Apparent alcoholic production from *Ananas comosus* and *Passiflora edulis* peel hydrolysates fermented with *Saccharomyces cerevisiae*

Producción alcohólica aparente a partir de hidrolizados de cáscaras de *Ananas comosus* y *Passiflora edulis* fermentados con *Saccharomyces cerevisiae*

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ABSTRACT

In this study, we compared the apparent alcoholic response of *Ananas comosus* and *Passiflora edulis* peel hydrolysates fermented with *Saccharomyces cerevisiae* in a 3 × 2 factorial design. Fermentation time (24, 36 and 72 h) and raw material were evaluated using three technical fermentation replicates per treatment and a fixed dose of 1 g L^{-1} commercial dry yeast. The peel flours were characterized by proximate analysis, and the hydrolysates were obtained by controlled dilute-acid treatment, neutralization and pasteurization. The response variables were final °Brix, apparent relative reduction in soluble solids, apparent alcoholic percentage and apparent productivity. Both flours contained more than 80 g 100 g^{-1} of carbohydrates. Time, raw material and their interaction significantly affected the apparent alcoholic percentage ($p < 0.001$, $p < 0.001$ and $p = 0.013$, respectively). Pineapple hydrolysate reached the highest observed response at 72 h, with 4.93 ± 0.06% v/v apparent alcohol and 86.70 ± 0.68% apparent relative reduction in soluble solids; passion fruit reached 3.50 ± 0.26% v/v and 72.85 ± 0.48%. The factorial model showed $R^2 = 96.42\%$, adjusted $R^2 = 94.92\%$ and predicted $R^2 = 91.93\%$. A quadratic model predicted an exploratory optimum close to 69 h for pineapple hydrolysate, at approximately 4.96% v/v, but this condition was not independently validated. Ethanol was not confirmed chromatographically; the reported values therefore represent apparent alcoholic percentage after distillation and densimetric measurement. This study provides preliminary comparative evidence for laboratory-scale valorization of fruit-peel hydrolysates.

Keywords: *apparent alcoholic percentage,* Ananas comosus, Passiflora edulis, Saccharomyces cerevisiae, *peel hydrolysate*

RESUMEN

Este estudio comparó la respuesta alcohólica aparente de hidrolizados de cáscaras de *Ananas comosus* y *Passiflora edulis* fermentados con *Saccharomyces cerevisiae* mediante un diseño factorial 3 × 2. Se evaluaron el tiempo de fermentación (24, 36 y 72 h) y la materia prima, con tres réplicas técnicas de fermentación por tratamiento y una dosis fija de 1 g L^{-1} de levadura seca comercial. Las harinas se caracterizaron mediante análisis proximal y los hidrolizados se obtuvieron mediante tratamiento ácido diluido, neutralización y pasteurización. Las variables de respuesta fueron °Brix final, reducción relativa aparente de sólidos solubles, porcentaje alcohólico aparente y productividad aparente. Ambas harinas presentaron más de 80 g 100 g^{-1} de carbohidratos. El tiempo, la materia prima y su interacción afectaron significativamente el porcentaje alcohólico aparente ($p < 0.001$, $p < 0.001$ y $p = 0.013$, respectivamente). El hidrolizado de piña alcanzó la mayor respuesta observada a 72 h, con 4.93 ± 0.06% v/v de alcohol aparente y 86.70 ± 0.68% de reducción relativa aparente de sólidos solubles; el maracuyá alcanzó 3.50 ± 0.26% v/v y 72.85 ± 0.48%. El modelo factorial presentó $R^2 = 96.42\%$, R^2 ajustado = 94.92% y R^2 predicho = 91.93%. Un modelo cuadrático predijo un óptimo exploratorio cercano a 69 h para el hidrolizado de piña, con aproximadamente 4.96% v/v, pero esta condición no se validó de manera independiente. El etanol no se confirmó cromatográficamente; los valores corresponden a un porcentaje alcohólico aparente determinado después de la destilación y mediante medición densimétrica. El estudio aporta evidencia comparativa preliminar para la valorización a escala de laboratorio de hidrolizados de cáscaras de frutas.

