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Impact of Blending Ratios on Fermentation Kinetics and Bioactive Enrichment in Lychee–Pineapple Co-Fermented Functional Cider

Newitchaya Wutthinithisanand [1] and Jakapan Rodsup [2,*]

[1] Division of Food Science and Technology, School of Agriculture and Natural Resources, University of Phayao, Phayao 56000, Thailand; newitchaya.wu@up.ac.th
[2] Department of Food Science and Technology, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Phitsanulok 65000, Thailand
* Correspondence: krodsup@rmutl.ac.th

Abstract

This study investigated the development of functional ciders through lychee–pineapple co-fermentation as a strategy for juice matrix optimization. Five formulations were evaluated, ranging from 100% pineapple juice to 100% lychee juice, using *Saccharomyces cerevisiae* EC-1118. Fermentation kinetics were monitored by cumulative CO_2 mass loss, while total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacities (DPPH and ABTS), sensory attributes, and multivariate analyses were evaluated. Lychee incorporation significantly enriched the functional profile, increasing TPC, TFC, DPPH, and ABTS by up to 49%, 117%, 69%, and 82%, respectively, compared to 100% pineapple juice. Although the lychee-rich matrix extended the fermentation lag phase, this effect was mitigated through co-fermentation. Fermentation further enhanced bioactive properties, with final ciders showing increases of up to 14% (TPC), 17% (TFC), 38% (DPPH), and 16% (ABTS) relative to their corresponding juices. Multivariate profiling differentiated lychee-dominant ciders by high bioactive density and floral notes, whereas pineapple-dominant ciders exhibited higher acidity and clarity. In conclusion, lychee–pineapple co-fermentation effectively optimizes fermentation performance, bioactive enrichment, and sensory quality without exogenous acidification.

Keywords: fruit cider; co-fermentation; bioactive potential; synergistic blending; organoleptic properties; *Litchi chinensis*; *Ananas comosus*

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1. Introduction

Northern Thailand is an important production region for tropical fruits, particularly lychee (*Litchi chinensis*) and pineapple (*Ananas comosus*). In 2024, Phayao Province was identified as one of the major lychee-producing areas in Upper Northern Thailand, and its 'Mae Jai' lychee has been recognized as a Geographical Indication (GI) product [1,2]. Similarly, Chiang Rai Province is renowned for its GI-certified 'Phulae' and 'Nang Lae' pineapples [2]. Both fruits are rich in vitamins, phenolic compounds, and antioxidants, making them attractive raw materials for functional beverage development [3,4]. Furthermore, the conversion of locally produced tropical fruits into fermented beverages represents a promising value-added strategy for improving resource utilization and reducing postharvest losses associated with highly perishable fruit crops [5]. However, despite their nutritional and economic value, they are still predominantly marketed as fresh fruits or conventional juices.

The growing demand for health-oriented and value-added beverages has stimulated interest in fruit-based ciders, a category that has expanded beyond traditional apple cider to include a wide range of fruits such as pears, plums, guavas, and dragon fruits [6–9]. These fermented fruit-based ciders are increasingly appreciated for their sensory characteristics and naturally occurring bioactive compounds [10,11]. Furthermore, consumer preference for novel flavors has encouraged the development of mixed-fruit cider formulations, creating new opportunities for the utilization of tropical fruit resources [12,13].

Recent studies have demonstrated the feasibility of using tropical fruits as substrates for alcoholic fermentation. Pineapple has been extensively investigated for fruit wine production due to its desirable sugar content and ester-derived aroma profile [14–16]. However, pineapple wines often exhibit excessive sourness after fermentation because citric acid remains the dominant organic acid after fermentable sugars are depleted [16]. In contrast, lychee is valued for its floral aroma and phenolic composition, with volatile compounds such as linalool and rose oxide contributing characteristic rose-like notes [17]. Owing to its high perishability and seasonal overproduction, lychee has also attracted increasing attention for fermentation-based value addition [17,18]. Nevertheless, lychee fermentation frequently requires pH adjustment to achieve suitable conditions for yeast growth and fermentation performance [19–21].

To overcome the limitations associated with single-fruit fermentations, co-fermentation has emerged as a promising strategy for combining complementary physicochemical, functional, and sensory attributes. Previous studies involving fruit–fruit combinations, such as banana–pineapple [22] and watermelon–pineapple [23], have demonstrated improvements in fermentation performance and volatile composition. Similar benefits have also been reported for fruit–herb and fruit–flower fermentations, including lychee–yerba mate [19], pear–flower [24], and rose–mulberry [25]. These studies showed that co-fermentation can enhance phenolic accumulation, antioxidant activity (e.g., DPPH and FRAP), and the formation of desirable aroma compounds, thereby improving overall sensory acceptance. Collectively, these findings suggest that strategically combining substrates with complementary chemical compositions may provide a practical approach for simultaneously enhancing the functional and sensory quality of fermented beverages. In this context, pineapple and lychee represent a particularly attractive combination. The naturally lower pH of pineapple juice may provide an alternative to chemical acidification during lychee fermentation, while lychee juice may reduce the excessive acidic perception commonly associated with pineapple wines. Such an approach may enhance aroma complexity, antioxidant potential, and overall sensory quality while reducing the need for external chemical additives [16,17].

Fermentation performance and cider quality are also influenced by microbial activity. *Saccharomyces cerevisiae* is widely used as a starter culture because of its robust fermentative capacity and desirable aroma production [26]. Fresh fruit juices naturally contain indigenous yeasts and bacteria that may contribute to spontaneous fermentation; however, uncontrolled microbial populations can lead to inconsistent fermentation and undesirable sensory characteristics [27]. Therefore, pre-fermentation pasteurization is commonly applied to reduce indigenous microorganisms before inoculation with *S. cerevisiae* [26].

Although lychee and pineapple have each been individually investigated for fermented beverage production, information regarding their co-fermentation remains limited. To the best of our knowledge, no previous study has systematically evaluated the effects of lychee–pineapple blending ratios on fermentation kinetics, bioactive enrichment, and sensory characteristics in a functional cider system. Therefore, this study aimed to investigate the influence of five lychee-to-pineapple juice ratios on fermentation performance, bioac-

tive composition, and sensory quality to develop a naturally balanced and value-added functional cider.

2. Materials and Methods

2.1. Materials

Commercial active dry yeast, *Saccharomyces cerevisiae* (Lalvin EC-1118; Lallemand, Fredericia, Denmark), and yeast nutrients (Vitamon® Combi; Erbslöh, Geisenheim, Germany) were employed in this study. For the raw materials, 'Hong Huay' lychee (*Litchi chinensis* Sonn.) was sourced from the Huai Pa Kluay Quality Lychee Production Conservation Group, Mae Chai District, Phayao, Thailand, and selected at the fully ripe stage (bright red skin, flattened tubercles) with a pH of approximately 4.2 and 16 °Brix TSS. 'Phulae' pineapple (*Ananas comosus* L. Merr.) was purchased from a local market in Mueang District, Chiang Rai, Thailand, and selected at 30–50% shell yellowing, presenting a pH of approximately 3.8 and 17 °Brix TSS.

