

Article

Co-Fermentation with Cider By-Products for Enhanced Beverage Quality: Kinetics, Functionality, and Sensory Impact

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Abstract

This study evaluated a new co-valorization strategy using apple pomace and a nitrogen-rich yeast lees extract to correct low concentrations of amino acids and phenolic compounds that can impair fermentation kinetics, aroma formation, and antioxidant activity, thereby improve cider quality and support the principles of the circular economy. Apple must (Gala) was supplemented with nitrogen extract (80 mg/L), dehydrated apple pomace (47.3 g/L), or both, and fermented with *Saccharomyces cerevisiae*. Fermentation kinetics, physicochemical parameters, individual phenolic profile (HPLC), antioxidant activity (DPPH, ABTS, FRAP, and CUPRAC), color (CIELAB), and sensory attributes were assessed. Nitrogen supplementation accelerated fermentation, increasing the maximum rate from 9 to 16 g CO₂/L/day when combined with apple pomace. While nitrogen extract alone reduced phenolic levels, its combination with pomace resulted in a balanced phenolic profile and superior fruity aroma. Apple pomace addition increased titratable acidity (1.6 times) and ethanol content (>1% v/v) and enhanced total phenolics (up to 20%) and antioxidant activity (up to 46% by FRAP). Sensory analysis indicated that the combined treatment achieved the highest overall acceptance. The integrated use of apple pomace and yeast lees extract represents a sustainable new strategy to enhance cider bioactivity, fermentation performance, and sensory quality while valorizing agro-industrial residues.

Keywords: apple pomace; nitrogen-rich extract; fermentation kinetics; phenolic compounds; activity antioxidant; sensory quality



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1. Introduction

Cider is an alcoholic beverage produced through the total or partial alcoholic fermentation of apple must and is the second most consumed fruit wine worldwide, after grape wine [1]. Its growing popularity is associated with a refreshing sensory profile, moderate alcohol content, gluten-free characteristics, and the presence of bioactive compounds, particularly polyphenols, which have been linked to antioxidant activity and potential health benefits [2]. Currently produced in more than 35 countries [3,4], cider exhibits considerable diversity resulting from differences in apple cultivars and processing practices. These

factors directly influence its chemical composition and sensory attributes, including aroma, color, turbidity, acidity, astringency, sweetness, carbonation, and foam stability [4–7].

Despite the expansion of cider production, the use of dessert apple cultivars remains a challenge due to their relatively low phenolic content, which may be up to five times lower than that found of traditional European cider apple. This limitation often results in beverages with lower antioxidant potential and reduced sensory complexity. Phenolic compounds contribute significantly to important quality attributes such as color, bitterness, astringency, and aroma development. However, a substantial proportion of these compounds remain in apple pomace after juice extraction. Previous studies have reported that approximately 42–58% of the total phenolic content can be retained in this product, mainly because of hydrophobic interactions and molecular interaction between phenolic compounds and cell wall components [3,8].

Apple pomace accounts for approximately 25–30% of the original apple mass processed and has been generated worldwide at an estimated volume of 7.5 million tons annually [9]. Composed primarily of peel and flesh residues (95%), together with seeds (2–4%) and stems (1%), this material is a valuable source of bioactive compounds. Apple pomace contains phenolic substances with recognized antioxidant activity, anti-inflammatory, antimutagenic, and antimicrobial properties, making it a promising ingredient for applications in the food, pharmaceutical, and cosmetic sectors [10,11].

However, apple pomace is often discarded in landfills. Its high moisture content (>70%) and elevated biodegradable organic load can contribute to environmental concerns when inadequately managed [12]. Consequently, specialized waste management is required, increasing disposal costs for juice processors. Therefore, the development of safe and efficient strategies for apple pomace utilization is essential, particularly given its large production volume and potential for value-added applications in the food and nutraceutical industries [11,13].

In addition to phenolic composition, cider quality is strongly influenced by its aroma profile, which depends on both characteristics of the apple must and yeast metabolic activity during fermentation. Fruity and floral aromas are particularly important attributes associated with consumer acceptance [4,7]. Among the factors affecting aroma formation, the availability of assimilable nitrogen plays a critical role. Insufficient nitrogen levels may result in sluggish or incomplete fermentations and can significantly reduce the formation of desirable volatile compounds [3].

Yeast lees constitute another important by-product generated during cider production. They are formed as sediment during fermentation and storage and consist of both liquid and solid fractions. The solid fraction is composed mainly of microbial biomass, including yeast and bacterial cells, together with insoluble carbohydrates and other precipitated material [14]. Depending on the type of fermented beverage, lees generation may range from approximately 2 to more than 60 kg per 1000 L of product with a moisture content between 85 and 90% [15]. Although yeast lees are commonly used as animal feed after treatments to eliminate viable cells, their potential remains underexploited. This by-product contains approximately 50% protein and can serve as a valuable source of nutrients for biotechnological applications. Moreover, its valorization may contribute to reducing environmental impacts associated with disposal practices, particularly when residues are discarded without prior stabilization or thermal treatment [16].

Apple pomace and yeast lees are the main solid residues generated by the cider industry. Within the framework of the circular economy, their recovery and reutilization represent an attractive strategy to increase process sustainability and resource efficiency. Apple pomace may provide a source of phenolic compounds capable of enhancing cider

bioactivity and sensory quality, whereas extracts obtained from yeast lees can supply assimilable nitrogen to support fermentation performance and aroma formation [17,18].

Previous studies have demonstrated that low phenolic content in dessert apples must be associated with reduced antioxidant capacity and lower sensory acceptance of cider. In addition, assimilable nitrogen is essential not only for yeast growth but also for the biosynthesis of aroma-active compounds [4,19]. Nitrogen concentrations below 100 mg/L, particularly regarding amino acids such as aspartic acid, asparagine, glutamic acid, and alanine, may negatively affect alcoholic fermentation kinetics and impair the formation of esters and higher alcohols which contribute substantially to the characteristic fruity aroma of cider [20].

Therefore, the present study evaluated the combined use of apple pomace and nitrogen-rich extract obtained from yeast lees during cider processing. The objective was to assess their potential to improve fermentation kinetics, enhance sensory quality, and functional properties, and promote the sustainable valorization of major cider-processing by-products.

2. Materials and Methods

2.1. Materials

Experiments were conducted using 54 kg of commercial apples (Category III), variety Gala, with a ripeness index between 4 and 5, as defined by Reid et al. [21]. The fruits were obtained from the local market of Ponta Grossa, Paraná, Brazil. Alcoholic fermentation for cider production was carried out using the yeast *Saccharomyces cerevisiae* Fermol Reims Champagne (ref. PB 2002, AEB Group, Brescia, Italy). Around of 5 kg of *Saccharomyces cerevisiae* yeast lees (Levteck Company, Ale type, batch 1P23102015, Florianópolis, Santa Catarina, Brazil), with 80% moisture content, was obtained from the Koch Bier microbrewery located in Ponta Grossa, Paraná, Brazil.

The reagents used included Folin–Ciocalteu, DPPH (2,2-diphenyl-2-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), TPTZ (2,4,6-tri(2-pyridyl)-s-triazine), and Trolox standard (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, 97% purity). All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The phenolic standards used in this study included hydroxycinnamic acids, flavan-3-ols, dihydrochalcones, and flavonols. Specifically, hydroxycinnamic acids comprised caffeic acid (98%), chlorogenic acid (95%), and p-coumaric acid (98%). Flavan-3-ols included (+)-catechin (98%), (–)-epicatechin (98%), procyanidin B1 (98%), and procyanidin B2 (98%). The dihydrochalcone phloridzin (99%) was also used. Flavonol standards comprised quercetin derivatives, namely quercetin-3-D-galactoside (hyperoside) (97%), quercetin-3-β-D-glucoside (isoquercetin) (90%), quercetin-3-O-rhamnoside (quercitrin) (78%), quercetin-3-rutinoside (rutin) (94%), and quercetin-O-α-L-arabinofuranoside (avicularin) (purity ≥ 90%). These were purchased from Sigma-Aldrich (Steinheim, Germany). Sugar analysis standards included D-glucose and sucrose (99%) (Sigma-Aldrich, Steinheim, Germany), as well as fructose and glycerol (99%) (Merck, Darmstadt, Germany). Analytical-grade reagents and solvents used in the study comprised acetic acid (≥99.7%) and acetonitrile (99.9%) (J. T. Baker, Phillipsburg, NJ, USA), and ethanol (99.5%) (Anidrol, Diadema, SP, Brazil). Ultrapure water (Type 1) was obtained using a Millipore system (Barueri, SP, Brazil).