Palabras clave: *porcentaje alcohólico aparente,* Ananas comosus, Passiflora edulis, Saccharomyces cerevisiae, *hidrolizado de cáscara*

1. Introduction

Fruit-processing chains generate peel-rich residues containing soluble carbohydrates and structural polysaccharides that can support hydrolysis and fermentation. Global pineapple exports increased by 3.9% in 2023 to approximately 3.2×10^6 t (FAO, 2024). Peru produced 90,754 t of passion fruit in 2022, with an average yield of 12,828 kg ha^{-1}. Production was concentrated in the Lima, La Libertad, Lambayeque and Piura regions of the country (MIDAGRI, 2026, Tables 3, 4 and 539). Crop production indicates a potential source of processing residues, although locally recoverable peel quantities were not estimated as part of this study. Regional feedstock inventories are needed to assess residue availability for bioethanol production (Basaglia *et al*., 2021). The International Energy Agency (IEA) (2024, 2025) projects growth in renewable transport fuels, while the United States Department of Agricultural Foreign Agricultural Service (USDA FAS) (2025, p. 1) forecast for Peruvian ethanol production in 2025 was 250 million liters. Economic and environmental performance must be assessed for each specific conversion system, as illustrated by Duarte *et al*. (2021) in the case of coffee residues. While the United Nations Environment Programme (UNEP, 2024) addresses food-waste measurement in retail, food service and households, its estimates do not quantify industrial fruit-peel availability or the performance of this process.

Pineapple peel contains soluble sugars and lignocellulosic fractions. Celis Cabeza *et al*. (2024, Table 1) reported 30.56% holocellulose in Perolera pineapple peel, with cellulose and hemicellulose accounting for 54.90% and 45.09% of the isolated holocellulose, respectively. These two percentages describe the composition of the holocellulose fraction, rather than independent percentages of the whole peel. Salafia *et al*. (2022) obtained 30.77 g L^{-1} ethanol through simultaneous saccharification and fermentation. Phong *et al*. (2022) reported 36.85 g L^{-1} ethanol from optimized pineapple-waste hydrolysate, while Mgeni *et al*. (2024) confirmed the fermentability of pineapple fruit-waste juice with baker's yeast. Pineapple waste has also shown a high biodegradable organic load in anaerobic biohythane systems (Chu *et al*., 2020). These studies indicate that pineapple residues contain fractions accessible to microbial conversion; however, their analytical methods and process conditions differ from the present screening approach.

Passion fruit peel contains holocellulose, pectin and soluble compounds that influence hydrolysis and fermentation. Megawati *et al*. (2020) reported 64% holocellulose, 11.36% pectin and 16.23% essential oil, and obtained up to 66.00 g L^{-1} glucose after enzymatic hydrolysis and 8.9% v/v ethanol after fermentation. Silva *et al*. (2021) reported an acid-detergent fiber fraction comprising 29.50% cellulose and 8.94% lignin, with 0.36% ash, in yellow passion fruit peel used as a microbial substrate. Borges *et al*. (2021) described this residue as a biorefinery raw material, whereas Borah and Goud (2025) showed that dilute-acid pretreatment, detoxification and a xylose-fermenting yeast improved conversion of spent passion fruit rind. These routes differ in both pretreatment and yeast substrate utilization. Salafia *et al*. (2022) observed residual pentoses after *Saccharomyces cerevisiae* fermentation, whereas Borah and Goud (2025) used a xylose-fermenting yeast. Phong *et al*. (2022) quantified organic acids and furfural in acid-treated pineapple hydrolysate, illustrating the need to characterize both sugars and potential inhibitors.

Pretreatment and fermentation conditions must be distinguished when comparing residue-conversion studies. Saleem *et al*. (2022) and Chaudhary *et al*. (2024) optimized the dilute-acid hydrolysis of pomegranate and melon peels, respectively, whereas Chaudhary *et al*. (2025) evaluated alkaline treatment of pomegranate peel. Rico *et al*. (2023) combined melon-peel autohydrolysis, enzymatic saccharification and alternative fermentation configurations. Sharma and Sharma (2024) applied response-surface methods to lignocellulosic pretreatment and bioethanol production. Fermentation studies separately address ethanol tolerance in an industrial yeast strain (Varize *et al*., 2022), pH and oxygen conditions in mixed fruit-peel hydrolysates (Shirvanyan *et al*., 2025) and repeated-batch operation in a rotary biofilm reactor using beet molasses (Roukas and Kotzekidou, 2020). Response-surface optimization has also been applied to stress-tolerant wild yeasts and molasses fermentation (Tadesse *et al*., 2024; Altınışık *et al*., 2025). These studies provide examples of factor selection and modeling, rather than validation of the present design. Fruit-juice concentration and millet supplementation (Emmanuel *et al*., 2025; Mtashobya *et al*., 2025), and pomegranate-residue fermentation with raisin extract (Kyriazis *et al*., 2026) represent further process routes whose substrate composition differs from the peel hydrolysates compared in this study.

Arsenic bioadsorption is an alternative biomass-valorization route: Hoyos-Alayo (2025a) reviewed fruit residues, and Hoyos-Alayo *et al*. (2026) compared *Citrus aurantifolia* and *Passiflora edulis* peel flours. These water-treatment studies provide context only, rather than evidence of fermentative performance. This study addresses the specific comparison of pineapple and passion fruit peel hydrolysates under the same fixed *Saccharomyces cerevisiae* dose and fermentation times. The objective, therefore, was to compare their apparent alcoholic response at 24, 36 and 72 h and to examine the time × raw material interaction. The working hypothesis was that the hydrolysate matrix would modify the temporal response under identical fermentation conditions.

