Grasas y Aceites 76 (1)
January-March 2025, 2209
ISSN-L: 0017-3495, eISSN: 1988-4214
https://doi.org/10.3989/gya.0758241.2209

Influence of rootstock types and yeast strains on the quality attributes of ‘fuji’ apple cider

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Submitted: 08 July 2024; Accepted: 26 February 2025; Published: 14 October 2025

SUMMARY: The study evaluated the impact of rootstocks (M9, M26, and P2) and yeast strains (Safcider and Safale) on the physicochemical properties, antioxidant activity, and fatty acid composition of apple cider made from ‘Fuji’ apples. Apple juice was fermented with Safcider or Safale, while unfermented juice served as a control. We analyzed physicochemical properties (pH, titratable acidity, and soluble solids content), antioxidant properties (total phenolic content, total flavonoid content, DPPH radical scavenging activity, and FRAP ferric reducing antioxidant power), and fatty acid composition. Results revealed that rootstocks and yeast strains significantly influenced cider quality. M26 and P2 rootstocks fermented with Safcider exhibited superior antioxidant properties and fatty acid composition compared to other combinations. This research provides valuable guidance for selecting optimal rootstock-yeast combinations to produce high-quality cider with desirable characteristics.

KEY-WORDS: *Antioxidant properties; Fatty acid composition; Fermentation;* Malus domestica.

RESUMEN: ***Influencia de los tipos de portainjertos y cepas de levaduras en los atributos de calidad de la sidra de manzana ‘fuji*’. En este estudio se evaluó el impacto de los portainjertos (M9, M26 y P2) y las cepas de levadura (Safcider y Safale) sobre las propiedades fisicoquímicas, la actividad antioxidante y la composición de ácidos grasos de la sidra de manzana elaborada a partir de manzanas ‘Fuji’. El jugo de manzana se fermentó con Safcider o Safale, mientras que el jugo sin fermentar sirvió como control. Analizamos las propiedades fisicoquímicas (pH, acidez titulable y contenido de sólidos solubles), las propiedades antioxidantes (contenido fenólico total, contenido total de flavonoides, actividad de eliminación de radicales DPPH y poder antioxidante reductor férrico FRAP) y la composición de ácidos grasos. Los resultados revelaron que los portainjertos y las cepas de levadura influyeron significativamente en la calidad de la sidra. Los portainjertos M26 y P2 fermentados con Safcider exhibieron propiedades antioxidantes y composición en ácidos grasos superiores en comparación con otras combinaciones. Esta investigación proporciona una valiosa guía para seleccionar combinaciones óptimas de portainjerto y levadura para producir sidra de alta calidad con características deseables.

PALABRAS CLAVE: *Composición en ácidos grasos; Fermentación;* Malus domestica*; Propiedades antioxidantes.*

Citation/Cómo citar este artículo: Tolera Geleta B, Heo JY. 2025. Influence of Rootstock Types and Yeast Strains on the Quality Attributes of ‘Fuji’ Apple Cider. *Grasas Aceites* **76** (1), 2209. https://doi.org/10.3989/gya. 0758241.2209

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1. INTRODUCTION

Apple cider is a fermented beverage known for its rich composition of biologically active compounds, including phytochemicals, minerals, and fatty acids, which offer various health benefits (Tsoupras *et al*., 2024). It has been reported to reduce the risk of allergic reactions, support cardiovascular health and type 2 diabetes management, as well as function as a probiotic (Zhang *et al*., 2021). Fermentation not only preserves the nutritional value of apples but also creates value-added products, making apple cider a promising avenue for diversification in the apple industry.

Apples (*Malus domestica* Bork) are a leading fruit crop in South Korea, accounting for 32,000 hectares of cultivation area and producing approximately 425,000 tons annually. In South Korea, most apples are consumed fresh, highlighting the underdevelopment of the apple processing industry. Moreover, the lack of diversification in apple cultivars leads to simultaneous harvesting periods, contributing to price volatility and financial losses for some farmers. Exploring alternative uses for apples, such as cider production, could mitigate these challenges by enhancing market stability and creating additional revenue streams.