2.2. Processing of Apple Must and Apple Pomace

The apples were carefully selected, weighed, and sanitized prior to processing. They were then crushed and pressed (AGM Máquinas, Bento Gonçalves, RS, Brazil) at 294 kPa for 5 min to obtain the integral apple must, with an average physical yield (η_p) of 71.7%.

The must was subsequently depectinized using a pectinolytic enzyme (Pectinex® Ultra Clear, LNF Bento Gonçalves and Novozymes Latin America LTDA, Araucária, Brazil) at a dosage of 3.0 mL/hL for 2 h at room temperature (25 ± 2 °C). After depectinization, the apple must was racked and stored at -18 °C until cider processing.

The apple pomace obtained after processing was dehydrated at 60 °C for 12 h in a forced-air circulation oven (Marconi, model MA035/5, Piracicaba, SP, Brazil). Subsequently, the material was ground using a knife mill (IKA Werke, M20, Wilmington, NC, USA) and sieved (>20 mesh), yielding particles with diameters greater than 0.84 mm. The processed material was then packaged in plastic bags. Moisture content analysis of the residue was performed in triplicate, according to the methodology described by AOAC [22], by drying the samples at 105 °C, using 5 g of sample per crucible.

2.3. Production of Nitrogen Extract from Yeast Lees

Brewer's yeast slurry was selected for this study to satisfy the required volume, given the similarity between brewer's and cider yeast biomasses. The brewer's slurry (Figure 1A) was washed three times with distilled water (20:80, *w/v*) and centrifuged at $3475 \times g$ for 5 min (Hitachi Himac CR21GII, Tokyo, Japan) (Figure 1B). The washed yeast slurry was then treated using an ultrasonic device (Sonics Vibra-Cell, 500 W, 20 kHz; model VC505, Newtown, CT, USA) for the extraction of nitrogenous compounds. The extraction was carried out for 30 min, at an amplitude of 70%, with a yeast-to-water ratio of 55:45 (*w/v*). The temperature was maintained at 20 °C by circulating chilled water through the jacket surrounding the treatment flask. Subsequently, the mixture was centrifuged again ($3475 \times g$ for 5 min) (Figure 1C), and the supernatant was collected and stored at -20 °C for further use. Total nitrogen content in the supernatant was determined in triplicate using the Kjeldahl method, according to AOAC [22].

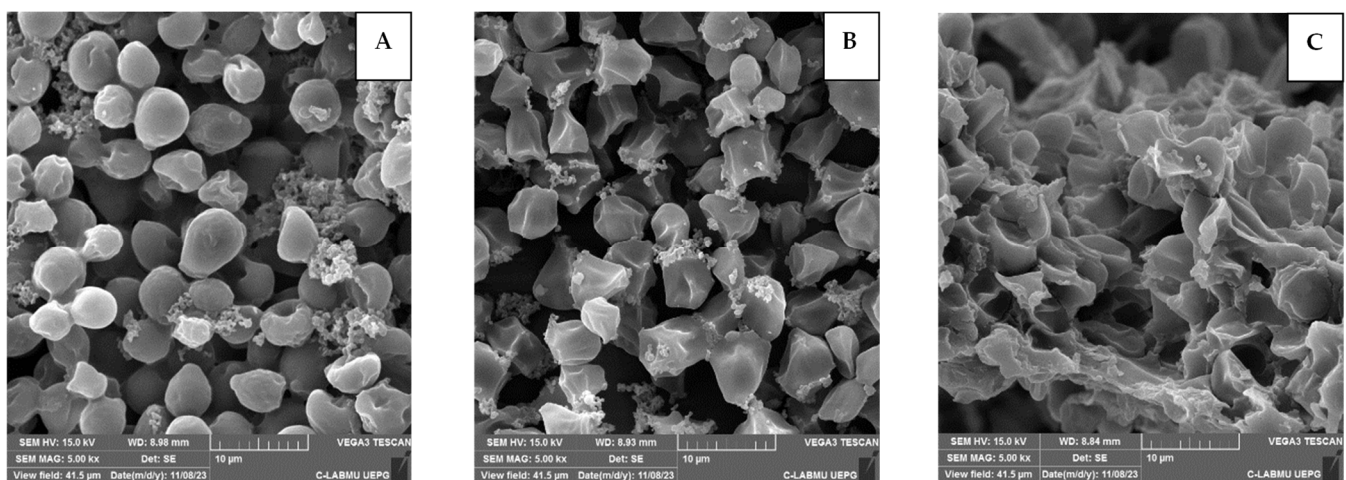


Figure 1. Scanning electron microscopy (SEM) images of yeast lees before and after treatments. Note: (A) yeast lees, (B) washed and centrifuged yeast lees, (C) yeast lees after ultrasound treatment.

2.4. Apple Must Treatments and Cider Production

Apple must was thawed at room temperature (20 ± 2 °C), homogenized, and distributed into 24 fermenters with a working volume of 450 mL (500 mL total volume) and four fermenters with a working volume of 1500 mL (1600 mL total volume). The fermenters were assigned to four treatments: (1) Apple must (M); (2) Apple must supplemented with nitrogen extract (80.0 mg/L) (ME); (3) Apple must supplemented with apple pomace (47.3 g/L) (MB); and (4) Apple must supplemented with apple pomace (47.3 g/L) and nitrogen extract (80.0 mg/L) (MEB). The concentration of 47.3 g/L of dehydrated apple pomace corresponds to the amount generated from the production of one liter of apple

must. The addition of 80 mg/L, together with the low nitrogen levels present in the must (<100 mg/L), may prevent sluggish or stuck fermentations and contribute to a more standardized aromatic profile in cider production. Apple pomace was immobilized at a ratio of 47.3 g/L in molin bags (10 cm × 7 cm, height and length) with glass balls to ensure submersion. *S. cerevisiae* was rehydrated according to the manufacturer's instructions and inoculated at a rate of 20 g/hL. The fermenters were sealed with S-type airlocks containing a potassium metabisulfite solution at 300 mg/L. Alcoholic fermentation was conducted for 15 days at 20 ± 2 °C. Each treatment included fermentation stop points at 1, 2, 3, 6, 10, and 15 days. At this stage, each fermenter was stirred for 10 min using a magnetic stirrer to resuspend the yeast cells and obtain a representative sample for cell counting. The yeast biomass and cider were separated by centrifugation at $1917 \times g$ for 15 min at 4 °C, and the resulting supernatant was collected for subsequent analyses.

2.5. Fermentation Monitoring

2.5.1. Viable Cell Count

Viable cells (cells/mL) were determined using a Neubauer chamber according to Bonneu et al. [23].

2.5.2. Fermentation Rate

The alcoholic fermentation process was monitored by measuring density using a digital densimeter (accuracy ± 0.001 g/mL; DMA 35 V3 Basic, Anton Paar, Graz, Austria), as well as by tracking system mass loss resulting from CO₂ release. Sample weight was recorded every 4 h over a period of 15 days, using an analytical balance with a sensitivity of 0.01 g (BL3200h, Shimadzu, São Paulo, Brazil), following the methodology described by Roger et al. [24]. The fermentation rate was calculated based on the variation in carbon dioxide (CO₂) loss over time (Equation (1)), where V represents the fermentation rate (CO₂ g/L/day), ΔCO_2 is the change in CO₂ production (g/L), and Δt is the change in time (days).

$$V = \Delta\text{CO}_2 / \Delta t \quad (1)$$

2.5.3. Physicochemical Analyses

Total Kjeldahl nitrogen (TKN) of the extract, apple must, and ciders was determined using the micro-Kjeldahl method, according to AOAC [22]. Reducing and total sugar contents were quantified using the Somogyi–Nelson method, as described by Maldonado et al. [25]. Alcohol content was determined by ebulliometric measurement, following the methodology proposed by Curvelo-Garcia [26]. Titratable acidity was expressed as malic acid and determined by neutralization with 0.1 M NaOH to pH 8.33, using phenolphthalein as the indicator, according to AOAC [22]. Sample pH was measured using a bench-top pH meter (Tecnal, model TEC 3-MP, São Paulo, Brazil).