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2. Materials and methods

2.1. Experimental design, replicate structure and analytical approach

A complete 3 × 2 factorial design was used. Factor A was fermentation time (24, 36 and 72 h), and factor B was raw material (*Ananas comosus* peel hydrolysate and *Passiflora edulis* peel hydrolysate). The dry commercial yeast dose was fixed at 1 g L^{-1}. Eighteen flasks were analyzed, with three flasks per time × raw material treatment. The available laboratory records indicate that one hydrolysate batch was prepared for each raw material and then divided among the nine corresponding flasks. The three observations per treatment were therefore technical fermentation replicates, rather than independent biomass and hydrolysis batches. Each flask constituted the operational experimental unit, but the inferential analysis represents within-batch variability and does not estimate between-batch biological variation. Treatments were initiated in one experimental run, no day block was included, and no formal randomization schedule was retained. Flask codes and the prespecified sampling times were used to maintain traceability. This structure and its limitations were considered in the interpretation of the factorial ANOVA and exploratory quadratic model. Table 1 summarizes the design and the scope of inference.

2.2. Collection and conditioning of residual biomass

Fresh *Ananas comosus* and *Passiflora edulis* peels were collected from local fruit retail and processing points in Lambayeque. Only those peels with no visible signs of decomposition or attached foreign material were accepted. Samples with rot, inorganic residues, excessive soil, previous cooking or added preservatives were discarded. The samples were transported in clean containers and processed within 48 h.

For biomass characterization, the peels were washed with distilled water, cut into small pieces and dried at 65°C for 24 h. The dry material was milled and sieved to homogenize particle size. The flours were stored in airtight containers and protected from moisture and light before proximate analysis.

2.3. Proximate characterization of peel flours

Proximate characterization included moisture, ash, protein, total fat, carbohydrates and total energy. Moisture was determined by gravimetric drying using a criterion based on AOAC 925.10 (AOAC International, 2023c). Ash was determined by muffle incineration using a criterion based on AOAC 923.03 (AOAC International, 2023a). Protein content was determined using the Kjeldahl method with a conversion factor of 6.25. The available documentation does not identify an official method number or a specific standardized Kjeldahl procedure; therefore, no AOAC method number was assigned to the protein determination. Total fat was determined by gravimetric extraction using a criterion based on AOAC 920.39 (AOAC International, 2023b), carbohydrates by difference and total energy as kcal 100 g^{-1}. The results were expressed as g 100 g^{-1} of analyzed flour or kcal 100 g^{-1}. The cited official methods designed for flour moisture, ash and crude fat in animal feed were used here as the stated basis for analyzing peel flours. Structural lignocellulosic fractions were not measured directly, and carbohydrate-by-difference values were not interpreted as cellulose or hemicellulose measurements.

2.4. Must preparation and dilute-acid treatment

The fresh peels intended for fermentation were washed, cut and homogenized with distilled water at a 1:2 mass-to-volume ratio, defined on a fresh, wet-mass basis as 1 g of peel per 2 mL of distilled water. The suspension was filtered through a fine mesh to remove coarse solids. The exact fresh-peel mass and distilled-water volume used to prepare each hydrolysate batch were not retained in the available laboratory record and have therefore not been reconstructed from the post-filtration must volumes. Nominal post-filtration must volumes were

Table 1. Experimental design and scope of inference

Factor or condition	Levels or value	Role	Scope
Fermentation time	24, 36 and 72 h	Quantitative factor	Three levels
Raw material	Pineapple and passion-fruit peel hydrolysates	Categorical factor	Two hydrolysate batches, one per raw material
Technical fermentation replicates	Three flasks per treatment	Within-batch repeatability	18 flasks in total
Yeast dose	1 g L^{-1} dry commercial product	Fixed condition	Not standardized by viable-cell count
Primary response	Apparent alcoholic percentage (% v/v)	Comparative densimetric response	Measured for every flask
Operational controls	Initial pH target 4.5 ± 0.1; incubation 34 ± 2 °C	Batch-level process control	Final pH not recorded

approximately 1.2 L for pineapple and 1.0 L for passion fruit. Concentrated H_2SO_4 (98%) was diluted to 10 mL before addition. The pineapple must received 2.2 mL of concentrated acid and the passion fruit must received 2.0 mL. Based on a nominal acid density of 1.84 g mL^{-1} and the stated batch volumes, the calculated final H_2SO_4 concentrations were approximately 0.033 mol L^{-1} for pineapple and 0.036 mol L^{-1} for passion fruit, equivalent to approximately 0.33 and 0.36% m/v, respectively. These values are calculated nominal concentrations, because the final batch volumes were not recorded volumetrically.