The quality of apple cider is influenced by multiple factors, including apple variety, growing conditions, rootstocks, yeast strains, and fruit maturity (Littleson *et al*., 2022). Therefore, thorough evaluations of these factors are necessary to expand the utilization and applicability of apple cider. Rootstocks, in particular, play a crucial role in determining apple tree performance, nutrient uptake, and fruit quality (Butkeviciute *et al*., 2022). While the impact of rootstocks on grape wine quality is well-documented (Ausari *et al.,* 2024), their effects on apple cider production remain largely unexplored. Understanding these effects can provide valuable insights into optimizing rootstock selection to improve cider quality. Yeast strains are also critical in shaping the aroma, flavor, and overall sensory attributes of cider (Ferremi Leali *et al*., 2024). Different yeast strains exhibit diverse fermentation capabilities, affecting the metabolic profile of apple juice and, consequently, the quality of cider (Maicas, 2020). However, limited information exists regarding the impact of yeast strains on the fatty acid composition of apple cider, which plays a vital role in its health benefits and sensory characteristics.

Globally and in South Korea, the ‘Fuji’ apple cultivar is widely cultivated, with M9 and M26 being the most commonly used rootstocks. For cider fermentation, commercially available yeasts such as SafCider and Safale are frequently employed. Despite the widespread use of these rootstocks and yeasts, no studies have systematically examined their combined effects on the physicochemical properties, antioxidant activity, and fatty acid composition of apple cider. Filling these gaps in the available information is essential for optimizing cider production and enhancing its market competitiveness. This study aims to evaluate the combined effects of rootstocks and yeast strains on the fundamental qualities of apple cider, including its physicochemical properties, antioxidant capacity, and fatty acid composition. The findings are expected to contribute to the scientific understanding of cider production and provide practical guidance for the development of the apple cider industry in South Korea and beyond.

2. MATERIALS AND METHODS

2.1. Plant materials

In this study, the ‘Fuji’ apple cultivar grafted onto three different rootstocks (M9, P2, and M26) was used. The apple trees, which were six years old, were established at the orchard of Gangwon State Agricultural Research Extension Service (37°52’28.99″N, 127°44’3.01″E) in Chuncheon, Korea. The trees were trained in a slender spindle system and managed using consistent agronomic practices. 150 fully mature fruits were harvested from three different trees. The fruits were immediately transported to the Plant Breeding Laboratory at Gangneung-Wonju National University, Gangneung, Korea.

2.2. Cider making and treatments

The harvested fruits were thoroughly washed with tap water, and dried. The apples were randomly sliced into small pieces, and seeds were removed, and the apple pieces were used to extract juice. The juice was obtained using an apple crusher, and 300 mL of juice was set aside and refrigerated for physicochemical analysis. For fermentation, two commercial yeast strains, Safcider (*Saccharomyces bayanus*) and Safale (*Saccharomyces cerevisiae*), were used. Safcider is optimized for cider production, while Safale is commonly used in beer fermentation. For each rootstock, three replicates were prepared for each yeast treatment, and an untreated juice sample served as a control. To initiate fermentation, 0.15 g of Safcider or 0.65 g of Safale was added to 1-L glass jars containing 700 mL of juice. The mixtures were sealed with rubber stoppers equipped with valves and incubated in the dark at 20 °C for 10 days. After fermentation, the presence of bubbles confirmed active fermentation. The fermented cider was filtered through cheesecloth, transferred to new glass bottles, and stored at 4 °C for further analysis (Figure 1).

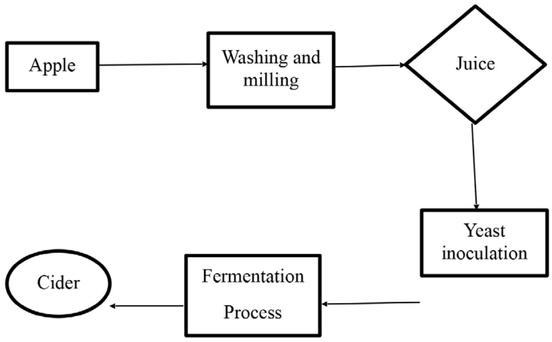

FIGURE 1. Fermentation procedure to produce apple cyder.

2.3. Physicochemical properties

The physicochemical properties of apple juice and cider, including soluble solid content (SSC), titratable acidity (TA), and pH, were analyzed using standardized methods. The SSC was measured using a hand-held digital refractometer (PAL-1; Atago, Tokyo, Japan), with results expressed as °Brix. TA was determined by diluting 1 mL of apple juice or cider with 39 mL of distilled water and measured using the Pal-Easy Acidity meter (Atago, Tokyo, Japan), with results expressed as malic acid equivalents. The pH was measured using a calibrated hand-held pH meter. These analyses followed consistent and validated methodologies as described by Geleta *et al*. (2023).