2.5.4. Phenolic Composition

Total phenolic compounds were determined according to Singleton and Rossi [27]. Absorbance was measured at 760 nm using an Epoch microplate spectrophotometer (Synergy-BioTek, Winooski, VT, USA), and concentrations were calculated using a chlorogenic acid calibration curve ($\text{TPC} = 1612.90 \times A_{760}$; $R^2 = 0.9932$). Results were expressed as milligrams of chlorogenic acid equivalents per liter (mg CAE/L), and all analyses were performed in triplicate.

Individual phenolic compounds were analyzed by HPLC (Waters Alliance 2695, Waters, Milford, MA, USA) according to Alberti et al. [6]. Prior to analysis, 10 mL of samples were frozen at -80 °C (Terroni, Cold 80, São Carlos, SP, Brazil) and lyophilized (Terroni, LS3000, São Carlos, SP, Brazil). The lyophilized material was reconstituted in a solution

of 2.5% acetic acid (*v/v*) and methanol (3:1, *v/v*), then filtered through a 0.22 µm nylon syringe filter (Waters). The chromatographic system consisted of a Waters Alliance 2695 equipped with a quaternary pump, degasser, and autosampler, coupled to a Waters PDA 2998 photodiode array detector and a Symmetry C18 column (4.6 × 150 mm, 3.5 µm; Waters), operated at 20 °C. Identification and quantification were performed by comparison of retention times and spectra with commercial standards.

2.5.5. Antioxidant Activity

In vitro antioxidant activities were assessed using the DPPH [28], ABTS [29], FRAP [30], and CUPRAC [31] methods. The concentration was obtained by comparing the absorbances with the standard curve of trolox of 100–1000 mol/L (DPPH = $22.72 \times A_{517}$, $R^2 = 0.9946$; ABTS = $4.48 \times A_{734}$, $R^2 = 0.9944$; FRAP = $1041.66 \times A_{595}$, $R^2 = 0.9986$; and CUPRAC = $3333.33 \times A_{450}$, $R^2 = 0.9969$).

2.5.6. Colorimetric Analysis

Cider color was expressed in the Commission Internationale de l'Éclairage (CIE) L^* , a^* , and b^* color space coordinates. Samples were analyzed using a CM-5-ID digital colorimeter (KONICA MINOLTA, Osaka, Japan) under Illuminant D65, with data processed using SpectraMagic NX software (CM-S100w, version 2.6, Osaka, Japan). The CIELAB coordinates (L^* , a^* , and b^*) were further converted into RGB, HEX, and CMYK color systems using NIX[®] color sensor software (version 3.0, Osaka, Japan). The chroma (C^*) parameter, representing color intensity, and the hue angle (h°), representing color tone, were calculated according to Equations (2) and (3), respectively.

$$C^* = (a^2 + b^2)^{1/2} \quad (2)$$

$$h^\circ = \tan^{-1} (b^*/a^*) + 180^\circ \text{ when } a^* < 0 \text{ and } h^* = \tan^{-1} (b^*/a^*) \text{ when } a^* > 0 \quad (3)$$

2.6. Scanning Electron Microscopy (SEM)

For scanning electron microscopy (SEM) analysis, samples of yeast biomass and apple pomace were frozen using an ultra-freezer (Nuair, model NU-9668GC, Plymouth, MN, USA) and subsequently freeze-dried (Terroni, model LS3000, São Paulo, Brazil). The dried samples were gold-sputtered and then examined using a scanning electron microscope (Vega 3, Tescan, Brno-Kohoutovice, Czech Republic).

2.7. Sensorial Analysis

The sensory attributes evaluated in the four cider treatments after 15 days of fermentation included sweetness, bitterness, alcohol flavor, astringency, acidity, aftertaste, color intensity, alcoholic odor, apple odor, yeast odor, fruity odor, and overall preference, according to a model adapted from Rodríguez Madrera et al. [32]. Sensory analysis was performed in triplicate by a trained panel of ten judges (four men and six women, aged 24–49), previously familiarized with the evaluated attributes and the use of the intensity scale through descriptive training sessions.

Samples (50 mL) were served at 15 °C in transparent 100 mL glasses coded with random three-digit numbers. The presentation order of samples was randomized for each evaluation session to minimize position and carry-over effects. Judges evaluated each attribute independently using a nine-point structured intensity scale, where 1 corresponded to very weak intensity, 5 to moderate intensity, and 9 to very strong intensity. For overall preference, the same nine-point scale was used, with higher scores indicating greater acceptance. Water was provided between samples to minimize sensory fatigue and carry-over

effects. The study was approved by the Research Ethics Committee of the State University of Ponta Grossa (CAAE 62047516.3.0000.0105).

2.8. Statistical Analysis

Data were expressed as mean $\pm$ sample standard deviation. Differences among samples were evaluated by analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) test, when applicable. Pearson's correlation analysis was also performed to assess relationships between individual phenolic compounds and antioxidant activities. All statistical analyses were performed using STATISTICA® software (version 13.3; TIBCO Software Inc., Palo Alto, CA, USA).

3. Results and Discussion

3.1. Effect of Nitrogen Extract and Apple Pomace on Fermentation Kinetics

The monitoring of alcoholic fermentation kinetics is shown in Figure 2 and Table S1 (Supplementary Material). The addition of nitrogen extract (ME) clearly enhanced both the fermentation rate and carbon dioxide (CO₂) production compared with untreated apple must (M) (Figure 2A), indicating that the initial yeast assimilable nitrogen (YAN) content of the must (~130 mg/L) was below the level required to sustain optimal yeast metabolic activity. Nitrogen availability is a key factor regulating yeast growth and fermentative performance; thus, nitrogen supplementation likely improved protein synthesis capacity and enzymatic activity, accelerating sugar catabolism and CO₂ release. In addition, the supplementation of the extract to the must may have increased dissolved oxygen levels in the mixture, thereby contributing to a higher rate of alcoholic fermentation [19].

The treatments containing apple pomace also promoted higher CO₂ release (Figure 2A), suggesting that, in addition to its compositional contribution, the pomace may have provided extra nutrients or fermentation-supporting compounds. In treatment MEB (nitrogen extract and apple pomace), a synergistic effect was observed, resulting in both the highest fermentation rate and cumulative CO₂ production. The maximum fermentation rate reached 16 g CO₂/L/day in MEB, whereas the unsupplemented or control apple must (M) showed a maximum rate of only 9 g CO₂/L/day (Figure 2B). The improvement observed in all supplemented treatments demonstrates that both nitrogen addition and pomace incorporate positively modulated fermentation kinetics, likely by alleviating nutritional limitations and improving yeast physiological performance.

YAN depletion occurred within the first two days of fermentation (Figure 2C), which corresponds to the exponential growth phase of yeasts, during which nitrogen is essential for amino acid and protein synthesis and, consequently, biomass formation. Residual non-assimilable nitrogen remained at approximately 50 mg/L. Total nitrogen consumption ranged from ~70 mg/L in treatments without supplementation to ~100 mg/L in those receiving nitrogen extract (Figure 2C). These results reinforce that the original YAN concentration of the apple must was insufficient to support maximum yeast growth and may also have limited the formation of volatile aroma compounds derived from specific amino acids [33].