After acid addition, each must was mixed manually for 3 min and held for 30 min at ambient laboratory temperature, nominally 24 ± 2°C, without external heating. The acid dilution was allowed to stand for 3–5 min before addition to dissipate dilution heat. After treatment, the reference values were pH 2.16 and 10.0 °Brix for pineapple and pH 2.34 and 7.8 °Brix for passion fruit. The hydrolysates were neutralized by dropwise addition of 1 mol L^{-1} NaOH to pH 4.5 ± 0.1. The exact cumulative NaOH volumes were not retained in the laboratory record and are therefore not reported. No post-neutralization precipitation-removal step was documented; soluble salts remained in the conditioned hydrolysate. The media were pasteurized at 65°C for 30 min and cooled before inoculation. Individual sugars, furfural, 5-hydroxymethylfurfural and organic acids were not quantified before or after treatment, and so hydrolysis efficiency and inhibitor formation were not determined directly.

2.5. Yeast preparation and fermentation

Commercial freeze-dried baker's yeast containing *Saccharomyces cerevisiae* was used. The dose of 1 g L^{-1} refers to the mass of dry commercial product added to the hydrolysate, not to a standardized viable-cell concentration. Manufacturer, commercial name, strain designation and lot number were not retained in the available record, thereby limiting exact product-level reproducibility. The yeast was reactivated for 15 min at 34 ± 2°C in a sterile portion of the corresponding hydrolysate and then incorporated into the fermentation flasks. Glassware was washed, disinfected and handled with clean utensils, and the hydrolysates were pasteurized before inoculation, in order to reduce contamination risk.

The nominal laboratory arrangement used approximately 100 mL working volume in 250 mL glass flasks, leaving headspace of approximately 150 mL. Fermentation proceeded statically at 34 ± 2°C under a loose sterile closure that allowed CO_2 release and limited, but did not eliminate, oxygen entry. The initial pH was controlled batchwise at 4.5 ± 0.1 after neutralization. Final pH was not measured for individual flasks. At 24, 36 or 72 h, the assigned flasks were removed and filtered to separate yeast biomass and residual solids. Final °Brix and apparent alcoholic percentage were then determined. Working volume, temperature, initial pH and closure arrangement were the operating conditions selected for this experiment. Strain, nutrient supply and oxygen availability were not included as factors in the design.

2.6. Distillation and apparent alcoholic measurement

The analytical record was reconstructed according to the laboratory protocol and standard distillation–densimetry practice. A nominal 100 mL portion of fermented liquid was transferred to a simple distillation assembly. The distillate was collected during the ethanol–water boiling interval, approximately 78–95°C, until approximately 90 mL had been recovered or the vapor temperature exceeded approximately 95°C. After cooling, the distillate was quantitatively transferred to a 100 mL volumetric flask and restored to the original volume with distilled water at 20°C. The reported percentage therefore refers to the original fermented-liquid volume after quantitative volume restoration and not to a concentrated distillate.

Apparent alcoholic percentage was obtained from alcoholmeter and densimeter readings and corrected to 20°C using international alcoholometric tables consistent with OIML R 22 (OIML, 1975) and the density-based approach described in OIV-MA-AS312-01:2021 (OIV, 2021). Three instrument readings were taken from each prepared distillate and averaged. The manufacturer, model and serial number of the alcoholmeter and densimeter were not retained. The result is described as apparent because densimetry after simple distillation does not specifically identify ethanol and can be influenced by other volatile compounds. Chromatographic confirmation was not performed.

2.7. Derived variables

Initial °Brix (B_i), final °Brix (Bf), apparent relative reduction in soluble solids (ARRSS), apparent alcoholic percentage (Aapp) and apparent productivity (Papp) were reported. ARRSS was calculated using Equation 1.

$$ARRSS\ (\%) = [(B_i - Bf) / B_i] \times 100 \quad (1)$$

The °Brix decrease was used only as an indirect comparative indicator. It was not interpreted as exact sugar consumption because refractive response in fermented matrices can be affected by ethanol, organic acids, minerals, pectins and other soluble compounds. Apparent productivity was calculated using Equation 2.

$$Papp\ (\%\ v/v\ h^{-1}) = Aapp / t \quad (2)$$

2.8. Statistical analysis

The 18 flask observations were analyzed by a two-factor fixed-effects ANOVA with time, raw material and their interaction at $\alpha = 0.05$. The general two-factor fixed-effects ANOVA framework is described by NIST/SEMATECH (2012, Section 7.4.3.8). Residual normality

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was evaluated by the Shapiro–Wilk test and homogeneity across the six treatment combinations by Levene's test. Tukey's honestly significant difference procedure was applied to the six time × raw material means. Because the interaction was significant, simple comparisons were reported for pineapple versus passion fruit at each time and among times within each raw material. The technical-replicate structure restricts inference to the tested hydrolysate batches.