2.2. Preparation of Juices

Both lychee and pineapple were initially cleaned by washing with tap water. For the lychee, spoiled fruits were manually removed, followed by peeling and de-seeding. The pineapple was peeled, de-eyed, and sliced into small pieces. The prepared fruit pulps were packed in 2 kg portions into polyethylene bags and stored at −18 °C until required. Prior to juice extraction, the frozen pulps were thawed in a refrigerator at 4 ± 1 °C overnight. Each fruit type was separately blended using a blender (HR2118, Philips Electronic (Thailand) Ltd., Bangkok, Thailand) at medium speed for 2 min, followed by filtration through a cheesecloth to obtain the clarified juice. A Completely Randomized Design (CRD) was employed to investigate five blending ratios to produce mixed juices of lychee (LJ) and pineapple (PJ): 0:100 (100PJ), 25:75 (25LJ:75PJ), 50:50 (50LJ:50PJ), 75:25 (75LJ:25PJ), and 100:0 (100LJ) (*v*/*v*). The resulting juice blends were pasteurized at 65 °C for 15 min and then cooled at room temperature. Initial physicochemical characteristics, including pH, total soluble solids (TSS), and titratable acidity (TA, expressed as % citric acid), were determined. The juice samples were stored at 4 ± 1 °C for further analysis of total polyphenols, total flavonoids, and antioxidant activities.

2.3. Cider Fermentation

To facilitate optimal yeast growth, each juice sample was supplemented with 150 mg/L of Vitamon® Combi prior to fermentation. The fermentation of the five mixed-juice ratios was conducted in triplicate using sanitized 620 mL green glass bottles, each filled with 500 mL of the prepared must. To maintain strictly anaerobic conditions while allowing gas release, each bottle was fitted with a sanitized airlock. The inoculum of *S. cerevisiae* EC-1118 was prepared following the manufacturer's instructions by rehydrating 3.75 g of active dry yeast in 50 mL of distilled water at 35 °C for 20 min. Each bottle was then inoculated with 2 mL of the suspension, yielding a final inoculation rate of 0.3 g/L (equivalent to an initial viable cell density of approximately 3×10^6 CFU/mL) once the temperature reached 30 °C. Post-fermentation, the bottles were stored at 4 °C for 7 days for cold settling. The ciders were then racked into fresh, sanitized 620 mL green glass bottles, maintaining the original volume (500 mL). Potassium disulfite (Merck, Darmstadt, Germany) was added to each sample to achieve a final SO_2 concentration of 80 mg/L. The bottles were sealed with crown caps and stored at 4 °C for subsequent quality analysis.

2.4. Determination of Fermentation Kinetics

Fermentation progress was monitored gravimetrically at 24 h intervals by measuring weight loss resulting from CO_2 evolution through the airlock. Cumulative CO_2 production was calculated from the difference between the initial bottle weight (0 h) and each subsequent measurement. Fermentation kinetics were evaluated using three parameters derived from the cumulative CO_2 production curves: (i) lag phase duration, defined as the period before detectable fermentation activity; (ii) maximum fermentation rate, calculated from the greatest increase in cumulative CO_2 production between two consecutive sampling intervals and expressed as g CO_2/L h; and (iii) fermentation completion time, defined as the time required to reach a constant weight, corresponding to a weight change of less than 1 g over two consecutive days.

2.5. Determination of Physicochemical Properties

The physicochemical properties of both the initial fruit juices and the fermented cider samples were determined in triplicate. The pH value was measured using a digital pH meter (SG2, Mettler-Toledo GmbH, Schwerzenbach, Switzerland), calibrated using standard buffer solutions (pH 4.0 and 7.0) before analysis. The total soluble solids (TSS) content was investigated using a digital handheld refractometer (Model PAL-3; Atago, Tokyo, Japan) with automatic temperature compensation to 25 °C; the results were expressed in °Brix. The titratable acidity (TA) was determined by titration against 0.1 N NaOH, following the procedure described by [28]. The results were expressed as a percentage of citric acid equivalents (% as citric acid).

2.6. Ethanol Content Analysis

The ethanol content of the mixed lychee–pineapple cider was determined using a gas chromatograph (Agilent 8890GC/FID, Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector. The analytical procedure was adapted from Ekowati et al. [29] with modifications for direct injection. Prior to analysis, samples were filtered through a 0.45 µm nylon syringe filter and diluted (1:10 v/v) with deionized water. Separation was achieved on a DB-BAC1 UI (Agilent Technologies, Inc., Santa Clara, CA, USA) capillary column (30 m × 0.32 mm × 1.8 µm). The oven temperature was initially held at 50 °C for 1 min, then increased to 150 °C at a rate of 10 °C/min, and maintained for 1 min. The inlet and detector temperatures were set at 200 °C and 250 °C, respectively. Helium served as the carrier gas, and 1 µL of the sample was injected with a split ratio of 25:1. The external standard method was employed for quantification (n = 3). The calibration curve showed linearity (r^2 > 0.995), and the results were expressed as % v/v.

2.7. Determination of Bioactive Compounds and Antioxidant Activities

2.7.1. Sample Extraction

Phytochemicals were extracted from the juice and cider samples (previously stored at −20 °C) following Bunea et al. [30], with the modified procedure. Briefly, a 20 mL aliquot of each sample was homogenized with 10 mL of 80% methanol for 5 min. The resulting homogenate was centrifuged at 5000 rpm for 15 min. The supernatants were collected and subsequently filtered through a 0.45 µm nylon membrane (Merck Chemical Technology Co., Ltd., Shanghai, China) and stored at −20 °C until further use. All extraction procedures were performed in triplicate.

2.7.2. Determination of Total Phenolic Content (TPC)

The TPC was quantified using the Folin–Ciocalteu colorimetric method as described by Bunea et al. [30]. A 25 µL aliquot of the extract was reacted with 120 µL of Folin–Ciocalteu

reagent. After 5 min, the mixture was neutralized with 340 µL of Na_2CO_3. Following a 90 min incubation in the dark at ambient temperature, the absorbance was measured at 750 nm. A standard curve was established using gallic acid (r^2 = 0.997), and the results were expressed as milligrams of gallic acid equivalents (GAE) per 100 mL (mg GAE/100 mL).

2.7.3. Determination of Total Flavonoid Content (TFC)

The TFC was determined following the method described by Dewanto et al. [31], with minor modifications. A 250 µL aliquot of the extract was diluted to a final volume of 1.5 mL with distilled water. Subsequently, 100 µL of 5% $NaNO_2$ was added and allowed to react for 5 min, followed by the addition of 300 µL of 10% $AlCl_3$. After 6 min, 2 mL of 1 N NaOH was added to the mixture. The absorbance was recorded at 510 nm against a reagent blank. TFC was expressed as milligrams of quercetin equivalents (QE) per 100 mL (mg QE/100 mL).

2.7.4. Determination of Antioxidant Activity

The antioxidant activity of extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity.

The DPPH assay was based on the protocol by Brand-Williams et al. [32], with modifications described by Thaipong et al. [33]. The DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol and maintained at −20 °C until required. A working solution was generated by diluting 10 mL of the stock with 45 mL of methanol to achieve an initial absorbance of 1.1 ± 0.02 units at 515 nm. To determine antioxidant activity, 150 µL of extract was reacted with 2850 µL of the DPPH solution and incubated in the dark for 24 h. Absorbance was then measured at 515 nm. Quantification was performed using a Trolox standard curve (25–800 µM) and final results were expressed as micromoles of trolox equivalents (TE) per mL (µmol TE/mL). Samples exceeding the linear range of the standard curve were subjected to further dilution.