However, nitrogen supplementation via extract appeared excessive, leaving an additional ~50 mg/L of residual assimilable nitrogen. Under this apple must condition, supplementation in the range of 30–50 mg/L would likely have been adequate to sustain fermentation without nutrient excess.

As shown in Figure 2D, nitrogen extract addition did not significantly affect cider acidity, indicating that nitrogen supplementation mainly influenced yeast metabolism rather than acid balance. In contrast, apple pomace addition increased final cider acidity by 62%. Apple pomace contains approximately 2.0 g/100 mL of residual acidity [11], and due

to the high solubility of malic acid, its transfer from the solid matrix to the liquid phase likely occurred during fermentation. This mass transfer process explains the increased titratable acidity of the final product and highlights the technological impact of pomace incorporation on cider chemical composition.

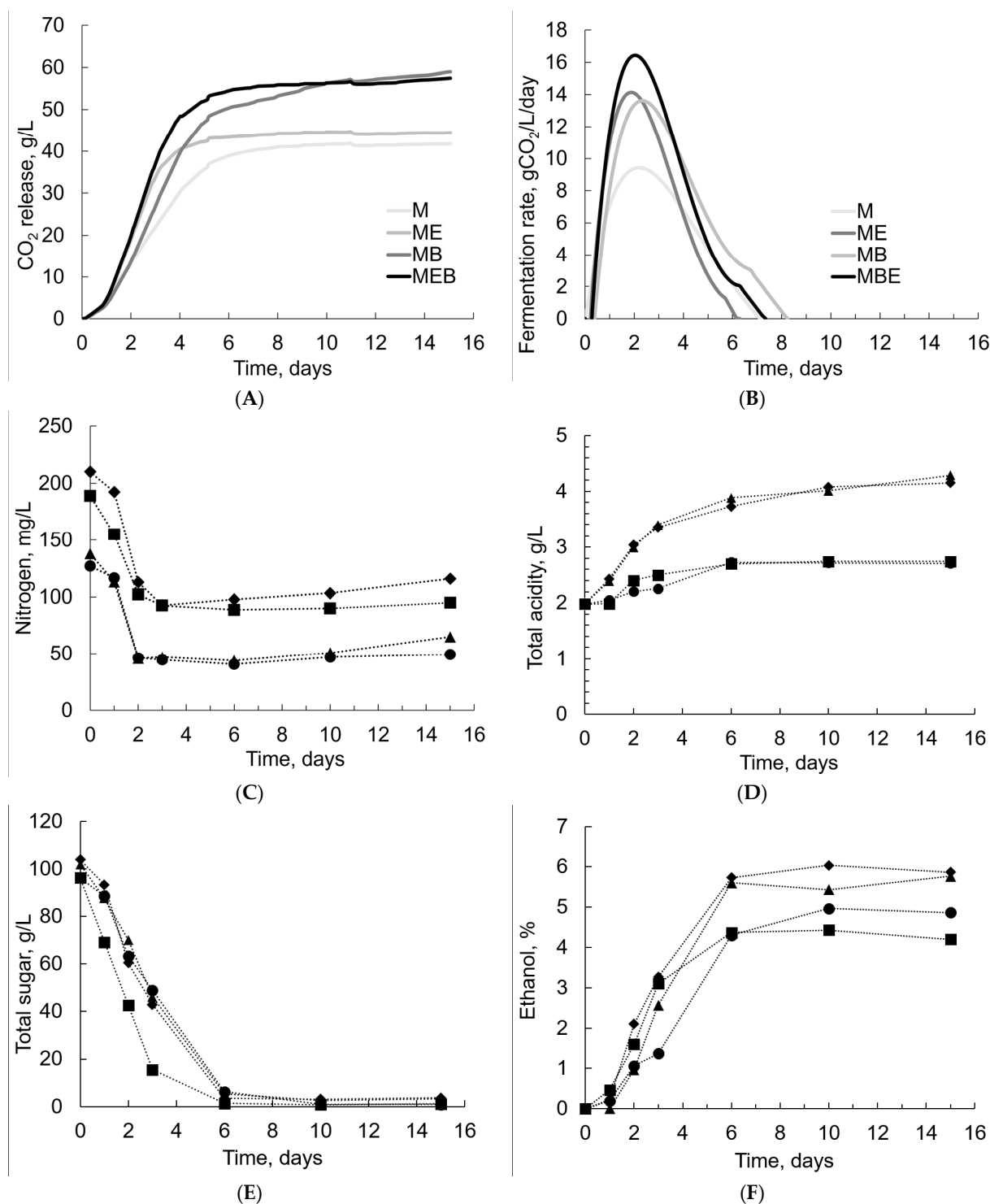


Figure 2. Kinetic parameters of the alcoholic fermentation of ciders supplemented with nitrogen extract from yeast lees and apple pomace. (A): CO₂ release; (B): Fermentation rate; (C): Nitrogen consumption; (D) Acidity; (E) Sugar consumption; and (F) Ethanol production. Note: (●) apple must (M); (■) apple must + nitrogen extract (ME); (▲) apple must + apple pomace (MB); and (◆) apple must + nitrogen extract and apple pomace (MEB).

The addition of immobilized apple pomace to apple must release residual sugars into the medium, leading to higher sugar levels in treatments supplemented with apple pomace (MB) and with both nitrogen extract and apple pomace (MEB). The free sugar content in apple pomace ranges from 39 to 62 g/kg [34,35], supporting its role as an additional fermentable substrate source.

Sugar consumption in the ME treatment was rapid during the first 3 days of alcoholic fermentation, followed by a slower depletion rate, with complete sugar exhaustion occurring by day 6 (Figure 2E). In this case, dissolved oxygen may have been the limiting factor for alcoholic fermentation, since assimilable nitrogen was still present in cider (Figure 2C). Fermentable sugars were depleted from the medium within approximately six days of alcoholic fermentation (Figure 2E). As shown in Figure 2F, ethanol concentration stabilized over the same period and differed as a function of apple pomace supplementation, with increases greater than 1.0% (*v/v*). These data confirm the incorporation and subsequent bioconversion of pomace-derived sugars into ethanol. Therefore, the combined supplementation of apple pomace and nitrogen extract (MEB) resulted in a synergistic effect, not only enhancing fermentation rate but also increasing the final alcohol content of the cider.

3.2. Yeast Immobilization and Color Evolution During Alcoholic Fermentation

In ciders supplemented with apple pomace (MB and MEB), yeasts were observed adhering to the pomace matrix, particularly to the cell wall structures of the residue, regardless of nitrogen extract addition (Figure 3). Therefore, yeast growth curves were not presented.

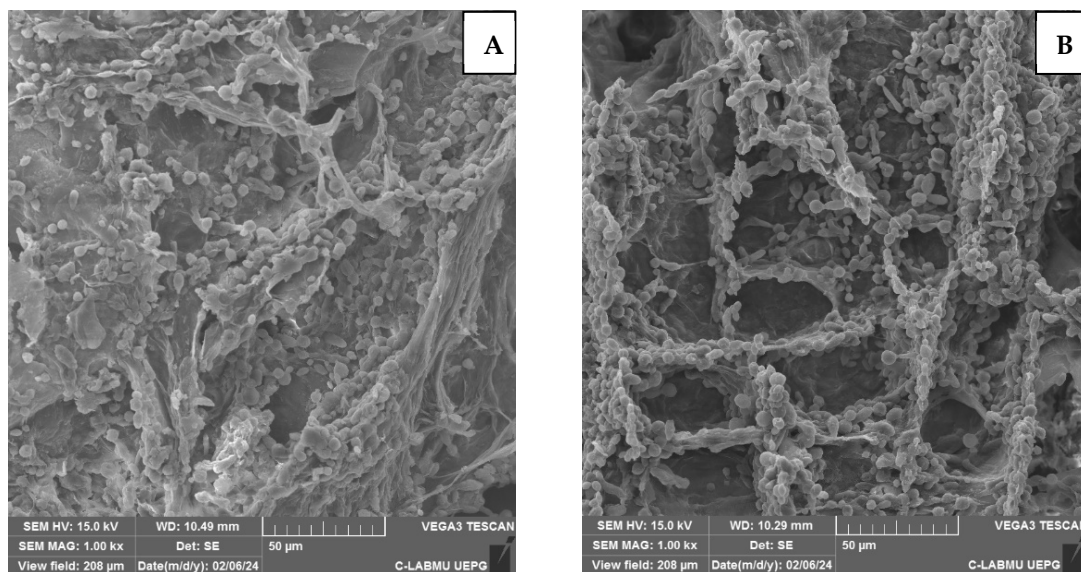


Figure 3. Scanning electron microscopy (SEM) images of yeasts adhered to the cell wall of apple pomace on the fifteenth day of cider alcoholic fermentation. Note: (A) apple pomace from cider fermentation without the addition of nitrogen extract (MB); (B) apple pomace from cider fermentation with the addition of nitrogen extract (MBE).