An exploratory quadratic model was fitted with time as a quantitative factor, raw material as a categorical factor, time^2 and the time × raw material interaction. Model quality was described using S, R^2, adjusted R^2, predicted R^2, term tests, lack of fit and residual plots. The predicted optimum was rounded to an operationally meaningful value and was not treated as experimentally validated; confirmation runs are required to evaluate model-selected settings (NIST/SEMATECH, 2012, Section 5.4.6). Analyses were performed in Minitab 22.3.1. The factorial model is represented by Equation 3 and the quadratic model by Equation 4.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (3)$$

$$Y = \beta_0 + \beta_1 t + \beta_2 t^2 + \beta_3 M + \beta_4 (t \times M) + \varepsilon \quad (4)$$

2.9. Analytical quality control and traceability

The pH meter was calibrated with pH 4.00 and 7.00 buffers, and the refractometer was verified with distilled water at 0.0 °Brix. The analytical balance was levelled and checked before weighing. Alcoholmetric glassware was cleaned and dried before use, and measurement temperature was recorded for correction to 20°C. Laboratory records included raw material, time, flask number, initial and final °Brix and apparent alcoholic percentage. Values were checked against the digital database before statistical analysis.

2.10. Safety, biosafety and waste management

H_2SO_4 and NaOH were handled using gloves, safety glasses and laboratory coats in a ventilated area. Acid was added to the water during dilution. Acidic and basic residues were neutralized before disposal. Fermentation residues were segregated as organic laboratory waste and kept separate from chemical residues.

2.11. Methodological scope and limitations

This study provides a controlled within-batch comparison of two hydrolysates. It did not include independent biomass or hydrolysis batches, individual sugar quantification, inhibitor analysis, chromatographic ethanol confirmation, a yeast-dose curve, final pH by flask, or independent validation of the predicted optimum. Product-level information for the commercial yeast and analytical instruments was not retained. Also, the study did not include life-cycle assessment, energy balance, economic analysis, carbon footprint, water footprint or industrial-scale testing. The results, therefore, are restricted to apparent alcoholic and soluble-solids responses in a preliminary laboratory screening system.

3. Results

3.1. Proximate characterization of peel flours

Both peel flours were carbohydrate-rich, with pineapple containing 83.29 g 100 g^{-1} and passion fruit 80.70 g 100 g^{-1}. Passion fruit peel flour had higher protein, ash and total fat contents, whereas pineapple peel flour had higher carbohydrate and moisture contents. The total energy was similar across matrices. These results are presented in Table 2 and Figure 1.

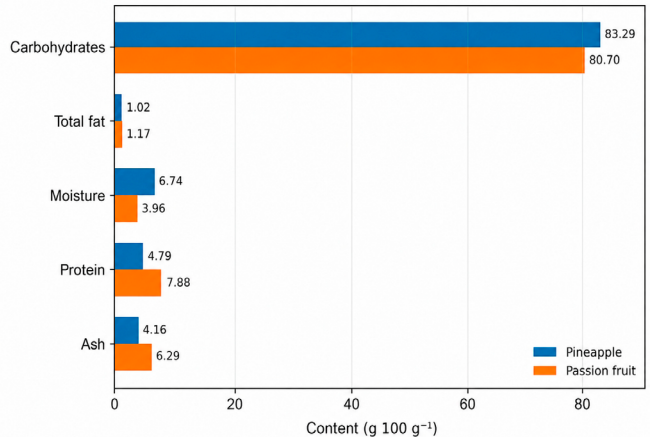


Figure 1. Comparative proximate composition of pineapple and passion fruit peel flours

Table 2. Proximate composition of pineapple and passion fruit peel flours

Parameter	Unit	Pineapple (*Ananas comosus*)	Passion fruit (*Passiflora edulis*)	Difference
Total energy	kcal 100 g^{-1}	361.50	364.85	-3.35
Carbohydrates	g 100 g^{-1}	83.29	80.70	2.59
Total fat	g 100 g^{-1}	1.02	1.17	-0.15
Moisture	g 100 g^{-1}	6.74	3.96	2.78
Protein	g 100 g^{-1}	4.79	7.88	-3.09
Ash	g 100 g^{-1}	4.16	6.29	-2.13

Note. Values are from reports IE-2026-2458 for pineapple peel flour and IE-2026-2457 for passion fruit peel flour.