The ABTS assay was determined according to the modified method described by Thaipong et al. [33]. Stock solutions of 7.4 mM ABTS and 2.6 mM potassium persulfate were combined (1:1 v/v) and reacted for 12 h in darkness to produce the ABTS radical cation. For each assay, the working solution was prepared by diluting the radical solution with methanol to achieve an absorbance of 1.1 ± 0.02 units at 734 nm. The reaction mixture, consisting of 150 µL of sample extract and 2850 µL of the ABTS solution, was incubated for 2 h in the dark prior to absorbance measurement at 734 nm. Antioxidant activity was determined using a Trolox standard curve (25–600 µM) and expressed as µmol TE/mL. Samples exceeding the linear range of the standard curve were subjected to further dilution with the appropriate solvent.

2.8. Sensory Evaluation

The sensory evaluation protocol received ethical approval from the University of Phayao Human Ethics Committee (Project No. HREC-UP-HSST 1.3/011/68). Quantitative Descriptive Analysis (QDA) was performed by ten trained panelists in a controlled environment at 25 °C under standardized white lighting. Cider samples (30 mL) were served at 10–12 °C in clear, lidded glasses, each identified by a unique three-digit random code and presented in a randomized order. The intensity of each sensory attribute was rated on a 5 cm unstructured linear scale (0 = not detectable, 5 = extremely strong). The evaluated attributes were categorized into appearance (clarity), aroma (fruity, floral, tropical fruit, complexity, and volatile acidity), and taste/flavor (bitterness, sweetness, and acidity).

2.9. Statistical Analysis

Data (analytical n = 3; sensory n = 10) were expressed as means ± SD. Statistical analysis was performed using IBM SPSS Statistics v.29.0.2.0 (IBM Corp., Armonk, NY, USA). One-way and two-way ANOVA followed by Duncan's Multiple Range Test were applied to physicochemical and sensory data, respectively. Relationships between juice ratios (X) and parameters (y) were evaluated using Pearson's correlation (r) and regression analysis, where the best-fit model (linear or quadratic) was selected based on r^2 and significance ($p < 0.05$). Principal component analysis (PCA) and K-means clustering were further applied to visualize multidimensional relationships among samples and quality attributes. Data visualization, including the correlation heatmap, was generated using Python (v.3.10) with *Seaborn* (v.0.13.2) and *Matplotlib* (v.3.10.0) libraries within the Google Colaboratory environment.

3. Results and Discussion

3.1. Physicochemical and Bioactive Profiles of Juice Formulations

3.1.1. Physicochemical Properties

The initial physicochemical and bioactive attributes of the juice formulations prior to inoculation are summarized in Table 1. Analysis indicated that the 100PJ provided a superior acidic environment for fermentation, with a significantly lower pH (3.78) and higher titratable acidity (TA, 0.64%) compared to the 100LJ (pH 4.10; TA 0.35%). These values are consistent with the established characteristics of pineapple juice (pH 3.70–3.84 and TA 0.49–0.67%), as documented by Chanprasartsuk et al. [34], Lu et al. [35], and Boondaeng et al. [15]. Furthermore, the lychee juice attributes in this study corroborated earlier findings by Pongjuntuk et al. [36]. Blending lychee juice with pineapple juice effectively reduced the pH to a range of 3.87–4.00 and elevated the TA from 0.35% (in 100LJ) to 0.45–0.54% in the mixed juices. Pineapple-derived organic acids served as a natural antimicrobial barrier, consistent with oenological principles regarding microbial suppression at low pH [37]. Although typical white and red wines maintain pH ranges of 3.0–3.4 and 3.3–3.7 [38], blending shifted the juice pH to 3.8–4.0. Despite deviating from traditional standards, this acidification sufficed to enhance microbial resistance relative to the raw substrate. Furthermore, this pH threshold preserved structural acidity, avoiding the sensory defects of 'flatness' or 'flabbiness' characteristic of high-pH viticultural products [37].

Table 1. Physicochemical properties, bioactive compounds, and antioxidant activities of various pineapple and lychee juice formulations.

Properties	100PJ	25LJ:75PJ	50LJ:50PJ	75LJ:25PJ	100LJ
pH	3.78 ± 0.00 ^{d}	3.87 ± 0.00 ^{c}	3.89 ± 0.09 ^{c}	4.00 ± 0.00 ^{b}	4.10 ± 0.00 ^{a}
TSS (°Brix)	17.1 ± 0.0 ^{a}	16.8 ± 0.1 ^{b}	16.6 ± 0.1 ^{c}	16.3 ± 0.1 ^{d}	15.9 ± 0.0 ^{e}
TA (% as citric acid)	0.64 ± 0.00 ^{a}	0.54 ± 0.03 ^{b}	0.51 ± 0.00 ^{b}	0.45 ± 0.00 ^{c}	0.35 ± 0.03 ^{d}
TPC (mg GAE/100 mL)	48.02 ± 2.18 ^{e}	55.37 ± 2.24 ^{d}	63.22 ± 2.16 ^{c}	71.72 ± 3.65 ^{b}	80.16 ± 5.09 ^{a}
TFC (mg QE/100 mL)	5.25 ± 0.27 ^{d}	7.81 ± 0.85 ^{c}	8.86 ± 0.48 ^{c}	11.43 ± 0.83 ^{b}	13.98 ± 1.18 ^{a}
DPPH (μmol TE/mL)	6.14 ± 0.10 ^{e}	7.66 ± 0.05 ^{d}	9.11 ± 0.11 ^{c}	10.39 ± 0.05 ^{b}	11.88 ± 0.21 ^{a}
ABTS (μmol TE/mL)	6.33 ± 0.13 ^{e}	8.02 ± 0.08 ^{d}	9.78 ± 0.16 ^{c}	11.52 ± 0.21 ^{b}	12.92 ± 0.28 ^{a}

Values are presented as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences according to Duncan's Multiple Range Test ($p < 0.05$). PJ, pineapple juice; LJ, lychee juice; TSS, total soluble solids; TA, titratable acidity; TPC, total phenolic content; GAE, gallic acid equivalents; TFC, total flavonoid content; QE, quercetin equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TE, trolox equivalents.

The TSS of pineapple juice (17.1 °Brix) and lychee juice (15.9 °Brix) were congruent with previously reported ranges of 14.0–18.1 °Brix [15,34,35] and 11.3–18.6 °Brix [18,36], respectively. While previous studies on pineapple [34,39] and lychee [18,20] fermentations

typically report the use of sucrose to adjust the TSS to 20–22 °Brix, this study prioritized a juice-blending strategy to optimize the fermentation matrix without exogenous sugar or acid supplementation. This approach is in line with the ideas of sustainable resource use and food security, as mentioned in the introduction. Through this blending process, the TSS was maintained within 16.3–16.8 °Brix, representing a slight enhancement over the 15.9 °Brix observed in 100% lychee juice (LJ100). This moderate sugar level ensures sufficient substrate availability for producing a beverage with moderate alcohol content while preserving the authentic sensory profile of the fruit matrix.