2.4. Determination of antioxidant properties

To evaluate antioxidant properties, 10 mL of apple juice or cider were centrifuged at 4000 rpm at 4 °C for 20 minutes. Total Phenolic Content, Total Flavonoid Content, DPPH Radical Scavenging Activity, Ferric Reducing Antioxidant Power and Vitamin C analyses were performed.

The total phenolic content was determined using the Folin-Ciocalteu method. Briefly, 100 µL of sample were mixed with 25 µL of 10% diluted Folin-Ciocalteu reagent. After three minutes at room temperature, 37.5 µL of 20% sodium bicarbonate and 62.5 µL of distilled water were added. The mixture was incubated in the dark at room temperature for one hour. Absorbance was measured at 765 nm using a Microplate reader (Mobi, MicroDigital, Seongnam, Korea). Results were expressed in mg of gallic acid equivalent per liter. Total flavonoid content was measured using the aluminum chloride colorimetric method. A mixture of 100 µL of sample, 75 µL methanol, 5 µL of 10% aluminum chloride, and 5 µL of 1 M sodium acetate was prepared. After stirring for 30 minutes in the dark at room temperature, absorbance was recorded at 415 nm using a Microplate reader (Mobi, MicroDigital, Seongnam, Korea). Results were expressed as mg of quercetin equivalents per liter. For the measurement of DPPH radical scavenging activity, a 200 µL sample was mixed with 2.8 mL of 0.004% DPPH solution and incubated in the dark at room temperature for 30 minutes. Absorbance at 517 nm was measured using a Microplate reader (Mobi, MicroDigital, Seongnam, Korea). The percentage inhibition was calculated using the formula:

DPPH Radical Scavenging Activity (%) = [(A_Control - A_Sample) / A_Control] × 100,
where A_Control is the absorbance of the control and A_Sample is the absorbance of the sample.

Ferric Reducing Antioxidant Power (FRAP) was measured following Benzie and Strain (1996). A fresh FRAP reagent was prepared using acetate buffer (300 mM, pH 3.6), 10 mM 2,4,6-Tripyridyl-S-Triazine (TPTZ) dissolved in 40 mM HCl, and 20 mM FeCl3. 100 µl of sample were mixed with 2.9 mL FRAP reagent, incubated at 37 °C for four minutes, and absorbance was measured at 593 nm using a Microplate reader (Mobi, MicroDigital, Seongnam, Korea). Results were expressed as µM Trolox/L. For Vitamin C content, a 2-mL sample was mixed with 10 mL of 15% metaphosphoric acid solution and centrifuged at 4500 rpm for 15 minutes. The supernatant (4 mL) was treated with 0.23 mL 3% bromine, 0.13 mL 10% thiourea, and 1 mL 2,4-Dinitrophenol (2,4-DNP). After incubation at 37 °C for three hours and cooling on ice, 5 mL of 85% sulfuric acid were added. Absorbance was measured at 521 nm using a Spectrophotometer (UV-5490, Neogen, Seoul, Korea). Calibration was performed using ascorbic acid as a standard, with results expressed as ascorbic acid mg/L.

2.5. Lipid extraction

In order to extract lipids, 100 mL of apple juice or cider were mixed with 100 mL of chloroform and 200 mL of methanol. The mixture was incubated with shaking for 1 hour, after which the lower organic phase was collected and washed sequentially with chloroform and distilled water. The lipid extract was subsequently methanolized by adding 2 mL of 2% sodium methoxide and heating at 95 °C for 10 minutes. This was followed by the addition of 2.17 mL of boron trifluoride (14%) and heating at 95 °C for 3 minutes. Fatty acid methyl esters (FAMEs) were extracted three times using hexane, concentrated in clean tubes, and prepared for gas chromatography (GC) analysis.

