing of Red Fuji, Golden Crown, and Snake Fruit ciders, the chlorogenic acid

level grew and then declined. This could be due to a combination of effects such as chlorogenic acid conversion to caffeic acid, chlorogenic acid oxidation, and other factors. Regardless of variance, when alcoholic fermentation is complete, the chlorogenic acid level in fresh apple cider tends to be in the lower range [41].

As can be observed in Figure 5D, the caffeic acid concentration of Gala, Sansa, and Red Jade ciders has been steadily increasing. In ciders prepared from Red Fuji, Golden Crown, and Snake Fruit, on the other hand, the caffeic acid level climbed, then declined. Because chlorogenic acid was changed to caffeic acid during the fermentation of cider, the caffeic acid level should rise. The drop in caffeic acid level in some samples at the end of fermentation could be due to varying degrees of oxidation of the caffeic acid. [42].

4. CONCLUSION

In this paper, we proposed an electrochemical platform for antioxidant property evaluation of apple cider. This platform was fabricated using chitosan and zinc ions medium. Hydroxyl radicals induced a depolymerization process that freed zinc ions from the complexes formed with the hydrogel. Then, the properties of depolymerization products and media can be used for the evaluation of the antioxidant performance of investigated apple ciders. The apple cider was tested using six different varieties of apples and three different yeast strains. The standard DPPH scavenging rate, superoxide anion scavenging rate, hydroxyl radical scavenging rate, and reducing capacity tests were also performed. The results showed that the proposed electrochemical approach can be utilized to quickly analyze the total antioxidant property of a sample, whereas standard methods can take a long time.

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