3.1.2. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Quantifying total phenolic content (TPC) and radical scavenging capacity is fundamental to elucidating both the sensory quality and health-promoting potential of fermented beverages [40]. Consequently, this study evaluated these parameters within the initial juice formulations, comprising pure lychee (100LJ), pure pineapple (100PJ), and their respective blends. As illustrated in Table 1, 100LJ exhibited the highest TPC at 80.16 mg GAE/100 mL, significantly surpassing the 48.02 mg GAE/100 mL recorded for 100PJ ($p < 0.05$) (Table 1). Within the blended formulations, TPC values exhibited a concentration-dependent increase relative to the proportion of lychee juice.

The TPC observed for 100LJ was lower than the previously reported range of 66–226 mg GAE/100 g for fresh lychee pulp [41]. This discrepancy is potentially attributable to the use of 80% acetone as an extraction solvent in the literature, compared to the aqueous methanol employed in the current study. Conversely, our results exceeded the values documented by Pongjuntuk et al. [36] (33 mg GAE/100 mL), despite both studies utilizing similar aqueous methanol extraction. Such variations may be ascribed to cultivar differences, mechanical juicing processes that leave phenolics bound to the pomace residue, or oxidative degradation. In contrast, the TPC of 100PJ (48.02 mg GAE/100 mL) was highly congruent with the 47–53 mg GAE/100 mL reported for 'Nanglae' and 'Phuket' cultivars [35]. The overall trend of 100LJ possessing higher phenolic content than 100PJ is consistent with the findings of [42], who reported that among various tropical fruits, litchi exhibited a substantially higher TPC (178 mg GAE/100 g) relative to pineapple (33 mg GAE/100 g).

Regarding the total flavonoid content (TFC), the results followed a trend congruent with the TPC findings, where 100LJ (13.98 mg QE/100 mL) significantly surpassed 100PJ (5.25 mg QE/100 mL). It is imperative to note that direct numerical benchmarking with previous literature is occasionally constrained by the diversity of reference standards and basis units (e.g., QE/100 mL vs. RE/100 g FW). Nevertheless, our findings align with the established flavonoid-rich profile of lychee relative to pineapple. This disparity is notably highlighted by Septembre-Malaterre et al. [42], who reported that litchi (53 mg QE/100 g) contains approximately 26.6-fold higher flavonoids than the negligible levels found in pineapple (2 mg QE/100 g). Specifically, the TFC observed in 100LJ is consistent with the findings of Wang et al. [43], who reported values of 24–26 mg RE/100 g FW for 'Nuomici' and 'Guiwei' lychee cultivars using a similar 80% methanol extraction. Correspondingly, the lower flavonoid levels in 100PJ are in qualitative agreement with the 11–13 mg RE/100 g FW documented by Lu et al. [35] for 'Nanglae' and 'Phuket' pineapple cultivars. Despite the variations in quantification standards (QE vs. RE) and sample matrices, the substantial flavonoid-to-phenolic ratio observed in the lychee–pineapple blends underscores their potential as a significant source of dietary antioxidants, supported by the standardized analytical framework [31]. TPC and TFC of the blends (25:75–75:25) increased proportionally, ranging from 55.37–71.72 mg GAE/100 mL and 7.81–11.43 mg QE/100 mL, respectively (Table 1). These values represent a 15–49% elevation in TPC and a substantial 48–117%

increase in TFC relative to the 100PJ baseline. This dose-dependent enhancement confirms that lychee incorporation effectively fortifies the pineapple-based matrix, significantly elevating its bioactive load and functional potential prior to fermentation.

3.1.3. Antioxidant Capacity

The antioxidant potential of the juices and their blends was elucidated using DPPH and ABTS assays (Table 1). Significant differences ($p < 0.05$) were found across all samples, primarily driven by the lychee juice (LJ) ratio. In the DPPH assay, 100LJ demonstrated the most scavenging capacity (11.88 μmol TE/mL), while 100PJ exhibited the minimum potential (6.14 μmol TE/mL). Adding LJ to the pineapple juice led to a dose-dependent increase in antioxidant activity; for example, the 75LJ:25PJ blend was 35% higher than the 25LJ:75PJ. These findings, these results, align with previous studies [35,36] that used 80% methanol extraction. Although reporting units are different—mg TE/L for lychee [40] and μmol TE/mL for pineapple [35]—the consistent potency of lychee over pineapple validates our results and suggests the observed activity is a characteristic of the fruit's phytochemistry rather than an extraction artifact.

ABTS results mirrored DPPH trends, with 100LJ reaching 12.92 μmol TE/mL. The slightly higher ABTS values are likely due to the radical's solubility in both aqueous and organic phases, allowing a more comprehensive assessment of hydrophilic and lipophilic antioxidants within the matrix.

The scavenging activity aligns with the total phenolic levels across all samples. As for 100LJ's peak activity (80.16 mg GAE/100 mL TPC), this relationship aligns with Huang et al. [44], who posit that the total phenolic profile often serves as a reliable predictor of a matrix's overall antioxidant potential. This relationship indicates that the dose-dependent increase in the blends is directly driven by the lychee-derived phenolic load. Thus, LJ fortification effectively bridges the bioactive deficit of pineapple juice, enhancing the cider's functional properties.

The antioxidant capacity of the juices and their blends, evaluated via DPPH and ABTS assays, increased proportionally with the lychee juice (LJ) ratio (Table 1). 100LJ exhibited the most robust scavenging activity, while 100PJ showed the minimum potential. This intrinsic superiority of lychee over pineapple is corroborated by Septembre-Malaterre et al. [42], who demonstrated that litchi consistently outperforms pineapple across multiple antioxidant platforms, including DPPH and Trolox equivalent capacity. Such findings validate our results, suggesting that the heightened activity is a stable phytochemical characteristic of the lychee matrix rather than an artifact of extraction.

Notably, the incorporation of LJ into the pineapple-based matrix led to a marked dose-dependent enhancement; the formulations exhibited a 24–69% elevation in DPPH radical scavenging activity and a substantial 26–82% increase in ABTS capacity relative to the 100PJ baseline. Mechanistically, ABTS values were consistently higher than those of DPPH. This is likely attributable to the dual solubility of the $ABTS^{+}$ radical in both aqueous and organic phases, facilitating a more comprehensive assessment of hydrophilic and lipophilic antioxidants compared to the sterically hindered DPPH radical [44]. Furthermore, the strong positive correlation between scavenging activity and total phenolic content (TPC) reinforces that the phenolic load is the primary driver of the observed bioactive potential [44]. As a result, fortifying LJ effectively reduces the bioactive deficit of pineapple juice, greatly increasing its functional load and health-promoting value before fermentation.

3.2. Fermentation Kinetics

During alcoholic fermentation, *S. cerevisiae* plays a pivotal role in converting primary fruit sugars into ethanol and carbon dioxide, while simultaneously generating essential by-

products such as volatile compounds, glycerol, and various bioactive components [45,46]. Monitoring the CO_2 evolution (Figure 1) revealed that the initial fermentation kinetics were significantly influenced by the fruit juice composition. The fermentation profiles further indicated that all treatments exhibited a relatively short lag phase (<24 h), followed by a rapid increase in CO_2 production during the active fermentation stage. The maximum fermentation rates were observed between 24 and 48 h. All pineapple-containing treatments (100PJ, 25LJ:75PJ, 50LJ:50PJ, and 75LJ:25PJ) exhibited comparable maximum fermentation rates ranging from 1.96 to 2.08 g CO_2/L h, whereas the 100LJ treatment showed a lower maximum rate of 1.76 g CO_2/L h, indicating slower early-stage fermentation kinetics. Despite these differences, all fermentations approached a plateau between 72 and 96 h and reached completion within 96 h. Pineapple juice is predominantly composed of sucrose, followed by glucose and fructose in an approximate ratio of 4:1:1 [35]. In contrast, lychee juice exhibits a distinct distribution characterized by a higher proportion of reducing sugars (glucose and fructose) and relatively low sucrose content [47]. While *S. cerevisiae* generally prefers glucose, the high sucrose concentration in 100PJ and its blends did not impede fermentation. These treatments demonstrated robust initial kinetics, entering active fermentation within the first 24 h and producing 19–25 g/L cumulative CO_2 during this period. This efficiency is attributed to the yeast's ability to secrete extracellular invertase, which rapidly hydrolyzes sucrose into fermentable monomers [48].