The natural clarification of cider during alcoholic fermentation is a typical phenomenon and may be associated with reactions inherent to the fermentative process, such as the consumption of sugars and nitrogen, as well as the retention of phenolic compounds on yeast cell walls [36]. The presence of apple pomace promoted not only yeast immobilization but also improved cider clarity and transparency in MB and MEB treatments. This effect may be associated with the physical retention of suspended particles and yeast cells

within the pomace matrix, contributing to a natural clarification process and influencing the physicochemical characteristics of the final beverage.

Consistent with this behavior, lightness (L^*) values increased, whereas a^* and b^* values decreased throughout cider fermentation. Supplementation of the apple must with nitrogen extract and/or apple pomace (ME, MB, and MEB) exerted a greater influence on these color parameters than the control must (M). Consequently, a similar trend was observed for the chroma (C^*) values, which showed a pronounced reduction in all treatments, indicating a substantial loss of color intensity as fermentation progressed (Table 1).

Table 1. Color parameters of cider samples supplemented with nitrogen extract from yeast lees and apple pomace.

Parameters	Treatments	Fermentation Time, Days					
		1	2	3	6	10	15
L^*	M	91.90 ^{Df} ± 0.04	92.67 ^{De} ± 0.05	92.87 ^{Dd} ± 0.03	94.52 ^{Dc} ± 0.03	96.07 ^{Db} ± 0.04	96.41 ^{Da} ± 0.05
	ME	96.11 ^{Af} ± 0.05	96.43 ^{Ae} ± 0.01	96.52 ^{Ad} ± 0.01	96.96 ^{Bc} ± 0.01	97.02 ^{Cb} ± 0.01	97.12 ^{Ca} ± 0.3
	MB	92.57 ^{Cf} ± 0.04	94.08 ^{Ce} ± 0.06	94.98 ^{Cd} ± 0.03	96.75 ^{Cc} ± 0.06	97.30 ^{Ab} ± 0.07	97.75 ^{Aa} ± 0.05
	MEB	94.14 ^{Bf} ± 0.04	95.75 ^{Be} ± 0.07	96.19 ^{Bd} ± 0.07	97.04 ^{Ac} ± 0.06	97.17 ^{Bb} ± 0.0	97.68 ^{Ba} ± 0.05
a^*	M	−4.11 ^{Ae} ± 0.02	−4.68 ^{Cf} ± 0.03	−4.02 ^{Bd} ± 0.01	−3.61 ^{Cc} ± 0.01	−3.31 ^{Cb} ± 0.02	−3.05 ^{Da} ± 0.02
	ME	−5.61 ^{Df} ± 0.02	−4.40 ^{Ae} ± 0.01	−3.83 ^{Ad} ± 0.01	−2.76 ^{Ac} ± 0.01	−2.39 ^{Ab} ± 0.01	−2.13 ^{Aa} ± 0.00
	MB	−5.29 ^{Cf} ± 0.02	−4.97 ^{Dd} ± 0.03	−5.24 ^{De} ± 0.01	−4.12 ^{Dc} ± 0.01	−3.56 ^{Db} ± 0.02	−2.93 ^{Ca} ± 0.01
	MEB	−5.15 ^{Be} ± 0.03	−4.48 ^{Bd} ± 0.03	−4.47 ^{Cd} ± 0.03	−3.23 ^{Bc} ± 0.02	−2.84 ^{Bb} ± 0.02	−2.45 ^{Ba} ± 0.01
b^*	M	60.56 ^{Aa} ± 0.01	54.18 ^{Ab} ± 0.02	47.58 ^{Ac} ± 0.02	32.50 ^{Ad} ± 0.03	25.22 ^{Ae} ± 0.04	22.89 ^{Af} ± 0.06
	ME	33.89 ^{Da} ± 0.06	28.82 ^{Db} ± 0.03	22.95 ^{Dc} ± 0.02	17.59 ^{Dd} ± 0.01	15.88 ^{De} ± 0.02	14.58 ^{Cf} ± 0.02
	MB	57.53 ^{Ba} ± 0.05	45.43 ^{Bb} ± 0.05	37.47 ^{Bc} ± 0.04	24.35 ^{Bd} ± 0.06	18.92 ^{Be} ± 0.08	15.38 ^{Bf} ± 0.05
	MEB	46.60 ^{Ca} ± 0.02	31.52 ^{Cb} ± 0.07	27.84 ^{Cc} ± 0.08	20.07 ^{Cd} ± 0.09	16.87 ^{Ce} ± 0.10	13.74 ^{Df} ± 0.07
C^*	M	60.70 ^{Aa} ± 0.01	54.38 ^{Ab} ± 0.02	47.75 ^{Ac} ± 0.02	32.70 ^{Ad} ± 0.03	25.44 ^{Ae} ± 0.04	23.09 ^{Af} ± 0.06
	ME	34.35 ^{Da} ± 0.06	29.15 ^{Db} ± 0.03	23.26 ^{Dc} ± 0.02	17.80 ^{Dd} ± 0.01	16.06 ^{De} ± 0.02	14.73 ^{Cf} ± 0.01
	MB	57.78 ^{Ba} ± 0.05	45.71 ^{Bb} ± 0.05	37.83 ^{Bc} ± 0.04	24.70 ^{Bd} ± 0.06	19.26 ^{Be} ± 0.07	15.66 ^{Bf} ± 0.05
	MEB	46.88 ^{Ca} ± 0.02	31.83 ^{Cb} ± 0.06	28.20 ^{Cc} ± 0.07	20.33 ^{Cd} ± 0.09	17.11 ^{Ce} ± 0.10	13.96 ^{Df} ± 0.06
h°	M	93.88 ^{Df} ± 0.02	94.93 ^{Dd} ± 0.03	94.82 ^{Ce} ± 0.03	96.33 ^{Dc} ± 0.02	97.48 ^{Db} ± 0.05	97.60 ^{Da} ± 0.06
	ME	99.40 ^{Ab} ± 0.03	98.68 ^{Ad} ± 0.01	99.46 ^{Aa} ± 0.00	98.91 ^{Cc} ± 0.02	98.57 ^{Ce} ± 0.02	98.31 ^{Cf} ± 0.02
	MB	95.26 ^{Cf} ± 0.02	96.25 ^{Ce} ± 0.03	97.96 ^{Bd} ± 0.02	99.61 ^{Ac} ± 0.05	100.65 ^{Ab} ± 0.09	100.79 ^{Aa} ± 0.08
	MEB	96.31 ^{Be} ± 0.03	98.10 ^{Bd} ± 0.07	99.14 ^{Bc} ± 0.07	99.15 ^{Bc} ± 0.08	99.56 ^{Bb} ± 0.10	100.09 ^{Ba} ± 0.07

Note: M: apple must; ME: apple must + nitrogen extract; MB: apple must + apple pomace; and MEB: apple must + nitrogen extract and apple pomace. Different lowercase letters in the same row indicate statistically significant differences among days ($p < 0.05$). Different uppercase letters in the same column indicate statistically significant differences among treatments ($p < 0.05$), and different lowercase letters in the same row indicate significant differences in the samples during fermentation ($p < 0.05$) according to Fisher's test.

During apple must processing, enzymatic oxidation of phenolic compounds by polyphenol oxidase (PPO) leads to the formation of brown pigments in the early stages, which are responsible for the initial must color [37]. In the present study, the apple must exhibit a hue angle (h°) of approximately 86° (yellowish green), which increased to around 99° (greenish yellow) during cider fermentation, indicating a shift in color tone.

The control apple must (M) maintained the highest color intensity throughout fermentation, whereas the must supplemented with nitrogen extract showed markedly lower color intensity from the first day. This behavior may be attributed to enhanced yeast metabolic activity promoted by nitrogen availability, which accelerates fermentation and favors the degradation, transformation, and precipitation of phenolic compounds and pigments [38]. In contrast, apple pomace addition contributed to maintaining color intensity, likely due to the release and retention of phenolic compounds, tannins, and pigments from the solid matrix [38].

An intermediate effect on color was observed in the treatment combining apple pomace and nitrogen extract, indicating antagonistic interactions between the two factors. While pomace supplementation tends to increase and stabilize color through its phenolic contribution, nitrogen extract stimulates yeast metabolism, promoting pigment transformation and removal from the medium. Over fermentation time, all treatments showed

decreasing a^* and b^* values, indicating a reduction in reddish and yellowish tones and the development of a lighter, less saturated color. Simultaneously, the increase in hue angle (h°) across treatments confirms a progressive shift toward pale yellow or yellowish-green hues during fermentation (Table 1).

3.3. Effect of Apple Must Supplements on Phenolic Compounds and Antioxidant Activity

As shown in Table 2, the MB and MEB treatments exhibited higher total phenolic content, 20% and 18%, respectively, and greater antioxidant activity (DPPH, FRAP, CUPRAC, and ABTS assays) from the first day until the end of alcoholic fermentation (15 days) when compared with the untreated apple must (M). Ciders produced with the MB treatment showed an increase in antioxidant activity after 15 days of alcoholic fermentation ranging from 4.4% (DPPH) to 28.7% (FRAP). However, the most pronounced improvements were observed in the MEB treatment, with increases ranging from 17.7% (CUPRAC) to 46.4% (FRAP). This difference may be attributed to more intense fermentative activity promoted by the nitrogen extract, which enhanced the extraction of other bioactive compounds, even though phenolic levels were lower than those observed in the MB treatment.

Table 2. Phenolic content and antioxidant activity of cider samples supplemented with nitrogen extract from yeast lees and apple pomace.

Parameters	Treatment	Fermentation Time, Days					
		1	2	3	6	10	15
TPC (mg CAE/L)	M	258.6 ^{Ba} ± 5.4	245.8 ^{Cb} ± 4.6	245.6 ^{Cb} ± 2.6	205.8 ^{Cc} ± 6.1	190.5 ^{Cd} ± 3.1	192.5 ^{Bd} ± 9.2
	ME	206.7 ^{Ca} ± 5.7	209.2 ^{Da} ± 2.2	193.1 ^{Db} ± 2.3	175.0 ^{Dc} ± 2.7	187.2 ^{Cb} ± 1.5	177.1 ^{Cc} ± 2.7
	MB	278.3 ^{Aa} ± 1.1	294.2 ^{Ab} ± 4.0	277.2 ^{Aa} ± 2.2	243.1 ^{Ac} ± 5.2	238.3 ^{Ac} ± 2.2	230.6 ^{Ad} ± 3.8
	MEB	282.8 ^{Aa} ± 2.8	262.2 ^{Bb} ± 2.2	265.8 ^{Bb} ± 1.7	228.6 ^{Bc} ± 1.9	220.5 ^{Bd} ± 2.4	227.2 ^{Ac} ± 2.1
	M	544.0 ^{Bc} ± 8.0	596.5 ^{Bb} ± 8.6	648.7 ^{Ba} ± 8.1	607.2 ^{Bab} ± 8.1	585.7 ^{Bbc} ± 4.9	609.8 ^{Bab} ± 6.4
DPPH (μmol ET/L)	ME	520.9 ^{Bcd} ± 8.0	519.7 ^{Ccd} ± 10.6	476.9 ^{Cd} ± 7.3	537.8 ^{Cc} ± 6.3	724.5 ^{Aa} ± 8.7	621.5 ^{Bb} ± 8.3
	MB	667.0 ^{Abc} ± 9.5	739.3 ^{Aa} ± 9.3	703.8 ^{ABab} ± 6.7	653.8 ^{Bbc} ± 6.5	659.3 ^{Bbc} ± 10.2	636.2 ^{Bc} ± 10.5
	MEB	695.5 ^{Abc} ± 9.1	627.7 ^{Bd} ± 8.1	663.9 ^{Ac} ± 6.9	707.6 ^{Abc} ± 8.7	732.6 ^{Ab} ± 4.5	813.9 ^{Aa} ± 5.9
	M	2988.7 ^{Bb} ± 7.8	3006.2 ^{Cb} ± 4.2	3172.5 ^{Ba} ± 3.8	3106.2 ^{Cab} ± 7.0	2976.2 ^{Bb} ± 2.5	2995.0 ^{Bb} ± 4.8
	ME	2420.0 ^{Cc} ± 2.6	2566.2 ^{Db} ± 4.5	2588.7 ^{Cb} ± 7.1	2685.0 ^{Dab} ± 4.9	2748.7 ^{Ca} ± 4.6	2582.5 ^{Cb} ± 6.5
CUPRAC (μmol ET/g)	MB	3257.5 ^{Ae} ± 3.3	4015.5 ^{Aa} ± 4.2	3787.0 ^{Ab} ± 2.0	3683.7 ^{Abc} ± 4.4	3578.7 ^{Ac} ± 4.3	3397.5 ^{Ad} ± 2.3
	MEB	3230.0 ^{Aa} ± 5.1	3359.6 ^{Bbc} ± 5.1	3679.6 ^{Aa} ± 12.5	3352.5 ^{Bbc} ± 2.3	3674.1 ^{Aa} ± 4.1	3526.2 ^{Aab} ± 2.6
	M	870.4 ^{Cab} ± 8.4	817.3 ^{Bbc} ± 7.3	862.3 ^{Bab} ± 7.1	889.0 ^{Ca} ± 7.2	804.8 ^{Dbc} ± 6.9	787.3 ^{Cc} ± 8.3
	ME	773.7 ^{Dbc} ± 6.5	716.0 ^{Cc} ± 9.0	799.5 ^{Bb} ± 6.9	783.1 ^{Db} ± 9.9	929.2 ^{Ca} ± 4.0	748.7 ^{Cbc} ± 6.7
	MB	970.4 ^{Bd} ± 5.6	1110.6 ^{Abc} ± 7.5	1141.7 ^{Ab} ± 10.3	1255.7 ^{Aa} ± 8.7	1078.1 ^{Bbc} ± 9.3	1013.3 ^{Bcd} ± 10.7
FRAP (μmol ET/L)	MEB	1049.3 ^{Ac} ± 9.5	1138.6 ^{Aabc} ± 11.6	1211.2 ^{Aab} ± 9.5	1104.5 ^{Bbc} ± 8.1	1216.5 ^{Aa} ± 6.9	1152.1 ^{Aabc} ± 9.4
	M	2300.7 ^{Ba} ± 3.8	2323.4 ^{Ba} ± 2.5	2297.4 ^{Ca} ± 4.8	2277.6 ^{Ca} ± 1.4	2051.5 ^{Cb} ± 5.2	2057.7 ^{Cb} ± 4.2
	ME	1963.0 ^{Ccd} ± 3.2	1999.0 ^{Cc} ± 24.8	2030.2 ^{Bbc} ± 7.0	2122.0 ^{Bab} ± 3.7	1890.4 ^{Dd} ± 3.6	2129.6 ^{BCa} ± 6.3
	MB	2666.5 ^{Aab} ± 5.9	2748.3 ^{Aa} ± 1.7	2564.4 ^{Ab} ± 4.1	2376.6 ^{Ac} ± 3.5	2367.3 ^{Bc} ± 5.9	2229.3 ^{Bd} ± 4.3
	MEB	2583.1 ^{Aab} ± 2.8	2689.1 ^{Aa} ± 3.7	2549.6 ^{Abc} ± 7.2	2354.8 ^{Ad} ± 3.8	2491.2 ^{Abc} ± 3.0	2456.9 ^{Ac} ± 4.7

Note: TPC: total phenolic compounds; DPPH: determination of antioxidant activity by DPPH radical (2,2-diphenyl-1-picrylhydrazyl) scavenging; CUPRAC: determination of antioxidant activity by copper reduction; FRAP: determination of antioxidant activity by ferric ion reduction; ABTS: determination of antioxidant activity by ABTS cation decolorization (2,2'-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid). M: apple must; ME: apple must + nitrogen extract; MB: apple must + apple pomace; and MEB: apple must + nitrogen extract and apple pomace. CAE: chlorogenic acid equivalent. Different uppercase letters in the same column indicate significant differences among treatments, and different lowercase letters in the same row indicate significant differences in the samples during fermentation ($p < 0.05$) according to Fisher's test.

Correlation analysis demonstrated positive and significant associations between total phenolic content and all antioxidant assays, particularly ABTS ($r = 0.8663$; $p < 0.001$), CUPRAC ($r = 0.7087$; $p < 0.001$), and FRAP ($r = 0.6086$; $p = 0.002$), reinforcing the major contribution of phenolic compounds to the antioxidant capacity of the ciders. A moderate but significant correlation was also observed with DPPH ($r = 0.4235$; $p = 0.039$), indicating that different antioxidant mechanisms may respond differently to the phenolic profile of the samples. Moreover, strong and significant correlations were observed among the antioxidant assays themselves, particularly between CUPRAC and FRAP ($r = 0.8912$; $p < 0.001$),

and ABTS and CUPRAC ($r = 0.8167$; $p < 0.001$). Significant correlations were also found between DPPH and CUPRAC ($r = 0.7291$; $p < 0.001$), DPPH and FRAP ($r = 0.7364$; $p < 0.001$), and FRAP and ABTS ($r = 0.7451$; $p < 0.001$). These results indicate good agreement among the different antioxidant methods despite their distinct reaction mechanisms. While DPPH and ABTS are primarily based on radical scavenging reactions, FRAP and CUPRAC evaluate the reducing capacity of antioxidants. The consistent correlations observed among these assays suggest that the phenolic compounds extracted from apple pomace contributed simultaneously to both electron-transfer and radical-quenching mechanisms, reinforcing the robustness of the antioxidant response observed in the ciders.

Although oxidative browning reactions, chemical associations, adsorption onto yeast cell walls, and precipitation of phenolic compounds may occur during fermentation [10,39,40], the extraction of phenolic compounds from apple pomace, favored by the increasing ethanol concentration, was predominant and persisted throughout the 15 days of fermentation. Adsorption may occur through interactions between phenolic hydroxyl groups and structural components of the yeast cell wall, particularly β -glucans and mannoproteins, reducing the concentration of free phenolics in the liquid phase. In addition, yeast metabolism and residual enzymatic activities may promote the biotransformation of native phenolic compounds into derivatives with different chemical structures and antioxidant properties. Phenolics may also be removed from the medium through complexation and precipitation reactions involving proteins, polysaccharides, and cell wall fragments generated during fermentation. Nevertheless, the higher concentrations of total phenolics observed in MB and MEB indicate that the continuous release of compounds from the pomace matrix exceeded this removal mechanisms under the conditions evaluated. In contrast, the treatment supplemented only with nitrogen extract (ME) exhibited lower total phenolic content and antioxidant activity than the control cider (M). This behavior may be associated with the higher fermentative activity observed in this treatment, which could have intensified adsorption, biotransformation, and precipitation processes, thereby reducing the concentration of phenolic compounds remaining in the final cider (Table 2) [41].

Table 3 shows a reduction in individual phenolic compounds from apple must to the control cider after 15 days of fermentation. Consistent with the results observed for total phenolics, the ME treatment exhibited the lowest concentration of individual phenolic compounds (82 mg/L), whereas the MB treatment showed the highest levels (177 mg/L), likely due to the continuous contribution of phenolics from the apple pomace matrix. These findings reinforce the role of apple pomace as a relevant source of bioactive compounds and as a key factor modulating the chemical composition of cider.

Epicatechin was the main phenolic compound detected in apple must; however, after 15 days of fermentation, its concentration was markedly reduced, and the compound was detected only in the MB treatment (Table 3). This result indicates that alcoholic fermentation substantially affected the availability of epicatechin, while the continuous release of phenolic compounds from apple pomace may have partially compensated for its reduction in the MB cider. Similar changes in the concentration of individual phenolic compounds during cider fermentation have been previously reported, reflecting modifications in the phenolic profile throughout the process [39]. Furthermore, interactions between phenolic compounds and other constituents of the fermentation medium, including proteins, suspended particles, and yeast biomass, may contribute to their removal from the soluble fraction [40]. Therefore, the lower concentrations of epicatechin observed in the final ciders likely result from a combination of precipitation and transformation phenomena occurring during fermentation.

Table 3. Concentration of individual phenolic compounds in apple must and cider samples supplemented with nitrogen extract from yeast lees and apple pomace.

Phenolic Compounds, mg/L	Apple Must	Ciders, 15 Days			
		M	ME	MB	MEB
Phloretin-2-xyloglucoside	11.33 ^b ± 0.02	10.00 ^c ± 0.12	8.22 ^d ± 0.02	3.85 ^e ± 0.03	13.27 ^a ± 0.03
Phloridzin	13.59 ^e ± 0.05	24.71 ^d ± 0.07	30.40 ^c ± 0.02	64.57 ^a ± 0.16	46.81 ^b ± 0.05
Epicatechin	63.89 ^b ± 0.14	nd	nd	4.09 ^a ± 4.09	nd
Chlorogenic acid	40.18 ^a ± 0.05	31.48 ^b ± 0.03	24.07 ^c ± 0.64	24.18 ^c ± 0.09	21.41 ^d ± 0.08
Caffeic acid	6.72 ^{ab} ± 0.06	7.33 ^{ab} ± 0.03	5.65 ^b ± 0.36	7.74 ^{ab} ± 0.05	10.14 ^a ± 4.61
p-Coumaric acid	3.34 ^c ± 0.16	3.48 ^b ± 0.01	2.89 ^d ± 0.01	3.93 ^a ± 0.03	3.26 ^c ± 0.01
Rutin	nd	nd	nd	8.14 ^a ± 0.03	7.22 ^b ± 0.05
Hyperoside	2.97 ^c ± 0.14	2.50 ^d ± 0.02	2.23 ^e ± 0.02	22.20 ^a ± 0.08	11.45 ^b ± 0.03
Quercetin	2.28 ^c ± 0.34	1.66 ^d ± 0.01	1.66 ^d ± 0.01	6.35 ^a ± 0.04	4.10 ^b ± 0.02
Avicularin	2.86 ^c ± 0.30	2.59 ^d ± 0.00	2.40 ^e ± 0.02	5.64 ^a ± 0.03	3.10 ^b ± 0.02
Quercitrin	5.98 ^c ± 0.77	4.90 ^d ± 0.00	3.67 ^e ± 0.01	15.45 ^a ± 0.07	8.54 ^b ± 0.05
Quercetin pentoside	nd	nd	nd	2.75 ^a ± 0.04	1.18 ^b ± 0.03
Quercetin xyloside	1.33 ^c ± 0.40	0.99 ^d ± 0.05	0.74 ^e ± 0.05	8.23 ^a ± 0.04	3.10 ^b ± 0.03
Σ of compounds	154.44	99.64	81.93	177.12	133.58

Note: Different letters in the same row indicate significant differences among samples ($p < 0.05$). nd = not detected. M: apple must; ME: apple must + nitrogen extract; MB: apple must + apple pomace; and MEB: apple must + nitrogen extract and apple pomace.

Phloridzin and chlorogenic acid were the phenolic compounds present at the highest concentrations in the ciders, exceeding the levels detected in apple must. These compounds are not formed or synthesized during alcoholic fermentation; rather, their increased concentrations can be attributed to enhanced solubility and extraction of matrix-bound compounds in the presence of ethanol, as well as to an apparent concentration effect during fermentation [39,41]. The latter may result from CO₂ loss, precipitation of solids and lees, and changes in effective volume, leading to an apparent increase in concentration. Additionally, the increase in phloridzin concentration during fermentation may be attributed to its synthesis, possibly through the glycosylation of phloretin catalyzed by yeast glycosyltransferases, as suggested by [42].

Quercetins glucosides, compounds known for their high antioxidant activity, were present at higher concentrations in the MB treatment (Table 3), which helps explain the greater antioxidant activity observed in this treatment. The higher abundance of these compounds, together with the elevated levels of chlorogenic acid and phloridzin, further supports the strong correlations observed between total phenolic content and antioxidant capacity, indicating that both the concentration and composition of phenolic compounds contributed to the enhanced functional properties of the ciders produced with apple pomace supplementation.

3.4. Sensory Impact of Nitrogen Extract and Apple Pomace Supplementation

Figure 4 presents the mean intensity scores assigned by the trained panel to each sensory attribute using the nine-point structured scale. Higher scores indicate greater perceived intensity of the evaluated attribute, whereas overall preference reflects the degree of acceptance of the cider samples.

Treatments involving apple pomace addition significantly and positively influenced the sensory profile of the cider (Figure 4). Ciders produced under ME and MB conditions showed increased astringency perception compared with the control and MEB treatment. This characteristic can be considered desirable, as moderate astringency contributes to cider body and mouthfeel [43]. However, when both supplements were combined (MEB), astringency perception was attenuated, possibly due to increased sensory complexity, where multiple attributes may have balanced or masked this sensation. The higher acidity

perceived by panelists in pomace-supplemented treatments is consistent with the analytical titratable acidity results (Figure 2D). Acidity is closely related to freshness perception and plays a key role in cider sensory quality [39].

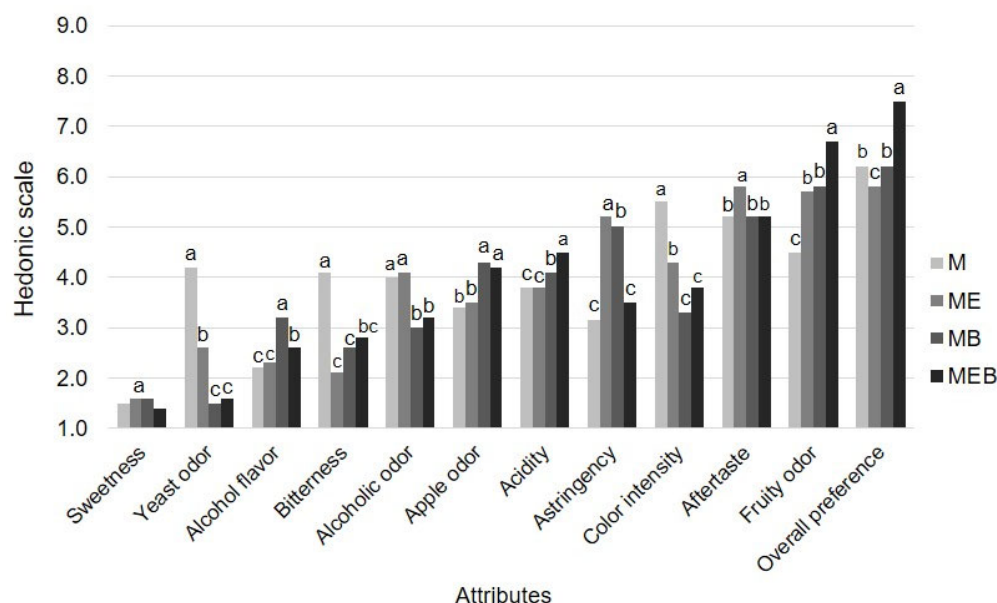


Figure 4. Sensory profile of cider samples supplemented with nitrogen extract from yeast lees and apple pomace. Note: M: apple must; ME: apple must + nitrogen extract; MB: apple must + apple pomace; and MEB: apple must + nitrogen extract and apple pomace. Different letters within each sensory attribute indicate significant differences ($p < 0.05$).

In treatments containing apple pomace (MB and MEB), negative odors were detected at low intensity, while positive aromatic attributes were significantly enhanced (Figure 4). Consequently, the MEB treatment exhibited the most attractive sensory profile and the highest overall acceptance. Although the ME and MB treatments individually improved several attributes compared with the control, such as (<) bitterness, (>) astringency, (<) yeast-related odor, and (>) fruity aroma, their combined application resulted in a synergistic effect, leading to superior overall sensory quality of the cider. The extraction of phenolic compounds in MB and MEB (Table 2) did not significantly affect the sensory perception of astringency and bitterness, possibly due to differences in the phenolic profile extracted during alcoholic fermentation (Table 3).

The most prominent attribute in the MEB treatment was fruity aroma (Figure 4). This effect may be associated with a higher availability of amino acids, such as asparagine, aspartic acid, glutamic acid, and alanine, which are key precursors in the synthesis of higher alcohols and esters responsible for fruity aromatic notes [38]. These amino acids are the first to be consumed during the yeast growth phase, which leads to a reduction in their concentration or their depletion in the stationary phase, when the highest production of volatile compounds responsible for fruity aroma occurs [4].

This study demonstrated the feasibility of valorizing two major by-products of the cider industry: apple pomace and yeast lees. Although both residues required prior processing before application, their positive impact on cider quality clearly outweighed the associated treatment costs.

Following alcoholic fermentation, the immobilized apple pomace remained within the system. This material, depleted of fermentable sugars and nutrients, can be dehydrated to remove residual ethanol and subsequently repurposed as ruminant feed. Due to the absence of readily fermentable substrates, the increased protein content resulting from

yeast biomass incorporation, and its naturally high dietary fiber content, the resulting pomace represents a value-added feed ingredient.

4. Limitations

This study was conducted using the dessert apple cultivar Gala, which typically has a chemical composition that is less favorable for high-quality cider production. This reflects the reality in many apple-producing countries, where dessert cultivars predominate in commercial orchards. Nevertheless, the use of industrial cultivars specifically intended for cider production may yield different results and should be explored in future research.

Another aspect to consider is the use of dehydrated apple pomace, which may increase production costs compared to fresh or wet pomace. Previous studies have reported comparable results using fresh pomace. In the present study, dried pomace was selected to ensure greater experimental control and standardization.

Furthermore, the nitrogen extract must be produced by a company equipped with appropriate technology and quality monitoring procedures, as was ensured in this study.

5. Conclusions

The combined use of apple pomace and nitrogen extract from yeast lees represents a novel and sustainable approach to improving cider quality while simultaneously valorizing important agro-industrial by-products. Nitrogen supplementation enhanced fermentation kinetics, increasing CO₂ production, fermentation rate, and ethanol yield, whereas apple pomace contributed additional fermentable sugars, organic acids, and phenolic compounds.

Ciders supplemented with apple pomace showed higher total phenolic content and antioxidant activity throughout fermentation. The combined treatment (apple pomace and nitrogen extract) resulted in a synergistic effect, providing both improved fermentative performance and enhanced functional properties.

Sensory analysis indicated that the combined supplementation produced the most attractive cider, characterized by enhanced fruity aroma, balanced acidity and astringency, and the highest overall acceptance.

Overall, this novel approach improves the technological, functional, and sensory attributes of cider produced from dessert apples while supporting the sustainable valorization of apple pomace and yeast lees within a circular economy framework.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/beverages12070076/s1>, Table S1: Physicochemical characteristics of cider samples supplemented with nitrogen extract and apple pomace.

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