FAME analysis was performed using a gas chromatograph equipped with a flame ionization detector (GC-FID) and a DB FastFAME column (Youngin Chromass, Anyang, Korea). Prior to injection, the autosampler syringe underwent three cycles of solvent washing followed by two cycles of sample washing to minimize cross-contamination. A 1 µL aliquot of each FAME sample was injected using a CTC Analytics PAL autosampler (Youngin Chromass, Anyang, Korea). Hydrogen was used as the carrier gas, and the GC temperature program started at 70 °C, increasing to 250 °C over a period of 30 minutes. The flame ionization detector was maintained at 280 °C, with hydrogen flow set to 30 mL/min, air flow at 300 mL/min, and nitrogen makeup flow at 20 mL/min. FAMEs were identified by comparing their retention times and peak shapes to those of authentic standards from a Supelco 37 Component FAME Mix (Sigma Aldrich, Darmstadt, Germany). Quantitative analysis was performed using calibration curves generated from these standards, ensuring precise determination of the fatty acid compositions in the samples.

2.6. Statistical analysis

One-way ANOVA was carried out to compare the effect of rootstock on physicochemical properties, antioxidant activity and fatty acid composition traits of apple juice. A combined ANOVA was performed to check the interaction effects of the factors on the variables of apple cider. Mean differences in the data were separated using Duncan's multiple range test (DMRT). A principal component analysis was performed to identify their contribution and their correlation between the variables. All analyses were carried out using SPSS version 28 (IBM, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of rootstock on basic quality and antioxidant properties of apple juice

The quality attributes of apple juice, including soluble solids content (SSC), titratable acidity (TA), and pH, exhibited significant variation based on rootstock type. SSC values ranged from 14.10 to 15.70 °Brix, with the highest levels observed in juices from apples grown on P2 rootstock, followed by M26, while M9-derived apples showed the lowest SSC (Table 1). The superior SSC levels in P2 and M26-derived juices suggest these rootstocks enhance sugar transport and metabolism during fruit development. Although TA differences among the rootstocks were not statistically significant, the variations in SSC resulted in distinct SSC/TA ratios. This ratio, a critical indicator of flavor balance, was highest in P2 and M26-derived juices, indicating enhanced sweetness and consumer appeal. These results align with the European Cider and Fruit Wine Association’s recommended SSC range of 10–15 °Brix and TA values of 0.1% to 0.22% (w/v) for optimal cider apple profiles (Calugar *et al*., 2021). Significant differences in pH were also observed among the rootstocks, with M9-derived juices exhibiting the highest pH, while P2-derived juices had the lowest. In terms of antioxidant properties, including total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, and ferric-reducing antioxidant power (FRAP), P2 and M26-derived juices demonstrated superior performance compared to M9-derived juices, which showed the lowest TPC and FRAP values. Interestingly, vitamin C content was highest in M9 and M26-derived juices, while P2-derived juices exhibited the lowest levels.

TABLE 1. Effect of rootstock on basic quality of apple juice.

Rootstocks	Soluble solid content (ºBrix, SSC)	Titratable acidity (%, TA)	SSC/TA	pH
M9	14.10±0.10c	0.18±0.02ns	79.02±9.39ns	4.04±0.01a
M26	14.80±0.00b	0.18±0.04ns	81.74±17.16ns	4.02±0.04ab
P2	15.70±0.17a	0.20±0.04ns	80.86±16.35ns	3.98±0.01b

The results are expressed as means of 3 replicates (n = 3).
Parameters with different letters are significantly different according to Duncan’s test ($p < 0.01$).
Values are presented as mean ± standard deviation.
ns indicates not significant.

TABLE 2. Effect of rootstock on antioxidant properties of apple juice.

Rootstocks	Total phenolic content (mg GAE/L)	Total flavonoid content (mg QE/L)	DPPH Radical scavenging activity (%)	Ferric reducing antioxidant power (µM Trolox/L)	Vitamin C (mg AA/L)
M9	2.80±0.31b	0.93±0.04ns	35.21±0.71ns	4.44±0.93b	2.97±0.52a
M26	4.29±0.33a	0.90±0.02ns	34.68±0.75ns	6.12±0.07a	2.49±0.06a
P2	4.05±0.34a	0.95±0.01ns	36.64±0.48ns	7.86±1.27a	1.61±0.03b

The results are expressed as means of 3 replicates (n = 3).
Parameters with different letters are significantly different according to Duncan’s test ($p < 0.01$).
Values are presented as mean ± standard deviation.
ns indicates not significant.