3.2. Apparent reduction in soluble solids and apparent alcoholic response

In both hydrolysates, final °Brix decreased and apparent alcoholic percentage increased with fermentation time. Pineapple decreased from 5.50 ± 0.10 °Brix at 24 h to 1.30 ± 0.10 °Brix at 72 h, corresponding to 86.70 ± 0.68% ARRSS. Passion fruit decreased from 4.70 ± 0.10 to 2.20 ± 0.10 °Brix, corresponding to 72.85 ± 0.48% ARRSS. The highest observed apparent alcoholic percentage was 4.93 ± 0.06% v/v for pineapple at 72 h; passion fruit reached 3.50 ± 0.26% v/v. Table 3 summarizes the treatment means. ARRSS is an indirect index and is not equivalent to measured sugar consumption.

The temporal profiles indicate a progressive increase in apparent alcoholic percentage for both hydrolysates, with the difference between raw materials becoming clearer at 36 and 72 h, as shown in Figure 2.

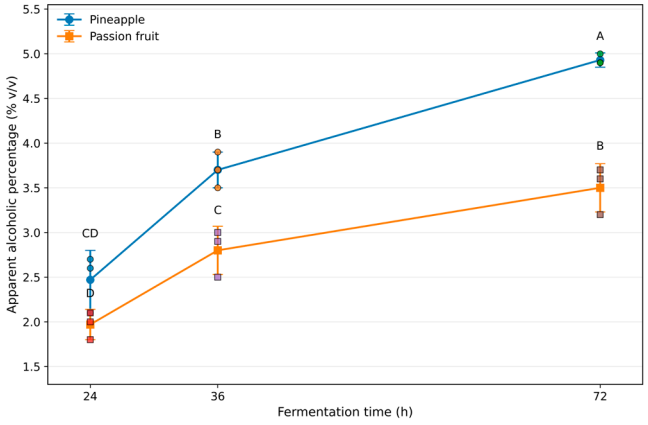


Figure 2. Apparent alcoholic percentage by fermentation time. The colored points show the three individual technical replicates, the circles and squares show treatment means, and the bars show standard deviation (n = 3). Different letters indicate Tukey HSD groups across the six treatment combinations at α = 0.05.

The apparent relative reduction in soluble solids also increased with time in both hydrolysates, while pineapple showed the largest additional reduction between 36 and 72 h, as shown in Figure 3.

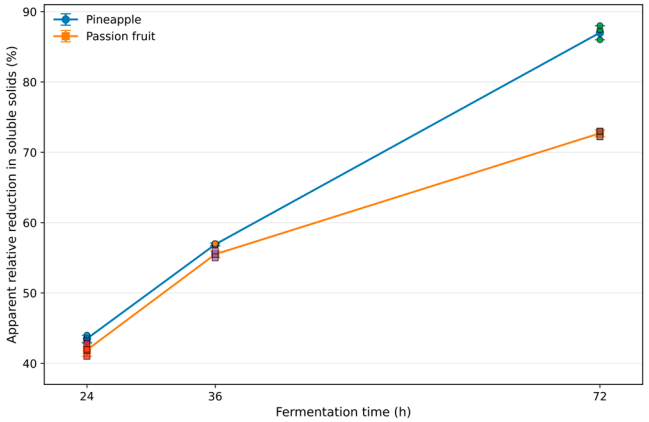


Figure 3. Apparent relative reduction in soluble solids by fermentation time. The colored points show the three individual technical replicates, the symbols show treatment means, and the bars show standard deviation (n = 3). The index is based on °Brix and is not an exact measurement of sugar consumption.

3.3. Factorial effects and simple comparisons

The factorial model was significant, $F(5,12) = 64.55$, $p < 0.001$. Time, raw material and their interaction were significant, confirming that the temporal response was dependent upon the hydrolysate matrix. Model performance was $R^2 = 96.42\%$, adjusted $R^2 = 94.92\%$ and predicted $R^2 = 91.93\%$, with $S = 0.2273$. The complete ANOVA is shown in Table 4.

Table 4. Two-factor ANOVA for apparent alcoholic percentage.

Source	DF	Adjusted SS	Adjusted MS	F	p
Model	5	16.6761	3.33522	64.55	< 0.001
Time	2	12.0044	6.00222	116.17	< 0.001
Raw material	1	4.0139	4.01389	77.69	< 0.001
Time × raw material	2	0.6578	0.32889	6.37	0.013
Error	12	0.6200	0.05167		
Total	17	17.2961			

Table 3. Apparent fermentative response of peel hydrolysates

Raw material	t (h)	B_i (°Brix)	Bf (°Brix)	ΔB (°Brix)	ARRSS (%)	Aapp (% v/v)	Papp (% v/v h^{-1})
Pineapple	24	9.77 ± 0.25	5.50 ± 0.10	4.27 ± 0.15	43.68 ± 0.45	2.47 ± 0.32	0.1028 ± 0.0134
Pineapple	36	9.77 ± 0.25	4.20 ± 0.10	5.57 ± 0.15	56.99 ± 0.15	3.70 ± 0.20	0.1028 ± 0.0056
Pineapple	72	9.77 ± 0.25	1.30 ± 0.10	8.47 ± 0.15	86.70 ± 0.68	4.93 ± 0.06	0.0685 ± 0.0008
Passion fruit	24	8.10 ± 0.26	4.70 ± 0.10	3.40 ± 0.17	41.96 ± 0.85	1.97 ± 0.15	0.0819 ± 0.0064
Passion fruit	36	8.10 ± 0.26	3.60 ± 0.10	4.50 ± 0.17	55.55 ± 0.50	2.80 ± 0.26	0.0778 ± 0.0073
Passion fruit	72	8.10 ± 0.26	2.20 ± 0.10	5.90 ± 0.17	72.85 ± 0.48	3.50 ± 0.26	0.0486 ± 0.0037

Note. ARRSS = apparent relative reduction in soluble solids; Aapp = apparent alcoholic percentage; Papp = apparent productivity. °Brix reduction was used as a comparative indicator and not as an exact sugar consumption measurement. Mean ± standard deviation, n = 3 technical fermentation replicates.

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Residuals were compatible with normality (Shapiro–Wilk W = 0.968, p = 0.755), and variance homogeneity across the six treatment combinations was not rejected (Levene F = 0.349, p = 0.873). The Tukey comparisons in Table 5 show that pineapple and passion fruit did not differ significantly at 24 h, but pineapple was higher at 36 and 72 h. Within each raw material, all three fermentation times differed significantly.

3.4. Exploratory quadratic model

The quadratic model was significant, $F(4,13) = 83.81$, $p < 0.001$, with $S = 0.2229$, $R^2 = 96.27\%$, adjusted $R^2 = 95.12\%$ and predicted $R^2 = 92.92\%$. Lack of fit was not significant, $F(1,12) = 0.50$, $p = 0.495$. Time, time^2 and the time × raw material interaction were significant. Table 6 presents the ANOVA and Table 7 the unstandardized coefficients.

The fitted equations in uncoded units were:

Passion fruit (*Passiflora edulis*): $Aapp = -0.985 + 0.1510t - 0.001235t^2$

Pineapple (*Ananas comosus*): $Aapp = -0.849 + 0.1694t - 0.001235t^2$

The model predicted an optimum near 69 h for pineapple hydrolysate, with an apparent alcoholic percentage of approximately 4.96% v/v and composite

Table 5. Simple comparisons from Tukey HSD across the six time × raw material means.

Comparison	Mean difference (% v/v)	95% CI	Adjusted p	Interpretation
Pineapple – passion fruit at 24 h	0.500	−0.123 to 1.123	0.148	Not significant
Pineapple – passion fruit at 36 h	0.900	0.277 to 1.523	0.004	Pineapple higher
Pineapple – passion fruit at 72 h	1.433	0.810 to 2.057	< 0.001	Pineapple higher
Passion fruit: 36 – 24 h	0.833	0.210 to 1.457	0.008	36 h higher
Passion fruit: 72 – 24 h	1.533	0.910 to 2.157	< 0.001	72 h higher
Passion fruit: 72 – 36 h	0.700	0.077 to 1.323	0.025	72 h higher
Pineapple: 36 – 24 h	1.233	0.610 to 1.857	< 0.001	36 h higher
Pineapple: 72 – 24 h	2.467	1.843 to 3.090	< 0.001	72 h higher
Pineapple: 72 – 36 h	1.233	0.610 to 1.857	< 0.001	72 h higher

Table 6. ANOVA for the exploratory quadratic model.

Source	DF	Adjusted SS	Adjusted MS	F	p
Model	4	16.6505	4.1626	83.81	< 0.001
Linear terms	2	16.4903	8.2451	166.02	< 0.001
Time	1	12.0000	12.0000	241.62	< 0.001
Raw material	1	4.4903	4.4903	90.41	< 0.001
Time^2	1	1.0503	1.0503	21.15	< 0.001
Time × raw material	1	0.6321	0.6321	12.73	0.003
Error	13	0.6456	0.0497		
Lack of fit	1	0.0256	0.0256	0.50	0.495
Pure error	12	0.6200	0.0517		

Table 7. Unstandardized coefficients for the exploratory quadratic model

Term	Coefficient	SE	t	p	95% CI
Intercept (passion fruit)	−0.9846	0.5637	−1.75	0.104	−2.202 to 0.233
Pineapple indicator	0.1359	0.2498	0.54	0.596	−0.404 to 0.676
Time	0.1510	0.0268	5.64	< 0.001	0.093 to 0.209
Time × pineapple	0.0184	0.0052	3.57	0.003	0.007 to 0.030
Time^2	−0.001235	0.000269	−4.60	< 0.001	−0.001816 to −0.000655

desirability of 0.988. The predicted mean had a 95% confidence interval of 4.705–5.216% v/v and a 95% prediction interval of 4.416–5.505% v/v. This condition was not independently validated and, therefore, should be interpreted as an exploratory estimate within the 24–72 h interval. It does not establish that 69 h is experimentally superior to the observed 72 h treatment.

The fitted curves summarize the substrate-specific temporal response and locate the exploratory pineapple maximum close to the upper experimental range, as illustrated in Figure 4.

Model adequacy was examined through residual normality, dispersion around zero and observation-order behavior before interpreting the predicted optimum, as shown in Figure 5.

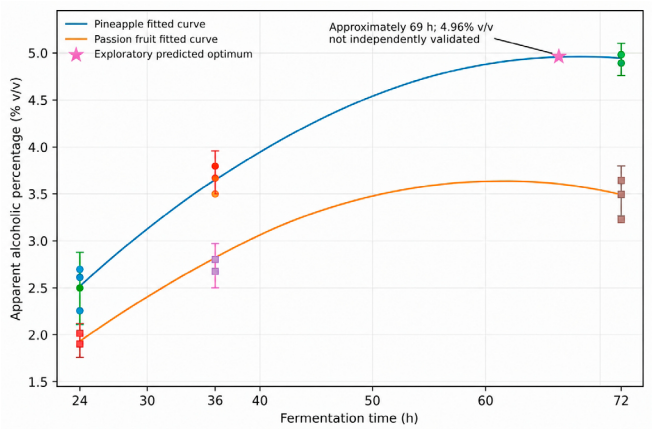


Figure 4. Observed treatment values, fitted quadratic curves and exploratory predicted optimum. The points show individual observations, the bars show standard deviation for treatment means (n = 3), and the star identifies the unvalidated model prediction near 69 h.

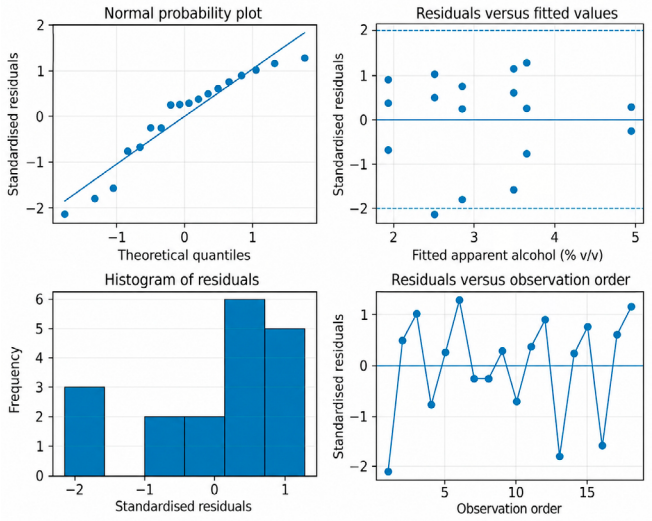


Figure 5. Residual diagnostics for the exploratory quadratic model: normal probability plot, residuals versus fitted values, histogram and residuals versus observation order. The plots showed no systematic pattern requiring model rejection; one standardized residual was slightly beyond −2.

3.5. Individual fermentation observations

Table 8 presents the 18 individual fermentation observations underlying the treatment summaries and statistical analyses. The accompanying notes distinguish batch-level operating conditions from unavailable per-flask measurements.

4. Discussion

Under the tested protocol, pineapple hydrolysate produced a higher apparent alcoholic percentage than passion fruit hydrolysate at 36 and 72 h. This difference should be interpreted in relation to the measured starting conditions. Pineapple entered fermentation at 9.77 ± 0.25 °Brix, compared with 8.10 ± 0.26 °Brix for passion fruit, indicating a larger initial pool of soluble material in the pineapple hydrolysate, although °Brix does not specifically quantify fermentable sugars. At the flour level, total carbohydrate contents were similar, at 83.29 and 80.70 g 100 g^{-1} for pineapple and passion fruit, respectively. The nominal dilute-acid concentrations also differed slightly, at approximately 0.033 mol L^{-1} for pineapple and 0.036 mol L^{-1} for passion fruit. The higher apparent alcoholic percentage observed for pineapple represents a within-protocol response of the prepared hydrolysates and should not be interpreted as evidence of greater fermentative efficiency or conversion yield per unit of fermentable sugar. