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Integrated Processing of Dragon Fruit and Cashew Apple Juice for High-Quality Fruit Wine: A Biotechnological Approach

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ABSTRACT

Fruit wine production is a promising strategy for enhancing the value of tropical fruits in Vietnam. This study developed a fermented beverage from red-fleshed dragon fruit (*Hylocereus costaricensis*) and cashew apple (*Anacardium occidentale*), investigating the effects of hydrolysis and fermentation conditions on product quality. The juice blend was enzymatically hydrolyzed with pectinase under varying pH, temperature, time, and enzyme levels. Optimal hydrolysis was achieved at pH 4.5, 50°C, 150 min, and 0.2 g/L enzyme, resulting in the highest juice clarity. Fermentation was carried out using *Saccharomyces cerevisiae* across different dilution ratios and yeast concentrations. The best results were observed at a 1 : 1 dilution and 1 g/L yeast dose, producing 12.54% ethanol. The final product showed high antioxidant capacity and polyphenol content while meeting safety standards. These findings demonstrate the potential of dragon fruit–cashew apple wine as a functional and economically viable product.

1 | Introduction

Vietnam's tropical monsoon climate and diverse soil conditions offer ideal environments for cultivating a wide range of tropical fruits. Among them, red-fleshed dragon fruit (*Hylocereus costaricensis*) and cashew apple (*Anacardium occidentale*) stand out not only for their economic significance but also for their nutritional value and potential in food processing applications [1]. However, the highly seasonal nature and limited postharvest shelf life of these fruits often lead to substantial losses if not promptly processed [2]. Fermentation, particularly into fruit wines, represents a sustainable approach to enhancing their economic value while expanding their functional food applications [3].

Red-fleshed dragon fruit is rich in anthocyanins, vitamin C, and minerals, such as potassium and magnesium, along with significant levels of polyphenolic compounds that exhibit strong antioxidant activity [4]. The fruit's juice typically has a total soluble solids ranging from 12 to 16°Brix and a natural pH between 4.5 and 5.0 [4]. However, its high pectin content results in high viscosity, which can hinder fermentation efficiency unless pretreated through enzymatic hydrolysis [5]. Conversely, the cashew apple—the swollen receptacle of the cashew flower—is often underutilized despite being a potent source of ascorbic acid (AA) (200–300 mg/100 g), tannins (> 2000 mg/100 g), and polyphenols [1]. It possesses a lower pH (3.8–4.2) and contains high levels of reducing sugars and

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organic acids, which support yeast activity and enhance the flavor profile of fermented products [6].

Blending these two fruit juices can offer a synergistic effect: The dragon fruit provides visual appeal and a mild sweetness, while the cashew apple enhances acidity, antioxidant potential, and sensory complexity [7]. Current food fermentation literature suggests that optimizing factors, such as juice blend ratio, pH, temperature, enzyme concentration, and yeast inoculation rate, is essential for achieving desirable fermentation performance, ethanol yield, and product quality [8]. Enzymatic hydrolysis using pectinase is widely adopted in fruit juice clarification, particularly for pectin-rich fruits [9]. Pectinase exhibits optimal activity at pH 4.0–4.5 and temperatures between 45°C and 50°C, effectively breaking glycosidic bonds in pectin molecules [10]. This process reduces viscosity, enhances juice clarity, and facilitates fermentation by improving yeast accessibility to fermentable sugars [11].

The yeast strain *Saccharomyces cerevisiae* is commonly employed in fruit wine production for its robust ethanol production under anaerobic conditions. It performs optimally at temperatures of 25°C–32°C with inoculum levels ranging from 0.5 to 2.0 g/L, typically yielding ethanol concentrations of 10%–14% v/v over a 5- to 10-day fermentation period [12]. Total soluble solids are usually adjusted to 22–24°Brix to support optimal fermentation kinetics [12]. Beyond ethanol, fermentation produces beneficial byproducts, such as organic acids (e.g., lactic and acetic acids), volatile esters that contribute to aroma, and biosynthesized B vitamins. However, poor fermentation control may result in undesirable compounds, such as excess acetic acid, methanol, or off-flavors, posing potential safety and quality concerns. In line with contemporary functional food trends, fruit wines are expected not only to meet alcohol content standards but also to demonstrate high antioxidant activity, desirable clarity and taste, and minimal synthetic additives. Therefore, the development of a fermentation protocol for red-fleshed dragon fruit wine fortified with cashew apple juice requires a comprehensive optimization strategy encompassing raw material treatment, fermentation conditions, and final product quality control.

2 | Material and Methods

2.1 | Plant Material

Red-fleshed dragon fruits (*Hylocereus costaricensis*) cultivated in Binh Thuan Province (10.9332°N, 108.1021°E) were harvested approximately 30 days after flowering. Only fruits were fully matured, undamaged, and morphologically intact. Cashew apples (*Anacardium occidentale*) sourced from Binh Phuoc Province (11.7512°N, 106.7235°E) were harvested approximately 3 months after flowering, coinciding with the nut harvest period. The selected samples displayed green, red, or yellow skin, and were firm, juicy, and structurally intact. The physicochemical properties of the raw materials (reducing sugar content, pH, titratable acidity (TA), and total soluble solids) are presented in Section 3.1. In addition, the raw materials needed to be processed within 24 h after harvest.

2.2 | Chemicals and Reagents

Sodium carbonate (99.8%), Axit 3,5-finitrosalicylic (> 98%), indigo carmine (reagent), potassium permanganate (> 99%), potassium chloride (> 99%), sodium acetate (> 99%), sodium

hydroxide (> 96%), AA (99.7%), 2,2-diphenyl-1-1 picrylhydrazyl (DPPH) (95%), Folin–Ciocalteu reagent (FCR), ethanol (96%), potassium iodide (≥ 99.5%), potassium iodate (≥ 99.7%), sulfuric acid (≥ 98%), formaldehyde (99.8%), AA (99.7%), and phenolphthalein were purchased in Merck (Germany), and starch slurry (99%) was purchased in Xilong (China).

2.3 | Processing

The juice extracted from red-fleshed dragon fruit (*Hylocereus costaricensis*) and cashew apple (*Anacardium occidentale*) was blended at a ratio of 7 : 3 (w/w) [13]. The pH of the mixture was adjusted to a range of 3.5–5.0 using citric acid and sodium carbonate. Pectin hydrolysis was conducted at temperatures ranging from 40°C to 50°C, for durations between 60 and 150 min, with pectinase enzyme concentrations varying from 0.1 to 0.4 g/L. Following hydrolysis, the optimal conditions identified were applied to the fermentation process. The hydrolyzed juice mixture was diluted with water at ratios ranging from 1 : 0.5 to 1 : 2 (w/w) and supplemented with sucrose to achieve a total soluble solids of 23 ± 1°Brix [14–16]. *Saccharomyces cerevisiae* (Angel strain) was inoculated at concentrations ranging from 0.5 to 2.0 g/L, and the fermentation was carried out for 7 days at 30 ± 2°C under anaerobic conditions. The product obtained from the process was pasteurized at 85 ± 2°C for 15 min and then naturally cooled to 25 ± 2°C before storage.

2.4 | Analysis Methods

2.4.1 | Total Polyphenol Content

A total of 50 cashew apples or 25 red-fleshed dragon fruits were prepared; each fruit was cut longitudinally, and one-quarter of the fruit was collected. The pooled samples were homogenized using a blender (SHD5114, Sunhouse, China). A total of 5 g of the homogenized mixture or of the fermented product (10 L per batch) was taken and extracted with 50 mL of 96° ethanol, and then sequentially filtered through 210 µm filter cloth and 11 µm filter paper. For analysis, 0.1 mL of the filtrate was mixed with 0.5 mL of 10% FCR and 0.4 mL of 7.5% sodium carbonate in an incubation tube. The mixture was vortexed and incubated in the dark for 60 min. The absorbance was then measured at 765 nm, and the total phenolic content was calculated using a gallic acid standard calibration curve $y = 127.2584x - 17.3250$ ($R^2 = 0.9994$) [17].

2.4.2 | Total AA

AA content was determined via a redox titration method. The iodine reagent was prepared by dissolving 5 g of potassium iodide and 0.268 g of potassium iodate in 30 mL of 3 M sulfuric acid and then diluting to 500 mL with distilled water. For each 30 g sample, prepared in the same manner as described in Section 2.4.1 (solid samples for raw materials and liquid samples for the final product), 50 mL of distilled water was used for extraction. A 20 mL aliquot of the extract was mixed with 10 drops of 1% starch indicator, followed by titration with iodine solution until a stable blue–black endpoint persisted for 30 s. A standard AA solution (0.1 g/mL) was titrated under identical conditions for calibration [18].

2.4.3 | Total Soluble Solids

Total soluble solids were measured using a refractometer (ATC, Alla, France), which determines sugar content based on the

refraction of light passing through the sample. The fresh fruit samples, after being homogenized using a blender as described in Section 2.4.1, were manually pressed through a filtering cloth with a pore size of 0.1 mm to obtain the juice. Then, two drops of either the extracted juice or the final product were placed on the prism for measurement [15].

2.4.4 | DPPH Antioxidant Activity

Each 5 g sample (prepared in the same manner as described in Section 2.4.1) was extracted with 50 mL of 96° ethanol. A 0.5 mL aliquot of the extract was mixed with 1.5 mL of DPPH solution using a vortex mixer at 1500 rpm for 30 s, followed by incubation in the dark for 30 min. Each 1 mL sample was placed in a quartz cuvette, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Antioxidant activity was quantified based on a standard curve constructed with AA equivalents $y = -36.75x + 28.31$ ($R^2 = 0.9990$) [19].

2.4.5 | Transmittance

To remove pulp and large suspended particles, 40 mL of juice was centrifuged at 3600 rpm for 10 min. The clarity of the supernatant was then assessed spectrophotometrically by measuring absorbance at 660 nm, and percent clarity was calculated accordingly [19].

2.4.6 | pH

The samples were prepared in the same manner as described in Section 2.4.3 to obtain the liquid extract. A total of 30 mL of the extracted juice or the final product was transferred into a glass beaker. The pH value of the samples was measured using a HI2211 pH/ORP meter (Hanna Instruments Ltd., USA) to ensure accuracy and consistency across all treatments [17].

2.4.7 | Ethanol

A total of 100 mL of sample was diluted with 100 mL of distilled water. Distillation was carried out at 85°C–90°C using a 600 mm condenser and continued until 90 mL of distillate was collected. The distillate was then made up to 100 mL with distilled water. Ethanol concentration was determined after simple distillation steps using a digital density meter (Snap 51, Anton Paar, USA) [15].

2.4.8 | Reducing Sugar

The reducing sugar content was determined based on the colorimetric reaction between reducing sugars and dinitrosalicylic acid reagent. A 10 mL portion of the initial sample is blended with 1000 mL of distilled water, thoroughly homogenized, and then passed through Whatman No. 1 filter paper (11 µm). The clarified filtrate is subsequently diluted by a factor of 3–5, depending on its sugar content. A 1 mL aliquot of this diluted solution is reacted with 2 mL of DNS reagent. The mixture is incubated in a water bath for 5 min and then allowed to cool to room temperature. Finally, the optical density of the resulting solution is recorded at a wavelength of 540 nm: $y = 0.2441x + 2.9172$ ($R^2 = 0.9992$) [15].

2.4.9 | TA

TA was evaluated using an automated titration unit equipped with potentiometric detection (TitroLine 7000, SI Analytics,

Oberbayern, Germany). A volume of 10 mL of the extract (Section 2.4.1) or the product was mixed with 50 mL of distilled water, and three drops of 0.1% phenolphthalein solution were added. Continuous agitation was maintained throughout the titration. The sample was then neutralized with 0.1 N NaOH until the indicator endpoint was attained. TA was calculated from the volume of NaOH consumed and expressed as g of citric acid equivalents per liter (gCAE/L), using an equivalent weight of 64.04 g/equivalent for citric acid [20].

2.4.10 | Tannin Content

Tannins were quantified through a titrimetric approach based on the Lowenthal principle. A total of 10 mL of the extract (Section 2.4.1) or the product was mixed with 1 mL of indigo carmine followed by the addition of 100 mL of distilled water. The resulting mixture was titrated with a KMnO₄ solution until the appearance of a yellow endpoint. A corresponding blank test was carried out using the same procedure, substituting distilled water for the sample extract [1].

2.4.11 | Total Anthocyanin Content

The total anthocyanin content was determined using the pH differential method. Samples were diluted with pH 1.0 (potassium chloride) and pH 4.5 (sodium acetate) buffers, and then centrifuged at 5000 rpm, 4°C for 15 min. The absorbance was measured at 520 and 700 nm, and TAC was calculated as cyanidin-3-glucoside equivalent (mgC3G/L) (An et al., 2024).

2.4.12 | Yeast Cell Counting

Yeast cell density and viability were quantified using a hemocytometer in conjunction with light microscopy. Before analysis, the yeast suspension was thoroughly homogenized using a vortex mixer, and a 10 µL aliquot was carefully loaded onto the counting grid. The chamber was then examined under a microscope equipped with a 40× objective lens and optimized illumination. Cell enumeration was performed across five designated large squares to ensure accuracy and statistical reliability [20].

2.4.13 | Methanol Content

A total of 5 mL of sulfuric acid (H₂SO₄, 97%) and 1 mL of potassium dichromate solution (K₂Cr₂O₇, 10% w/v) were added to each test sample containing a known mixture of methanol and ethanol in deionized water. After vortexing for 2 min, the resulting oxidation reaction yielded a chromogenic response. The absorbance was measured using a Shimadzu UV-1800 spectrophotometer across the range of 900–1100 cm⁻¹, with the peak at 970 cm⁻¹ corresponding to methanol. A calibration curve was constructed using partial least squares (PLS) regression, achieving a detection limit (LOD) of 0.5% and quantification limit (LOQ) of 1.5% methanol (w/v) [21].

2.4.14 | Sulfite Content (SO₂)

The sulfite content in wine samples was determined using the Monier-Williams distillation–titration method. A 25 mL wine sample was acidified with 10 mL of 0.5 M HCl and gently heated. The released SO₂ was captured in 25 mL of 3% hydrogen peroxide under a nitrogen stream for 20 min. After cooling, the absorbent solution was titrated with 0.01 N NaOH using phenolphthalein as

TABLE 1 | Characterization of the physicochemical and functional composition of red dragon fruit and cashew apple.

No.	Indicators	Red dragon	Cashew apple	Units
1	pH	4.79 ± 0.01	4.13 ± 0.12	—
2	Total soluble solids	14.00 ± 0.21	9.00 ± 0.52	°Brix
3	Titrateable acidity	5.41 ± 0.11	4.46 ± 0.14	gCAE/L
4	Reducing sugar	102.02 ± 0.32	72.17 ± 0.98	g/L
5	DPPH antioxidant activity	24.71 ± 0.22	24.44 ± 0.14	%
6	Tannin content	449.44 ± 0.45	4226.59 ± 0.37	mg/100 g
7	Total polyphenol content	742.86 ± 0.79	486.53 ± 0.89	mgGAE/100 g
8	Total ascorbic acid	361.97 ± 0.63	1321.13 ± 0.63	mgAA/100 g
9	Total anthocyanin content	193.64 ± 0.87	—	mg/g

Note: Data are expressed as mean ± standard deviation (SD) from three independent experiments ($n = 3$), each analyzed in triplicate ($n = 3$). Abbreviations: AA, ascorbic acid; CAE, citric acid equivalent; DPPH, 2,2-diphenyl-1-1 picrylhydrazyl; GAE, gallic acid equivalent.

an indicator. The volume of NaOH used was recorded to calculate the sulfite content and expressed as mgSO₂/L [22].

2.4.15 | Ochratoxin A (OTA) Content

The OTA content in samples was determined using high-performance liquid chromatography (HPLC) following immunoaffinity column clean-up, as described in the OchraTest manual. A 25 g sample was mixed with 5 g of sodium chloride and extracted using 100 mL of methanol–water (80 : 20, v/v) by shaking for 1 h. The extract was filtered, and 10 mL of the filtrate was diluted with 90 mL of phosphate-buffered saline (PBS, pH 7.4) and then centrifuged at 4000 rpm for 30 min. A 40 mL aliquot of the supernatant was applied to a preconditioned OchraTest immunoaffinity column, followed by sequential washes with 5 mL of PBS and 5 mL of distilled water. The OTA was eluted with 2 mL of methanol, evaporated under vacuum, and reconstituted in 200 µL of mobile phase. A 20 µL aliquot of this solution was injected into the HPLC system. The mobile phase consisted of acetonitrile, water, and acetic acid (42.6 : 56.4:1, v/v/v). Fluorescence detection was performed with excitation and emission wavelengths set at 330 nm and 460 nm, respectively [23].

2.4.16 | Lead Content (Pb)

The lead content in samples was determined using high-resolution continuum source graphite furnace atomic absorption spectrometry (HRCS-GFAAS). Each 0.25 g of sample was weighed into polytetrafluoroethylene digestion vessels. Samples were digested using a mixture of 7 mL concentrated nitric acid (HNO₃) and 1 mL hydrogen peroxide (H₂O₂) in a microwave digestion system under a two-step temperature program: ramping to 190°C in 10 min, followed by holding at 190°C for 30 min. The digested solution was evaporated to ~2 mL and then diluted to 50 mL with deionized water. For GFAAS analysis, 20 µL of the prepared solution was injected, with 10 g/L ammonium phosphate monobasic (NH₄H₂PO₄) used as a chemical modifier. The optimized pyrolysis and atomization temperatures for Pb were 900°C and 1500°C, respectively. Calibration was performed using nonlinear regression with standard solutions ranging from 0 to 80 mg/L [24].

2.4.17 | Statistical and Data Analysis

The final experimental results represent data obtained from three independent processing batches, with each analytical sample

measured in triplicate ($n = 9$). Data were analyzed using Excel 2016 (Redmond, WA, USA). ANOVA (95% confidence) and LSD tests determined significance (Plains, Virginia, USA). Graphs and optimizations were generated via JMP Pro 18 (SAS Institute, Cary, North Carolina, USA).

3 | Result and Discussion

3.1 | Evaluation of Physicochemical and Biochemical Properties of Raw Materials

The physicochemical and bioactive profiles of red-fleshed dragon fruit (*Hylocereus costaricensis*) and cashew apple (*Anacardium occidentale*) reveal complementary nutritional attributes, making them highly suitable for combined fermentation in functional beverage development. As shown in Table 1, the pH values of dragon fruit (4.79) and cashew apple (4.13) both fall within the slightly acidic range, conducive to yeast viability and microbial stability during fermentation. The total soluble solids, which reflect the fermentable sugar content, were higher in dragon fruit (14.00°Brix) than in cashew apple (9.00°Brix), suggesting a greater initial sugar reservoir in the former for ethanol conversion. TA results also demonstrate that dragon fruit (5.41 gCAE/L) possesses higher organic acid content compared to cashew apple (4.46 gCAE/L), which can influence both the buffering capacity and flavor complexity of the final product. Reducing sugar content, a direct indicator of fermentable monosaccharides and disaccharides, was significantly greater in dragon fruit (102.02 g/L) than in cashew apple (72.17 g/L), further supporting its primary role as a sugar donor in the fermentation process.

From a functional food perspective, both fruits exhibit strong antioxidant profiles. DPPH antioxidant activities were comparable (24.71% for dragon fruit and 24.44% for cashew apple), indicating equivalent potential for oxidative stress reduction. However, their phenolic compositions vary markedly. Cashew apple contains a notably higher tannin concentration (4226.59 mg/100 g) compared to dragon fruit (449.44 mg/100 mL), which may contribute to astringency and microbial inhibition if not properly balanced during formulation. The total polyphenol content was higher in dragon fruit (742.86 mgGAE/100 g), contributing to color stability and overall antioxidant capacity, while total AA (vitamin C) was markedly elevated in cashew

TABLE 2 | Influence of enzymatic hydrolysis parameters on product transparency.

Factors	Value	Transparency
pH	3.5	54.53 ± 0.33 ^d
	4.0	65.62 ± 0.68 ^c
	4.5	74.65 ± 0.43 ^a
	5.0	70.36 ± 0.27 ^b
Temperature (°C)	40	66.77 ± 0.77 ^c
	45	68.50 ± 0.43 ^b
	50	74.65 ± 0.69 ^a
	—	—
Time (min)	60	74.47 ± 0.54 ^b
	90	75.05 ± 0.72 ^b
	120	76.27 ± 0.58 ^a
	150	76.80 ± 0.44 ^a
Enzyme content (g/L)	0.1	61.05 ± 0.88 ^c
	0.2	76.80 ± 0.26 ^a
	0.3	66.68 ± 0.34 ^b
	0.4	66.57 ± 0.61 ^b

Note: The lowercase (a–d) letters or groups of letters indicated the difference in quality between fermentation conditions; (—): not evaluated; data are expressed as mean ± standard deviation (SD) from three independent experiments ($n = 3$), each analyzed in triplicate ($n = 3$).

apple (1321.13 mgAA/100 g), surpassing dragon fruit by nearly fourfold (361.97 mgAA/100 g). This makes cashew apple a potent source of natural vitamin C, which can synergistically enhance the antioxidant profile of the blended juice. In addition, several previous reports have suggested that the presence of vitamin C can enhance the survival efficiency of *Saccharomyces cerevisiae* during fermentation [25]. Another study successfully achieved vitamin C biosynthesis using wild-type *Saccharomyces cerevisiae*, further supporting the notion that vitamin C exerts a positive effect on the activity of *Saccharomyces cerevisiae* [26]. In addition, vitamin C has also been reported to exhibit potential inhibitory effects against several harmful bacteria, such as *S. aureus*, *Streptococcus*, and *P. aeruginosa* [27]. Therefore, a product with a high vitamin C content not only enhances yeast activity and provides antioxidant capacity but also inhibits harmful bacterial strains, thereby contributing to improved product safety. Notably, dragon fruit also provides a valuable concentration of anthocyanins (193.64 mg/g), natural pigments with strong antioxidant and anti-inflammatory effects, further enriching the bioactive matrix of the mixture.

Taken together, these findings indicated that blending dragon fruit and cashew apple juices provides a more balanced biochemical matrix—combining complementary sugars, organic acids, minerals, and phytochemicals. Although sugar and acidity were later standardized to ensure experimental consistency, the intrinsic improvements created by the blending process still contribute significantly to the nutritional value and functional properties of the final fermented beverage. Standardization also needs to be conducted at a sufficiently high total soluble solids level to improve ethanol production efficiency and meet wine quality standards. A previous report indicated that fermentation at the native TSS level (9–14°Brix) using *Saccharomyces cerevisiae* strains AR5 and GU3 resulted in a maximum ethanol concentration of only about 30 g/L, equivalent to approximately 7.5% ethanol. Ethanol kinetics stabilized with no significant changes

after around 20 h, and extending fermentation to 80 h did not yield any further notable increase in ethanol content [28].

3.2 | Hydrolytic Efficiency of Pectinase on Blended Fruit Juice Substrates

The hydrolysis efficiency of red-fleshed dragon fruit juice using pectinase was significantly influenced by pH, temperature, reaction time, and enzyme concentration, as evidenced by the clarity (transparency) of the treated juice (Table 2). Among the tested pH levels, a value of 4.5 resulted in the highest transparency (74.65%), confirming the optimal catalytic activity of pectinase under slightly acidic conditions. At this pH, the ionization state of amino acid residues in the enzyme's active site is maintained in a configuration that promotes maximum substrate binding and cleavage efficiency [29]. Deviations from this optimum, particularly at pH 3.5 or above 5.0, led to partial protonation or deprotonation of key functional groups, disrupting enzyme–substrate interactions and reducing hydrolysis performance [30]. Similarly, increasing the hydrolysis temperature from 40°C to 50°C enhanced molecular collisions between enzyme and substrate, thereby accelerating the breakdown of the pectin network and resulting in improved juice clarity, with transparency reaching a peak at 50°C (74.65%) [31]. This temperature likely approximates the thermal optimum of the pectinase used, beyond which enzymatic activity may decline due to denaturation. Reaction time also played a critical role; while transparency increased progressively from 74.47% at 60 min to 76.80% at 150 min, the marginal gains after 90 min suggest that the majority of the pectin had already been hydrolyzed by this point, with prolonged exposure contributing minimally to further clarification. The most pronounced improvement in clarity was observed when the enzyme concentration was adjusted to 0.2 g/L, yielding the highest transparency (76.80%). At this dosage, the enzyme-to-substrate ratio enabled efficient hydrolysis without excessive free enzyme molecules. In contrast, higher concentrations, such as 0.3 and 0.4 g/L, resulted in reduced clarity, likely due to enzyme saturation and the possible formation of intermediate hydrolysis products that interfered with light transmission [32]. These findings unequivocally demonstrate that pH 4.5, a temperature of 50°C, 150 min of hydrolysis, and an enzyme concentration of 0.2 g/L constitute the optimal conditions for pectin breakdown in red-fleshed dragon fruit juice, enabling maximal release of soluble compounds and enhancing the overall transparency of the juice.

3.3 | Influence of Dilution Ratios on the Fermentative Performance of Mixed Fruit Juice

The dilution ratio significantly influenced the physicochemical characteristics of the fermented product, particularly ethanol concentration, pH, TA, and yeast proliferation (Table 3). Throughout the 7-day fermentation process, the 1:1 dilution ratio consistently yielded the highest ethanol concentration, peaking at 12.54% v/v, while the 1:2.5 dilution resulted in the lowest ethanol content (8.09%). Although the total soluble solids were adjusted to the same level across all treatments before fermentation by sugar supplementation, the differing dilution ratios led to significant variations in fermentation outcomes. This trend is primarily attributed to the physiological effects of osmotic pressure. In more diluted systems (1:2.5), achieving the same TSS required the addition of significantly more sugar,

TABLE 3 | Impact of fermentation duration and dilution level with water on product physicochemical characteristics.

Days	Ratio (w/w)	TSS (°Brix)	pH	TA (g/L)	Ethanol (%)	Yeast (10 ⁶) (cells/mL)
1	1 : 1	21.50 ± 0.22 ^{Aa}	3.88 ± 0.09 ^{Aa}	1.68 ± 0.06 ^{Da}	1.76 ± 0.09 ^{Gc}	21.01 ± 0.11 ^{Fc}
	1 : 1.5	21.50 ± 0.36 ^{Aa}	3.99 ± 0.05 ^{Aa}	1.39 ± 0.03 ^{Dc}	1.96 ± 0.03 ^{Eb}	25.17 ± 0.23 ^{Fa}
	1 : 2	21.00 ± 0.16 ^{Aa}	3.83 ± 0.05 ^{Aa}	1.39 ± 0.03 ^{Cc}	2.11 ± 0.06 ^{Fa}	24.17 ± 0.26 ^{Eb}
	1 : 2.5	22.50 ± 0.42 ^{Aa}	3.78 ± 0.06 ^{Aa}	1.52 ± 0.05 ^{Cb}	1.74 ± 0.05 ^{Gc}	20.25 ± 0.31 ^{Dc}
2	1 : 1	17.00 ± 0.17 ^{Bb}	3.63 ± 0.02 ^{Ea}	3.37 ± 0.08 ^{Cb}	4.13 ± 0.02 ^{Fc}	31.58 ± 0.25 ^{Eb}
	1 : 1.5	17.50 ± 0.33 ^{Bb}	3.62 ± 0.06 ^{Da}	2.89 ± 0.09 ^{Cd}	4.80 ± 0.08 ^{Db}	31.58 ± 0.42 ^{Eb}
	1 : 2	18.00 ± 0.42 ^{Ba}	3.57 ± 0.02 ^{Ba}	3.44 ± 0.03 ^{Ba}	5.21 ± 0.02 ^{Ea}	32.58 ± 0.43 ^{Da}
	1 : 2.5	19.30 ± 0.38 ^{Ba}	3.46 ± 0.01 ^{Ea}	3.20 ± 0.23 ^{Bc}	4.21 ± 0.04 ^{Fc}	24.01 ± 0.56 ^{Dc}
3	1 : 1	14.00 ± 0.39 ^{Cd}	3.68 ± 0.04 ^{Da}	3.99 ± 0.07 ^{Ac}	7.76 ± 0.03 ^{Ea}	64.03 ± 0.53 ^{Ba}
	1 : 1.5	15.00 ± 0.41 ^{Cc}	3.64 ± 0.03 ^{CDa}	4.01 ± 0.12 ^{Ab}	6.93 ± 0.16 ^{Cb}	57.58 ± 0.64 ^{BCb}
	1 : 2	16.00 ± 0.51 ^{Cb}	3.56 ± 0.04 ^{Bb}	4.23 ± 0.14 ^{Aa}	6.37 ± 0.06 ^{Db}	49.50 ± 0.33 ^{Bc}
	1 : 2.5	17.00 ± 0.23 ^{Ca}	3.48 ± 0.06 ^{Db}	3.97 ± 0.12 ^{Ac}	5.97 ± 0.06 ^{Ec}	43.42 ± 0.42 ^{Bd}
4	1 : 1	12.00 ± 0.71 ^{Dd}	3.71 ± 0.02 ^{Ca}	3.79 ± 0.12 ^{ABa}	9.55 ± 0.03 ^{Da}	73.42 ± 0.17 ^{Aa}
	1 : 1.5	13.00 ± 0.13 ^{Dc}	3.64 ± 0.01 ^{Ca}	3.14 ± 0.23 ^{BCc}	8.66 ± 0.04 ^{Bb}	63.92 ± 0.32 ^{Ab}
	1 : 2	14.00 ± 0.42 ^{Db}	3.53 ± 0.04 ^{Cb}	3.56 ± 0.19 ^{Ba}	8.34 ± 0.04 ^{Cb}	60.02 ± 0.26 ^{Ac}
	1 : 2.5	15.00 ± 0.25 ^{Da}	3.50 ± 0.03 ^{Cb}	3.35 ± 0.25 ^{Bb}	7.37 ± 0.02 ^{Dc}	54.25 ± 0.52 ^{Ad}
5	1 : 1	10.50 ± 0.15 ^{Ed}	3.70 ± 0.04 ^{Ca}	3.54 ± 0.22 ^{BCa}	10.61 ± 0.08 ^{Ca}	67.01 ± 0.31 ^{Ba}
	1 : 1.5	11.00 ± 0.27 ^{Ec}	3.62 ± 0.12 ^{Ca}	2.99 ± 0.03 ^{BCc}	8.91 ± 0.08 ^{Ab}	59.83 ± 0.34 ^{Bb}
	1 : 2	12.00 ± 0.21 ^{Eb}	3.50 ± 0.04 ^{Db}	3.30 ± 0.09 ^{Bb}	8.61 ± 0.05 ^{Bb}	51.33 ± 0.23 ^{Bc}
	1 : 2.5	13.50 ± 0.24 ^{Ea}	3.47 ± 0.03 ^{Eb}	3.05 ± 0.22 ^{Bc}	7.87 ± 0.01 ^{Cc}	52.08 ± 0.34 ^{Ac}
6	1 : 1	9.00 ± 0.51 ^{Fd}	3.77 ± 0.02 ^{Ba}	3.63 ± 0.05 ^{BCa}	12.12 ± 0.03 ^{Ba}	56.50 ± 0.11 ^{Ca}
	1 : 1.5	10.00 ± 0.21 ^{Fc}	3.70 ± 0.02 ^{Ba}	3.14 ± 0.08 ^{BCb}	9.05 ± 0.04 ^{Ab}	55.75 ± 0.48 ^{Cb}
	1 : 2	10.50 ± 0.37 ^{Gb}	3.56 ± 0.09 ^{Bb}	3.40 ± 0.07 ^{Ba}	8.89 ± 0.01 ^{Ac}	46.42 ± 0.54 ^{Bc}
	1 : 2.5	12.50 ± 0.38 ^{Ga}	3.53 ± 0.02 ^{Bb}	3.32 ± 0.10 ^{Ba}	8.41 ± 0.04 ^{Ad}	45.01 ± 0.27 ^{Bc}
7	1 : 1	8.00 ± 0.42 ^{Gd}	3.79 ± 0.08 ^{Ba}	3.63 ± 0.04 ^{BCa}	12.54 ± 0.03 ^{Aa}	46.67 ± 0.41 ^{Db}
	1 : 1.5	9.00 ± 0.17 ^{Gc}	3.71 ± 0.05 ^{Ba}	3.28 ± 0.05 ^{Bc}	9.04 ± 0.03 ^{Ab}	49.17 ± 0.39 ^{Da}
	1 : 2	10.00 ± 0.06 ^{Gb}	3.57 ± 0.08 ^{Bb}	3.55 ± 0.17 ^{Bb}	8.68 ± 0.12 ^{Bc}	39.17 ± 0.42 ^{Cc}
	1 : 2.5	11.00 ± 0.06 ^{Ga}	3.54 ± 0.05 ^{Bb}	3.39 ± 0.24 ^{Bc}	8.09 ± 0.04 ^{Bd}	37.75 ± 0.73 ^{Cc}

Note: The uppercase (A–G) and lowercase (a–d) letters or groups of letters indicated the difference in quality between fermentation days and dilution ratios, respectively; data are expressed as mean ± standard deviation (SD) from three independent experiments ($n = 3$), each analyzed in triplicate ($n = 3$).

which in turn created a hyperosmotic environment. Elevated osmotic pressure imposes stress on yeast cells, impairing their membrane integrity, nutrient transport, and metabolic activity, thus inhibiting ethanol production despite the availability of fermentable substrates. In contrast, the more concentrated 1 : 1 medium maintained a more favorable osmotic balance, supporting efficient sugar uptake, yeast proliferation, and ethanol biosynthesis [15]. Residual total soluble solid values reinforce this interpretation, with the lowest total soluble solids observed at the 1 : 1 ratio (8.0°Brix), and the highest at 1 : 2.5 (11.0°Brix), indicating incomplete sugar utilization under high-osmotic conditions. Yeast population dynamics further mirror this trend: Cell densities increased rapidly during the initial three days, peaking on day 4 at 73.42×10^6 cells/mL for the 1 : 1 dilution, followed by a gradual decline. This trajectory reflects a classic microbial growth pattern modulated by nutrient availability and environmental stress, with ethanol accumulation potentially contributing to growth inhibition in later stages [33]. TA levels also aligned with ethanol trends, reaching the highest value at the 1 : 1 dilution (3.63 g/L) and the lowest at the 1 : 1.5 ratio (3.28 g/L). As acetic acid, a major component of TA in fruit wines, is generated through ethanol oxidation under semiaerobic conditions, the observed TA variation underscores the metabolic linkage between ethanol output and acid formation [34]. Meanwhile, final pH values were higher in more concentrated

samples (3.79 ± 0.08 for 1 : 1) and lower in more diluted ones (3.54 at 1 : 2.5), likely due to stronger buffering capacity and higher metabolic turnover in denser media. Taken together, these findings demonstrate that a 1 : 1 dilution ratio provides the most favorable fermentation environment in this red dragon fruit–cashew apple wine system. Despite equal initial total soluble solids, lower dilution levels mitigate osmotic stress, preserve yeast viability, and optimize substrate-to-product conversion. These results corroborate previous research on tropical fruit wine fermentations and highlight the importance of controlling not just sugar concentration, but also the physicochemical matrix in which fermentation occurs. The 1 : 1 dilution ratio thus represents a scientifically validated optimal condition for maximizing ethanol production and fermentation quality.

3.4 | Dynamic Variation in Yeast Cell Density Throughout Fermentation

The initial yeast inoculation level had a pronounced impact on the growth dynamics and population density of *Saccharomyces cerevisiae* throughout the fermentation process. As illustrated in Figure 1, yeast content increased rapidly from day 1 to day 4 for all inoculation levels, reaching peak cell densities before gradually declining toward day 7. A clear positive correlation was observed between initial yeast concentration and maximum

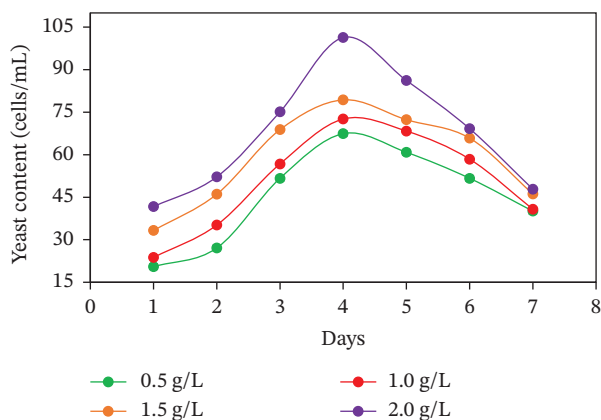


FIGURE 1 | Dynamic variation in yeast cell density throughout fermentation.

yeast population: At 0.5 g/L, yeast density increased from 20.50×10^6 to 67.42×10^6 cells/mL; at 1.0 g/L, from 23.75×10^6 to 72.58×10^6 cells/mL; at 1.5 g/L, from 33.25×10^6 to 79.33×10^6 cells/mL; and at 2.0 g/L, the highest growth was recorded, from 41.67×10^6 to 101.33×10^6 cells/mL. This trend unequivocally confirms that higher inoculation rates stimulate more robust yeast proliferation during the early exponential phase due to greater initial biomass and accelerated metabolic activation [35].

However, from day 5 onward, all treatments exhibited a steady decline in yeast density, attributed to nutrient depletion, ethanol accumulation, and the gradual shift to stationary and death phases. Notably, despite the rapid initial growth at 2.0 g/L, the residual yeast concentration on day 7 (47.83×10^6 cells/mL) was only moderately higher than that at 1.0 g/L (40.75×10^6 cells/mL), suggesting diminishing returns at higher inoculum levels. While a higher yeast dose can expedite fermentation by enhancing substrate conversion rates and shortening lag phases, excessive inoculation is not necessarily beneficial. Overcrowded environments may lead to competition for limited nutrients, elevated ethanol toxicity, and metabolic stress, which ultimately inhibit further cell division and compromise product quality [36].

Additionally, large-scale fermentation using high yeast concentrations demands extended propagation time and increased production cost. However, suboptimal inoculum levels (0.5 g/L) result in prolonged adaptation and fermentation time, lower ethanol output, and inferior product characteristics, consistent with previous findings on sluggish fermentations under low biomass conditions.

Considering both biological efficiency and practical feasibility, the data support that an initial yeast concentration of 1.0 g/L provides the most balanced and effective condition, enabling sufficient growth, adequate fermentation rate, and favorable final product quality without incurring unnecessary resource demands. This inoculation level ensures optimal yeast metabolic performance while avoiding overstimulation or premature stagnation due to environmental stress.

3.5 | Evaluation of Product Quality Attributes

Table 4 presents a comprehensive overview of the physicochemical attributes and food safety compliance of the final fermented wine product derived from red-fleshed dragon fruit and cashew apple juice. The values reflect favorable fermentation performance and nutritional retention, along with strict adherence to national safety standards. The pH value of 4.00 lies within the acceptable acidic range for fruit wines, supporting microbial stability and flavor preservation. The total soluble solids at 9.00° Brix indicate that a significant portion of the sugars was metabolized during fermentation, correlating well with the ethanol level and demonstrating efficient yeast activity. The TA of 2.46 g/L aligns with typical acidity levels in balanced fruit wines, contributing to freshness and shelf-life extension. Meanwhile, reducing sugars remains at 15.63 g/L, indicating partial residual sweetness that may enhance the sensory appeal without impairing product stability. From a functional nutrition standpoint, the product exhibits strong DPPH antioxidant activity (9.31%), coupled with high levels of polyphenols (596.85 mgGAE/100 mL) and tannins (430.34 mg/100 mL). These figures suggest potent antioxidant capacity, likely contributing to the product's bioactive profile and potential health-promoting effects. The AA (vitamin C) content of 137.11 mgAA/100 mL

TABLE 4 | Physicochemical composition and safety parameters of the final fermented wine product.

No.	Indicators	Units	Limit	Content
1	pH		—	4.00 ± 0.07
2	Total soluble solids	°Brix	—	9.00 ± 0.11
3	Titrateable acidity	gCAE/L	—	2.46 ± 0.32
4	Reducing sugar	g/L	—	15.63 ± 0.34
5	DPPH antioxidant activity	%	—	9.31 ± 0.14
6	Tannin content	mg/100 mL	—	430.34 ± 0.98
7	Total polyphenol content	mgGAE/100 mL	—	596.85 ± 0.58
8	Total ascorbic acid	mmAA/100 mL	—	137.11 ± 0.73
9	Ethanol content	% v/v	> 8.50	12.21 ± 0.64
10	Methanol content	mg/L	< 10.00	—
11	Sulfite content (SO ₂)	mg/kg	< 10.00	—
12	Ochratoxin A content	µg/kg	< 1.00	—
13	Lead content (Pb)	mg/L	< 0.01	—

Note: (—): not detected or not specified; data are expressed as mean $\pm$ standard deviation (SD) from three independent experiments ($n = 3$), each analyzed in triplicate ($n = 3$).

further enhances its functional food value, exceeding average values found in many commercial fruit beverages. The ethanol content at 12.21% v/v surpasses the regulatory minimum threshold of 8.50%, validating the product's classification as a standard alcoholic beverage and confirming optimal fermentation conditions. Besides, potential lead contamination in fruit wine fermentation commonly arises from raw materials, water quality, and contact with metallic components during enzymatic treatment or fermentation. In this study, all equipment in contact with juice or wine was made from food-grade stainless steel or glass, and purified water was used throughout the process. Fruits were thoroughly washed, and fermentation was conducted under closed conditions to prevent environmental contamination. These controls effectively eliminated all common contamination pathways. Indeed, no methanol, sulfite (SO₂), OTA, or lead (Pb) residues were detected, indicating full compliance with food safety standards as per QCVN 6-3:2010/BYT for alcoholic beverages, QCVN 8-1:2011/BYT for mycotoxin limits, and TCVN 7045:2013 for wine products. These data clearly confirm that the final fermented wine meets both physicochemical and toxicological safety requirements. The absence of methanol and heavy metals affirms that the fermentation process did not lead to any undesirable metabolic byproducts or contamination. The lack of detectable sulfites and OTA enhances consumer safety and supports clean-label claims.

In conclusion, the wine product exhibits a well-balanced profile, demonstrating both technological success in fermentation and nutritional-functional potential. The product not only meets but also exceeds several quality benchmarks for artisanal fruit wines, offering a promising avenue for commercialization with health-oriented positioning.

4 | Conclusion

This study successfully demonstrates the feasibility of producing a high-quality fruit wine from red-fleshed dragon fruit and cashew apple through enzymatic hydrolysis and controlled fermentation. Optimal hydrolysis conditions (pH 4.5, 50°C, 150 min, 0.2 g/L pectinase) significantly enhanced juice clarity (76.80%), while fermentation at a 1 : 1 dilution ratio with 1.0 g/L *Saccharomyces cerevisiae* yielded the highest ethanol content (12.54% v/v) and favorable physicochemical properties. The final product exhibited strong antioxidant potential, with 596.85 mgGAE/100 mL polyphenols, 137.11 mgAA/100 mL vitamin C, and 9.31% DPPH antioxidant activity, highlighting its functional value. Importantly, all safety parameters including methanol, sulfites, OTA, and lead were within acceptable limits or undetectable. These findings reinforce the potential of blending underutilized tropical fruits into value-added fermented beverages. Future research may focus on scaling the process for commercial production, enhancing flavor stability, and evaluating consumer acceptance to support broader market penetration of functional tropical fruit wines.

Author Contributions

Van Thinh Pham: software, supervision, project administration, and validation; Thi Thu Huong Tran: resources and visualization; Le Thi Bich Thom: methodology, data curation, and formal analysis; Nguyen Thanh Danh: methodology, data curation, and formal analysis; Ngoc Minh Truong:

supervision; Thi Ngoc Tram Ho: validation; Le Hanh Uyen: validation; Binh An Pham: conceptualization, formal analysis, and writing—original draft; Ngoc Duc Vu: conceptualization, formal analysis, funding acquisition, investigation, writing—original draft, and writing—review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All the data are available within this manuscript.

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