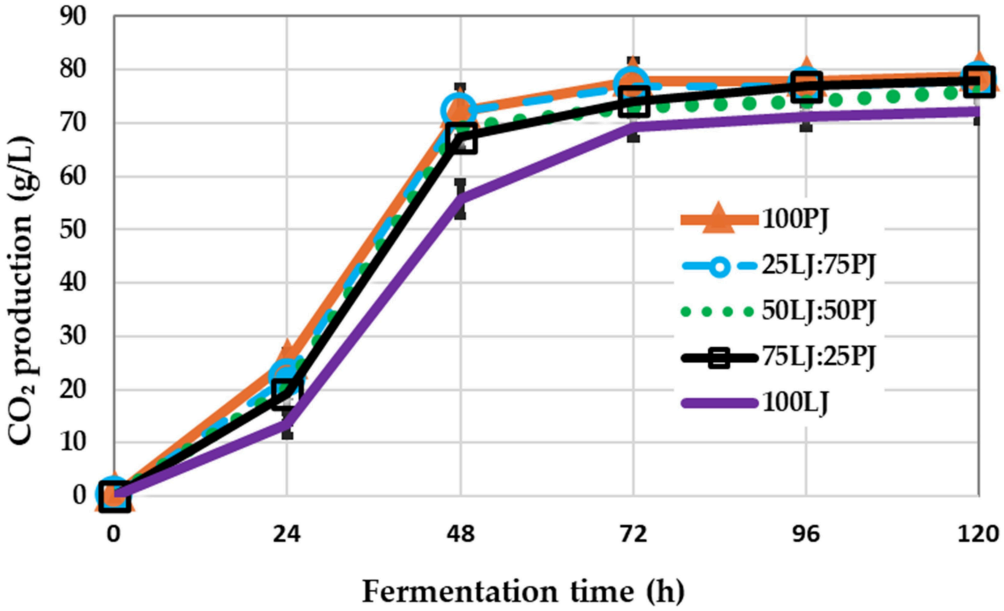


Figure 1. Fermentation kinetics of CO_2 production in mixed-fruit ciders with different proportions of lychee and pineapple juices. Note: Values are expressed as mean ± SD (n = 3 replicates). PJ, pineapple juice; LJ, lychee juice.

In contrast, a distinct kinetic suppression was observed in the 100LJ treatment, particularly during the initial and mid-stages (0–48 h). Despite its high proportion of reducing sugars, 100LJ exhibited a significantly lower CO_2 evolution slope compared to pineapple-based samples. This delay is likely driven by metabolic stress induced by lychee-derived hydroxycinnamic acids (HCAs). As evidenced in Table 1, 100LJ possessed the highest baseline of TPC and TFC, including ferulic acid and caffeic acid [18,43]—compounds identified by Baranowski et al. [49] and Herald and Davidson [50] as the most potent inhibitors in this class, capable of extending the lag phase even at low concentrations.

The mechanistic basis for this early-stage suppression involves plasma membrane perturbation [51] and the interference with amino acid metabolism [52]. In particular, Mekoue

Nguela et al. [51] demonstrated that polyphenol-induced membrane disorganization suppresses the TORC2 signaling complex—a key regulator of nutrient uptake. This signaling failure, combined with the metabolic burden of generating substantial antioxidant capacity to mitigate oxidative damage and free radical accumulation [52], likely forces a redirection of energy toward cellular maintenance rather than active fermentation during the first 48 h.

However, a discernible shift in kinetics occurred between 48 and 72 h, where the CO_2 evolution of 100LJ accelerated, showing a steeper slope that suggests a successful metabolic adaptation phase. This resilience was likely supported by the inclusion of exogenous nitrogen supplement prior to fermentation, which provided the necessary metabolic buffer for the yeast to eventually overcome the phenolic barrier [52,53]. While EC-1118 in this study demonstrated the resilience to finish the fermentation, the high HCA load in lychee-based formulations remains a significant consideration for modern co-inoculation strategies involving more sensitive microorganisms, such as non-*Saccharomyces* yeasts or malolactic bacteria, which may lack the same stress-tolerance mechanisms.

3.3. Physicochemical and Functional Characteristics of Fermented Ciders

3.3.1. Total Soluble Solids and Ethanol Content

The alcoholic fermentation transformed the fruit musts into ciders, with final physicochemical parameters summarized in Table 2. All treatments reached a stable fermentative endpoint, with total soluble solids (TSS) decreasing from initial levels (15.9–17.1 °Brix) to a final range of 6.33–6.60 °Brix. This attenuation indicates a complete fermentation where fermentable sugars were exhaustively utilized by *S. cerevisiae*. The resulting ethanol contents ranged from 9.15% to 10.08% (*v*/*v*). Despite the initial lag phase observed in the 100LJ treatment (Section 3.2), it reached full attenuation within 120 h without becoming sluggish. The variations in final ethanol concentrations directly reflect the initial sugar baselines of the respective fruit juices, with 100PJ yielding the highest ethanol content (10.08%) due to its higher initial TSS.

Table 2. Physicochemical and functional properties of ciders derived from various juice formulations.

Properties	100PJ	25PJ:75PJ	50LJ:50PJ	75LJ:25PJ	100LJ
pH	3.88 ± 0.01 ^{c}	3.97 ± 0.01 ^{b}	4.00 ± 0.03 ^{b}	4.07 ± 0.02 ^{a}	4.10 ± 0.01 ^{a}
TSS (°Brix)	6.4 ± 0.0 ^{b}	6.6 ± 0.0 ^{a}	6.6 ± 0.0 ^{a}	6.4 ± 0.1 ^{b}	6.3 ± 0.1 ^{b}
TA (% as citric acid)	0.80 ± 0.03 ^{a}	0.75 ± 0.02 ^{a}	0.65 ± 0.08 ^{b}	0.54 ± 0.04 ^{c}	0.48 ± 0.03 ^{c}
Ethanol (% *v*/*v*)	10.08 ± 0.66 ^{a}	10.07 ± 0.63 ^{a}	9.77 ± 0.75 ^{a}	9.33 ± 0.80 ^{a}	9.15 ± 0.85 ^{a}
TPC (mg GAE/100 mL)	50.82 ± 4.01 ^{d}	60.89 ± 2.54 ^{c}	69.54 ± 6.10 ^{c}	81.04 ± 5.80 ^{b}	91.62 ± 7.19 ^{a}
TFC (mg QE/100 mL)	6.15 ± 0.66 ^{e}	8.11 ± 0.31 ^{d}	9.01 ± 0.27 ^{c}	11.98 ± 0.35 ^{b}	13.77 ± 0.49 ^{a}
DPPH (µmol TE/mL)	6.88 ± 0.14 ^{e}	8.68 ± 0.15 ^{d}	10.51 ± 0.11 ^{c}	13.85 ± 0.09 ^{b}	16.35 ± 0.31 ^{a}
ABTS (µmol TE/mL)	6.96 ± 0.12 ^{e}	9.46 ± 0.06 ^{d}	10.76 ± 0.17 ^{c}	13.68 ± 0.15 ^{b}	15.00 ± 0.11 ^{a}

Note: Values are presented as mean ± standard deviation (n = 3). Different superscript letters within the same row indicate significant differences according to Duncan's Multiple Range Test ($p < 0.05$). PJ, pineapple juice; LJ, lychee juice; TSS, total soluble solids; TA, titratable acidity; TPC, total phenolic content; GAE, gallic acid equivalents; TFC, total flavonoid content; QE, quercetin equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TE, trolox equivalents.

3.3.2. Acidity and pH

Metabolic activity during fermentation led to an increase in titratable acidity (TA) across all formulations (Table 2). This rise is primarily driven by the yeast's production of secondary organic acids, such as succinic, acetic, and pyruvic acids [37,38,54]. As reported by Volschenk et al. [55], *S. cerevisiae* metabolism can shift carbon flux to increase final TA by 1–2 g/L, consistent with the trends in all samples.

However, a paradoxical increase in pH was observed (most notably in 100PJ), suggesting that the final pH is governed more by acid–base equilibrium than by total titratable acidity. According to the principles outlined by Ribéreau-Gayon et al. [54], the high con-

centration of endogenous potassium (K^+) in fruit matrices promotes "salification"—the formation of potassium salts of organic acids (e.g., citrate and malate). Supporting this, compositional data indicate that pineapple contains approximately 109 mg/100 g of potassium, while lychee exhibits higher levels ranging from 130.7 to 180.6 mg/100 g across cultivars [56,57]. This process reduces the concentration of free hydrogen ions and shifts the dissociation equilibrium, thereby increasing pH despite elevated TA. This interaction between potassium accumulation and pH regulation has been extensively reviewed in fruit systems [58] and remains a critical factor in determining the final acidity profile of fermented juices, a phenomenon similarly observed in the fermentation of other high-potassium matrices such as Chardonnay and Shiraz [59]. These results align with previous observations in tropical fruit wines, particularly in lychee wine fermentation, where an upward pH shift (e.g., from 3.52 to 3.63–3.79) has been consistently reported [21,60]. This trend is further corroborated by Li et al. [61], who reported a similar pH increase in kiwi wine despite high and stable titratable acidity. Consistent with these observations, Ye et al. [62] demonstrated that during the fermentation of 'Fuji' apple cider with *S. cerevisiae*, the pH rose from 3.29 to 3.50 while the titratable acidity remained relatively stable. Furthermore, recent findings by Xu et al. [63] indicated that 'Fuji' apple cider supplemented with organic nitrogen exhibited a simultaneous increase in both pH and titratable acidity, specifically in the amino acid-enriched treatments. Collectively, these findings confirm that the final pH is governed by the complex equilibrium between total acid synthesis and the juice's inherent mineral buffering capacity.

3.3.3. Total Phenolic, Total Flavonoid, and Antioxidant Capacity

Post-fermentation analysis revealed a significant enrichment in both phytochemical profiles and antioxidant capacities across all formulations (Table 2). Compared to the pre-fermented juices (Table 1), TPC and TFC increased within the range of 14.3–24.8% and 14.7–24.8%, respectively. The 100LJ cider exhibited the peak bioactive potential, reaching 91.62 mg GAE/100 mL (TPC) and 1.56 mg QE/100 mL (TFC). Conversely, the 100PJ formulation, while serving as the baseline matrix with the lowest initial values, demonstrated a notable response to the fermentation process. Specifically, the DPPH radical scavenging activity of 100PJ increased by 99.4%, rising from 1.76 to 3.51 μmol TE/mL, while its ABTS capacity remained relatively stable at 2.51 μmol TE/mL. Post-fermentation antioxidant capacities mirrored the phenolic enrichment across all samples, with DPPH and ABTS values increasing by 8.9–99.4% and 0.4–21.5%, respectively. Notably, a clear dose-dependent trend was observed throughout the study; the final functional potency of the ciders was directly proportional to the ratio of lychee juice (LJ) incorporated into the matrix. These findings indicate that while the initial fruit ratio dictates the baseline potential, the fermentation process by *S. cerevisiae* consistently acts as a biological driver that upgrades the functional and antioxidant value of the final beverage across all measured dimensions.

The significant enrichment of TPC and TFC observed in this study is consistent with previous reports on fruit-based fermentations. Specifically, Niyomvong et al. [64] demonstrated that *S. cerevisiae* fermentation enhanced the phenolic profile of plum wine, increasing total phenolics by 15.8–30.1%, which aligns with the upward trend observed in our cider formulations. Similarly, alcoholic fermentation has been shown to increase TPC from 8.37 to 28.7 mg/L in other fruit matrices [61], while in Kei-apple juice, phenolic acids such as gallic and ferulic acids increased by 25% to 328% post-fermentation [65]. These substantial increases across various studies reinforce our findings that *S. cerevisiae* effectively upgrades the phenolic density of the fruit matrix.

This liberation of phenolic compounds is primarily attributed to enzymatic biotransformations occurring during the fermentation process [64,66]. The synthesis of hydrolytic

enzymes, specifically β-glucosidase, facilitates the hydrolysis of bound glycosidic precursors into their free aglycone forms [54,64]. This mechanism is reported to be responsible for releasing phenolic aglycone moieties from plant matrices, thereby elevating the measurable free phenolic content [65]. Furthermore, the growth of microorganisms produces cellulolytic and pectinolytic enzymes that degrade plant cell wall structures, promoting the release of sequestered anthocyanins, flavonols, and hydroxycinnamic acids (HCA) into the medium [65,67].

The metabolic shifts observed in our matrix are further supported by the hydrolysis of HCA esters (such as caftaric and fertaric acids), which liberates free acids including *p*-coumaric and ferulic acids [68]. While certain compounds may undergo biotransformation or decline, such as protocatechuic acid, the continuous release of acids from precursors—exemplified by the hydrolysis of chlorogenic acid into caffeic acid—ensures a net increase in the total phenolic profile [65]. Collectively, these findings confirm that the enzymatic activity of *S. cerevisiae* serves as a critical driver for the enhanced bioactive and antioxidant potential observed in the final cider products [61,64,65].

In conclusion, the strategic blending of lychee and pineapple cultivars achieves a critical synergy: maximizing bioactive extraction while maintaining technological stability through indigenous acid-base equilibrium. This approach fulfills Clean Label and ESG principles by leveraging the natural properties of local agricultural matrices to eliminate the need for synthetic additives [65]. By utilizing the synergistic interaction between fruit-derived organic acids and microbial enzymes, this production model provides a sustainable pathway for developing high-value functional beverages that meet both nutritional standards and environmental responsibilities.

The blending ratios of lychee and pineapple juices significantly influenced most of the physicochemical and functional properties of ciders (Table 3). Linear regression analysis revealed that the lychee juice proportion (X) was a strong predictor for most parameters ($r^2 > 0.93$, $p < 0.01$), with the notable exception of TSS. The lack of significant correlation for TSS ($p \geq 0.05$, $r^2 = 0.222$) was primarily attributed to the similar initial TSS levels of the two raw juices, resulting in a consistent sugar profile regardless of the mixture ratio. As the proportion of lychee juice increased, a distinct linear rise was observed in pH, bioactive compounds (TPC and TFC), and antioxidant activities (DPPH and ABTS). Conversely, TA and ethanol content showed strong negative correlations with the lychee juice content, indicating a decrease in acidity and final alcohol concentration as the pineapple juice fraction was reduced.

Table 3. Linear regression analysis and Pearson's correlation of the effects of blending ratios on the physicochemical and functional properties of lychee–pineapple ciders.

Parameters	Pearson's *r*	Coefficient of Determination (r^2)	Linear Regression Equation
pH	0.984 **	0.968	$y = 3.896 + 0.002X$
TSS (°Brix)	−0.471 ^{ns}	0.222	none
TA (% as citric acid)	−0.993 **	0.985	$y = 0.814 - 0.003X$
Ethanol (% *v*/*v*)	−0.967 **	0.934	$y = 10.2 - 0.01X$
TPC (mg GAE/100 mL)	0.999 **	0.998	$y = 50.432 + 0.407X$
TFC (mg QE/100 mL)	0.989 **	0.979	$y = 5.982 + 0.076X$
DPPH (μmol TE/mL)	0.992 **	0.985	$y = 6.432 + 0.096X$
ABTS (μmol TE/mL)	0.993 **	0.986	$y = 7.112 + 0.081X$

Note: The independent variable (X) represents the percentage of lychee juice in the mixture, ranging from 0% (100PJ) to 100% (100LJ). Linear and quadratic models were evaluated for all variables. The linear model was selected for all significant parameters because the quadratic term (X^2) did not significantly improve the model fit ($p \geq 0.05$). ** indicates that correlation is significant ($p < 0.01$); ns indicates non-significant. PJ, pineapple juice; LJ, lychee juice; TSS, total soluble solids; TA, titratable acidity; TPC, total phenolic content; GAE, gallic acid equivalents; TFC, total flavonoid content; QE, quercetin equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TE, trolox equivalents.

The functional significance of these changes was further validated by Pearson's correlation analysis (Figure 2). Positive correlations (r 0.988, $p < 0.01$) were observed between antioxidant contents (TPC and TFC) and their respective radical scavenging activities (DPPH and ABTS). These results demonstrate that the antioxidant capacity of the blended cider is primarily driven by the phenolic and flavonoid contributions from the lychee juice, which can be precisely controlled through the blending ratio.

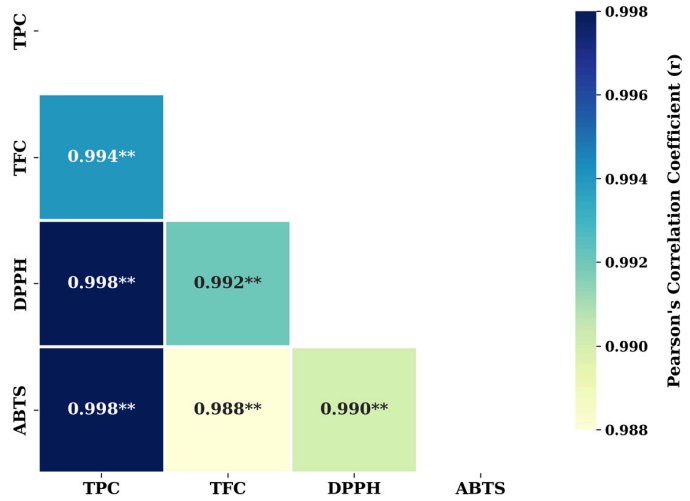


Figure 2. Correlation heatmap of antioxidant compounds and activities of lychee–pineapple ciders. Values represent Pearson's correlation coefficients (r). ** indicates correlation is significant ($p < 0.01$). TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

3.3.4. Descriptive Sensory Characteristics

The blending ratios significantly influenced the sensory profiles of the mixed fruit ciders (Figure 3). The 100PJ formulation exhibited the highest clarity and acidic odor, which are recognized as hallmark traits of pineapple-based fermented beverages. This acidity promotes the perception of freshness and directly modifies flavor components, which are features sought by consumers in modern wines [37,38]. Conversely, increasing LJ proportion significantly enhanced the fruity and floral notes ($p < 0.05$). The 100LJ sample achieved the highest scores for these attributes, which may be attributed to the concentration of volatile terpenes, specifically linalool and geraniol, reported to be inherent in lychee pulp [69]. These compounds are key aroma-active constituents providing the characteristic rose-like and sweet floral notes in lychee-based fermented beverages [17]. Notably, the 50LJ:50PJ and 75LJ:25PJ blends demonstrated significantly higher complexity scores than the pure ciders. This synergistic sensory effect suggests that the combination of PJ's acidic backbone and LJ's aromatic intensity creates a more balanced and sophisticated sensory profile.

To provide a comprehensive overview of the relationships between cider formulations and their quality attributes, principal component analysis (PCA) and K-means clustering were performed (Figure 4), explaining 93.37% of the total variance (*PC*1: 66.95%; *PC*2: 26.42%). *PC*1 effectively discriminated samples according to juice blending ratios. Lychee-rich formulations (100LJ and 75LJ:25PJ) clustered on the positive *PC*1 axis, showing strong associations with bioactive compounds (TPC, TFC), antioxidant activities (DPPH, ABTS), and aromatic sensory attributes (fruity, floral, and tropical fruit). In contrast, the pure pineapple cider (100PJ) was associated with higher TA, ethanol content, and clarity on the negative *PC*1 axis. The intermediate blends (25LJ:75PJ and 50LJ:50PJ) formed a distinct cluster representing a transitional quality profile. *PC*2 was primarily linked to

complexity and TSS, with the 50LJ:50PJ formulation exhibiting the highest loading for these attributes. These multivariate results confirm that the blending ratio is the primary driver for modulating the functional and sensory characteristics of the final cider, allowing for precise quality tailoring.

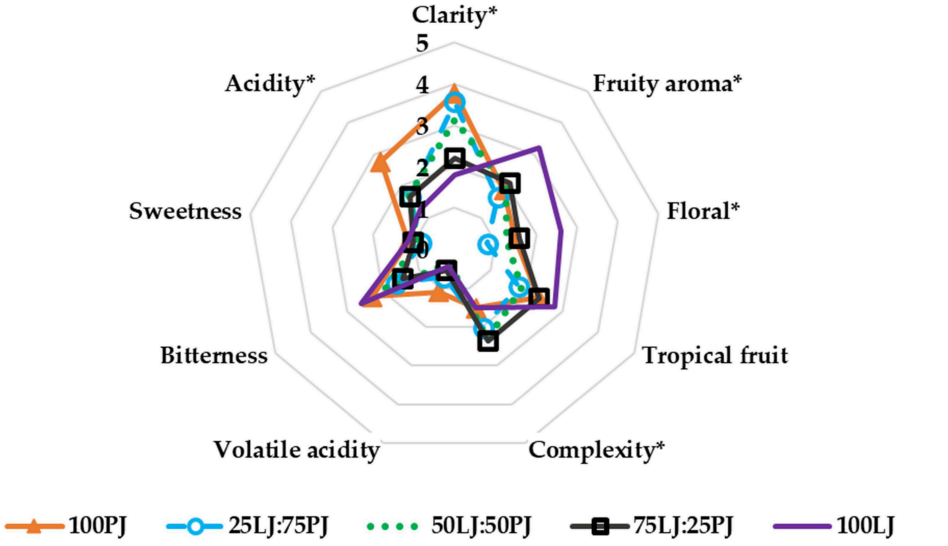


Figure 3. Sensory profiles of mixed-fruit ciders determined by Quantitative Descriptive Analysis (QDA). Values are expressed as mean ± SD (n = 10 trained panelists). Note: Asterisks (*) indicate attributes with significant differences between treatments ($p < 0.05$) based on two-way ANOVA. PJ, pineapple juice; LJ, lychee juice.

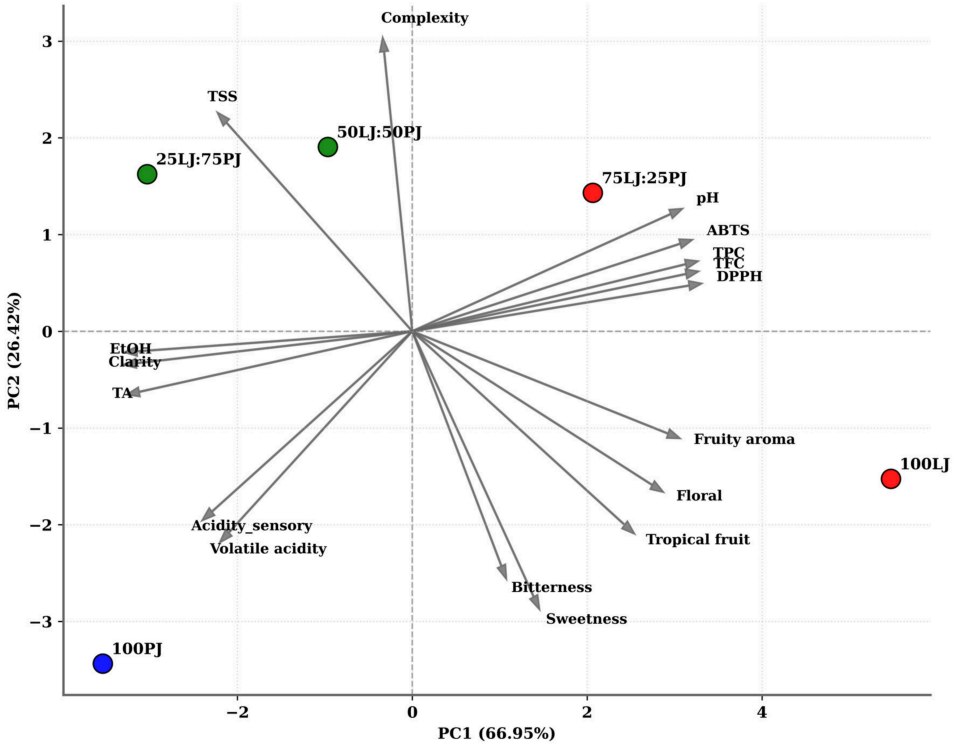


Figure 4. PCA biplot of cider formulations and quality parameters. Note: *PC*1 (66.95%) and *PC*2 (26.42%) account for 93.37% of total variance. Vectors and circles represent quality parameters and cider samples, respectively; colors indicate groups from K-means clustering. PJ, pineapple juice; LJ, lychee juice; TSS, total soluble solids; TA, titratable acidity; TPC, total phenolic content; TFC, total flavonoid content; EtOH, ethanol; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

4. Limitations

While this study demonstrates how yeast interacts with fruit matrices, there is some context to keep in mind. Our findings are based on local Northern Thai fruits—'Hong Huay' lychee and 'Phulae' pineapple—which have unique chemical compositions tied to local soil and climate. Therefore, results might vary if different varieties or regions are involved. Additionally, although the analysis identified high levels of TPC, TFC, and antioxidant activity (DPPH/ABTS) in lychee, we did not quantify individual phenolic acids (like HCAs) directly, relying instead on established literature to explain their inhibitory effects. While the resilient EC-1118 strain successfully completed the fermentation, more sensitive microbes might struggle with this metabolic stress. Although microbial population dynamics were not directly monitored in this study, several measures were implemented to minimize the influence of indigenous microorganisms. All fruit juices were pasteurized prior to fermentation, and a freshly opened commercial Saccharomyces cerevisiae EC-1118 starter culture was inoculated according to the manufacturer's recommended protocol. Given the well-documented fermentative robustness and competitive performance of EC-1118 in alcoholic beverage production, the observed fermentation behavior was expected to be primarily driven by the inoculated starter culture. Nevertheless, future studies incorporating microbial community analysis and proteomic approaches would provide deeper mechanistic insights into the relationships between microbial activity, metabolite formation, and sensory quality. Furthermore, because this research was conducted on a laboratory scale at room temperature, the fermentation kinetics and chemical interactions might behave differently during large-scale production. Regarding the pH rise, while we did not measure specific acid salts directly, our explanation is based on established theories and the observed data. Finally, while our sensory test provided a good overview of consumer appeal, future studies could dive deeper by identifying individual volatile compounds to better understand how this chemical matrix shifts the fruit cider's aroma profile.

5. Conclusions

This study demonstrated that blending of lychee (LJ) and pineapple (PJ) optimized the juice matrix for fermentation, eliminating the need for exogenous acids. This synergy successfully mitigated the phenolic-induced extended lag phase observed in 100LJ, while S. cerevisiae fermentation significantly enhanced bioactive content (TPC, TFC) and antioxidant capacities (DPPH, ABTS). Formulations at 50LJ:50PJ and 75LJ:25PJ provided an optimal equilibrium, balancing PJ-derived acidity and clarity with LJ's characteristic aroma to produce stable functional beverages with superior sensory profiles. By utilizing local GI fruits through this natural and chemical-free approach, the proposed process supports ESG principles, food security, and value addition to regional agricultural resources. Future work should validate the fermentation process at pilot and industrial scales while further investigating yeast–phenolic interactions to facilitate the commercialization of high-value functional ciders derived from local GI fruits.

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Institutional Review Board Statement: This study was conducted in accordance with the ethical guidelines of the University of Phayao Human Ethics Committee. The study protocol was approved by this committee (Approval Code: No. HREC-UP-HSST 1.3/011/68, approved on 18 February 2025).

Informed Consent Statement: All participants provided informed consent prior to their participation, and the study was conducted in accordance with the Declaration of Helsinki, the Belmont Report, CIOMS Guidelines, and the International Conference on Harmonization in Good Clinical Practice (ICH-GCP). All participants were informed about the nature of the study and provided written informed consent.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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Abbreviations

PJ	Pineapple Juice
LJ	Lychee Juice
TPC	Total Phenolic Content
TFC	Total Flavonoid Content
GI	Geographical Indication
ESG	Environmental, Social, and Governance
TSS	Total Soluble Solids
TA	Titratable Acidity
GAE	Gallic Acid Equivalents
QE	Quercetin Equivalents
DPPH	2,2-diphenyl-1-picrylhydrazyl radical scavenging activity
ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity
TE	Trolox Equivalents
QDA	Quantitative Descriptive Analysis
ANOVA	Analysis of Variance
PCA	Principal Component Analysis
EtOH	Ethanol

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