The observed variations in SSC, TA, and antioxidant properties can be attributed to the physiological and biochemical mechanisms regulated by rootstocks. Nutrient uptake and translocation efficiency are central factors in determining the quality of apple cider ingredients. It is well known that the absorption of micronutrients varies depending on the rootstock (Martínez-Ballesta *et al*., 2010). Potassium plays a crucial role in sugar assimilation, enabling the production of high-sugar fruits, while zinc and manganese, which are essential for enzymatic antioxidant production, influence antioxidant properties (Tian *et al*., 2023). Additionally, hormones such as cytokinins, transported from the rootstock to the scion, promote sugar accumulation during fruit ripening, whereas abscisic acid, a stress-response hormone, stimulates polyphenol biosynthesis, enhancing antioxidant properties (Hayat *et al*., 2023). Based on these results, it is presumed that the differences in mineral absorption efficiency and hormone transport in P2 and M26 rootstocks resulted in variations in juice quality. Additionally, genotype-specific interactions between rootstock and scion influence tree characteristics (Zebro and Heo, 2024). For instance, the specific canopy structure and tree vigor of certain rootstocks enhance light interception, which is a critical factor in sugar synthesis and antioxidant activity (Rasool *et al*., 2020). Although further investigation is necessary for a more precise understanding, these findings underscore the critical role of rootstock selection in determining the quality attributes and antioxidant potential of apple juice. Depending on production goals—whether prioritizing sweetness, acidity, or antioxidant activity—selecting the appropriate rootstock is essential for optimizing fruit quality.

3.2. Effects of yeast and rootstock on the basic quality, and antioxidant properties of apple cider

The fermentation process further influenced the quality traits and antioxidant properties of apple cider. The SSC of apple juice decreased significantly during fermentation due to sugar consumption by yeast, confirming the conversion of sugars into alcohol (Rosend *et al*., 2020; Wei *et al*., 2020). Among the tested yeasts, Safcider demonstrated a higher sugar assimilation capacity than Safale, leading to a greater SSC reduction across all rootstocks. The maximum SSC was recorded for M26 fermented with Safale, while M9 fermented with Safcider had the lowest SSC (Table 3). These findings indicate that both rootstock and yeast selection significantly affect cider sweetness and balance, offering opportunities for optimizing cider quality. Fermentation increased titratable acidity (TA) in cider compared to non-fermented juice, likely due to the conversion of organic acids into metabolites (Wei *et al*., 2020). TA levels also varied depending on yeast and rootstock combinations, with Safale generally producing higher TA except in M9, where Safcider showed the highest TA. For instance, cider from ‘Fuji’ apples grown on M26 and fermented with Safale achieved a TA of 0.57%, aligning with U.S. standards for high-quality hard ciders (Sommer *et al*., 2022).

TABLE 3. Effect of yeast and rootstock on basic quality attributes of apple cider.

	Rootstocks	Soluble solid content (^{O}Brix)	Titratable acid (Malic acid)	SSC/TA	pH
Safcider	M9	3.70±0.17e	0.35±0.01b	10.57±0.58d	3.83±0.02e
	M26	3.16±0.05f	0.21±0.03c	12.71±2.58c	4.12±0.02b
	P2	4.30±0.00d	0.25±0.02c	20.21±1.38b	4.21±0.00a
Safale	M9	6.00±0.00c	0.30±0.04b	19.78±2.54b	3.98±0.02d
	M26	8.30±0.00a	0.54±0.02a	15.19±0.56c	4.02±0.01c
	P2	6.96±0.05b	0.25±0.01c	27.57±1.84a	3.82±0.05e
Rootstock(R)		792.46***	43.32***	2.27ns	67.93***
Yeast(Y)		54965.06***	82.77***	165.45***	77.16***
R x Y		281.86***	74.37***	0.86ns	225.60***

The results are expressed as means of 3 replicates (n = 3).
Parameters with different letters are significantly different according to Duncan’s test (p < 0.01).
Values are presented as mean ± standard deviation.
ns indicates not significant.
*** Significant at p < 0.001.

Fermentation also caused minor pH variations, with values ranging from 3.82 to 4.21. Safcider fermentation generally yielded higher pH values across rootstocks, except for M9. Studies have shown that pH influences aroma profiles, with higher pH (> 3.98) enhancing cider quality and storage potential (Way *et al*., 2022). M26 rootstock fermented with Safale demonstrated excellent potential for long-term cider storage. The fermentation process improved the antioxidant properties of cider, as evidenced by increased phenolic content and DPPH radical scavenging activity compared to non-fermented juice. Safale consistently produced cider with higher antioxidant properties across all rootstocks, potentially due to enhanced phenolic breakdown and antioxidant enzyme activation during fermentation (Xu *et al*., 2021). Rootstock also significantly influenced antioxidant properties, with P2-derived cider showing the highest levels of most antioxidant traits except vitamin C, which was most abundant in M26-derived cider (Table 4). However, fermentation reduced vitamin C and total flavonoid content (TFC) in cider compared to juice, likely due to oxidation and phytochemical degradation (Kiczorowski *et al*., 2022). These reductions were consistent with findings in other fermented products, such as vegetables, where vitamin C levels declined post-fermentation. These findings highlight the complex interplay between rootstock and yeast in determining the basic quality and antioxidant properties of apple juice and cider. Rootstock selection not only influences the initial juice quality traits but also affects the final characteristics of cider through fermentation. Yeast choice further modulates these effects, providing opportunities to tailor apple juice and cider production to meet specific quality standards and consumer preferences.

TABLE 4. Effect of yeast and rootstock on antioxidant properties of apple cider.

		Total phenolic content (mg GAE/L)	Total flavonoid content (mg QE/L)	DPPH Radical scavenging activity (%)	Ferric reducing anti-oxidant power (μM Trolox/L)	Vitamin C (mg AA/L)
Safcider	M9	3.35±0.35b	0.68±0.04d	45.24±2.09ab	8.65±1.62c	0.39±0.35c
	M26	2.93±0.20c	0.82±0.01b	42.72±2.27b	8.65±1.94b	0.23±0.05d
	P2	3.00±0.12c	0.76±0.01c	45.98±2.18ab	10.41±0.14bc	0.23±0.03d
Safale	M9	4.22±0.10a	0.80±0.02bc	45.60±1.50ab	11.88±3.07ab	0.64±0.10b
	M26	4.21±0.34a	0.76±0.01c	47.57±1.08ab	12.15±0.25ab	1.04±0.02a
	P2	4.57±0.23a	1.06±0.01a	49.50±3.84a	14.05±1.62a	0.50±0.09c
Rootstock (R)		6.73**	68.38***	3.94*	3.85*	15.55**
Yeast (Y)		52.22***	107.43***	94.44***	39.93***	227.2***
R x Y		13.11***	19.27***	1.15ns	0.831ns	9.46***

The results are expressed as means of 3 replicates (n = 3).
Parameters with different letters are significantly different according to Duncan's test ($p < 0.01$).
Values are presented as mean ± standard deviation.
ns indicates not significant.
* Significant at $p< 0.05$
** Significant at $p < 0.01$.
*** Significant at $p <0.001$

3.3 Fatty acid composition of apple cider from different rootstock and yeast

A total of eleven fatty acids were identified in the cider samples, with unsaturated fatty acids predominating over saturated fatty acids. Generally, linoleic acid was the most abundant unsaturated fatty acid, followed by oleic acid, γ-linolenic acid, and α-linolenic acid. The concentration of linoleic acid varied significantly depending on the rootstock and yeast strain used, with the highest levels observed in ciders fermented with Safcider yeast from the M26 and P2 rootstocks. Although research on the relationship between fatty acids and cider quality is limited, studies on fermented foods provide valuable insights. Fatty acids are essential precursors for volatile compounds such as higher alcohols, esters, and aldehydes, which contribute to wine's aroma and flavor (Longo *et al*., 2021). Long-chain fatty acids undergo metabolic transformation into aldehydes and other volatiles, enhancing aromatic complexity (Zhang *et al*., 2023). In contrast, short-chain fatty acids impact volatile acidity, where low concentrations enhance freshness and fruitiness, while excessive levels may lead to undesirable flavors (Perestrelo *et al*., 2019). Given the importance of aroma in cider quality, these findings suggest that rootstock and yeast selection should be carefully considered in future cider production.

Distinct fatty acid profiles were observed among different rootstock and yeast combinations. The P2 rootstock fermented with Safale yeast exhibited the highest levels of butyric acid, whereas the M26 rootstock fermented with Safcider yeast displayed the highest concentrations of lauric, palmitic, and linoleic acids. Similarly, the M9 rootstock fermented with Safcider yeast yielded the highest amounts of caprylic, capric, cis-10 heptadecenoic, myristic, oleic, and γ-linolenic acids. Linoleic acid, the dominant fatty acid in apple cider, not only plays a critical role in aroma formation but is also linked to potential health benefits, including a reduced risk of atherosclerosis, hypercholesterolemia, and chronic diseases (Mercola and D'Adamo, 2023). Saturated fatty acids constituted 46.25% of the total fatty acid content, with palmitic acid (C16:0; 59.48%), cis-10 heptadecenoic acid (C17:0; 16.89%), and lauric acid (C12:0; 5.46%) being the major contributors. Interestingly, the highest palmitic acid levels were found in ciders from the M26 rootstock fermented with Safcider yeast, underscoring the impact of rootstock on fatty acid composition.

Fermentation led to increased fatty acid levels in cider compared to non-fermented apple juice, indicating that the fermentation process enhances fatty acid synthesis. This increase is likely linked to NAD+ regeneration during redox reactions, which facilitates fatty acid biosynthesis (Xing *et al*., 2023). Among the tested combinations, ciders from the M26 and P2 rootstocks fermented with Safcider yeast exhibited the most favorable fatty acid profiles. These findings provide crucial insights into the effects of rootstock and yeast selection on the sensory and nutritional characteristics of the cider, offering valuable guidance for optimizing cider production to enhance both consumer appeal and health benefits.

TABLE 5. Effect of yeast and rootstock on fatty acid composition of apple cider as expressed in mg/mL.

Treatment	Rootstock	Butyric (C4:0)	Caprylic (C8:0)	Capric (C10:0)	Lauric (C12:0)	Heptadecenoic Cis-10 (C17:0)	Myristic (C14:0)	Palmitic (C16:0)	Oleic C18:1cis (n9)	Linoleic C18:2cis (n6)	γ-Linolenic C18:3n6	α-Linolenic C18:3n3
	M9	1.04e	2.84c	2.25a	3.21bc	8.00b	5.09b	26.32d	8.11de	39.83c	4.38ab	0.23b
Control	M26	2.66abcd	5.527b	2.25a	3.21bc	4.98d	2.56d	23.83de	8.32de	36.80c	0.68e	ND
	P2	2.16bcde	1.62d	2.36a	3.83b	4.81d	2.93d	22.50e	8.57d	27.36de	2.73cd	1.05b
	M9	3.24ab	ND	1.92a	2.07de	13.03b	5.02b	21.30	7.59de	29.27de	1.81de	0.02b
Safale	M26	3.71ab	ND	2.26a	2.39cd	14.64b	5.02b	21.36e	7.59de	26.16e	2.36cd	1.21b
	P2	4.22a	1.56d	ND	1.45e	ND	ND	16.90g	6.08e	28.15de	2.73cd	0.55b
	M9	1.21de	6.22a	2.80a	3.20bc	26.30a	9.44a	53.10b	37.91a	31.03d	5.38a	0.14b
Safcider	M26	1.60cde	ND	1.55a	6.08a	14.83b	4.08c	69.67a	28.48b	108.01a	2.06cde	ND
	P2	3.11abc	ND	ND	2.59cd	ND	2.75d	49.89c	16.58c	87.06b	3.55bc	2.61a

The results are expressed as means of 3 replicates (n = 3).
Parameters with different letters are significantly different according to Duncan's test ($p < 0.01$).
Values are presented as mean ± standard deviation.
ND indicates not detected.
*** Significant at $p < 0.001$

3.4. Principal component analysis of apple cider quality attributes

A principal component analysis (PCA) was conducted to evaluate the contributions of yeast strains and rootstocks to the quality attributes of apple cider and to elucidate correlations among various variables. PCA illustrated the relationship between the initial juice composition and the chemical content of the cider, influenced by the yeast strains used for fermentation (Figure 2). Two principal components, the first principal component (PCAI) and the second principal component (PCAII), explained 32.25 and 23.58% of the total variance, respectively, providing a comprehensive representation of the data. The biplot revealed that PCAI was strongly associated with apples fermented with Safcider, while PCAII was linked to apples fermented with Safale and the initial apple juice. Rootstocks P2 and M26, when fermented with Safcider, contributed significantly to the production of major fatty acids, antioxidant activity, and fundamental cider quality attributes such as TA. In contrast, ciders fermented with Safale exhibited higher TPC, butyric acid, and α-linolenic acid levels across the three rootstocks. These findings underscore the metabolic variations among yeast strains, despite their belonging to the same species. Such variations are consistent with evidence that genetic diversity among yeast strains can lead to differences in metabolite synthesis (Breunig *et al*., 2014).

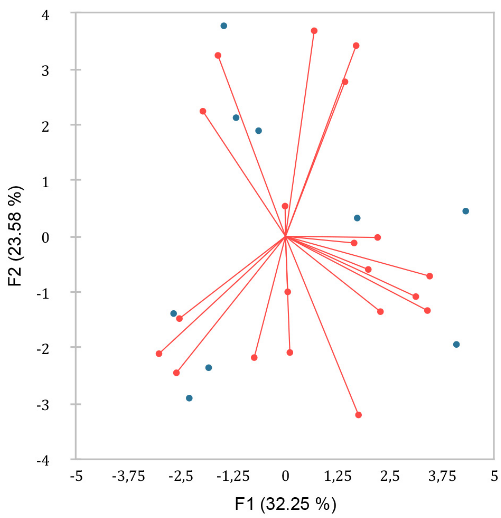

FIGURE 2. PCA analysis of cider quality characteristics; where, SC: Safcider; C:Control; SA:Safale.

The antioxidant properties were dispersed across the PCA plot, reflecting complex synthesis pathways. Antioxidants measured by DPPH and FRAP assays were positively correlated with TA, indicating that TA may enhance antioxidant properties. This aligns with findings that higher titratable acidity improves antioxidant activity in fermented milk and hot brewed coffee (Rao and Fuller, 2018). Moreover, major fatty acids, both saturated and unsaturated, exhibited positive correlations with one another, emphasizing the importance of a balanced fatty acid composition. Managing the polyunsaturated/saturated fatty acid ratio is crucial for maintaining optimal serum LDL-cholesterol levels (Guo *et al*., 2010). Additionally, some fatty acids positively correlated with antioxidant properties; for instance, butyric acid and α-linolenic acid were positively associated with total phenolic content, while others exhibited negative correlations. This finding is consistent with previous research showing that fatty acid content correlates positively with resveratrol and negatively with rutin in grape pomace samples (Szabó *et al*., 2021).

The study also revealed that apple juice fermented with Safale exhibited higher physicochemical and antioxidant properties, whereas juice fermented with Safcider presented elevated fatty acid levels. This variation may result from differences in the genetic composition of the yeast strains. Our findings indicate that apple juice fermented with Safcider exhibits high levels of physicochemical properties (such as acidity and pH), antioxidant properties (FRAP and DPPH), and major fatty acid composition, making it a promising choice for producing high-quality apple cider from the 'Fuji' cultivar.

4. CONCLUSIONS

This study demonstrated that the interaction between rootstocks and yeast strains significantly influences the quality attributes of apple cider, including physicochemical properties, antioxidant activity, and fatty acid composition. Specific combinations of rootstocks and yeast strains were found to enhance key cider quality parameters, emphasizing the importance of optimizing these factors in cider production. For instance, the M26 and P2 rootstocks fermented with Safcider exhibited superior fatty acid profiles, while Safale fermentation enhanced antioxidant properties, demonstrating the distinct contributions of yeast strains to cider quality. Additionally, the 'Fuji' apple cultivar was shown to be a potential source for cider production, with its juice meeting critical standards for apple cider, such as balanced acidity, pH, and phenolic content.

The results of this study provide valuable insights into the role of rootstocks and yeast strains in shaping the chemical and nutritional profiles of cider, which has practical implications for the apple industry. By leveraging the identified optimal combinations of rootstocks and yeasts, producers can tailor cider production to meet consumer preferences and market demands. Furthermore, this research highlights the potential for expanding the use of apples in Korea, particularly for value-added products like cider, which can contribute to the diversification and economic growth of the apple industry. Future research should explore a broader range of apple cultivars commonly grown in Korea to validate these findings and further refine the production process. Expanding this research will provide a more comprehensive understanding of how rootstock and yeast interactions influence cider quality and pave the way for innovative approaches to apple utilization in Korea and beyond.

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

AUTHORSHIP CONTRIBUTION STATEMENT

B. Tolera Geleta: Conceptualization, Methodology, Investigation, Writing – original draft. J.Y. Heo: Formal analysis, Writing – review & editing.

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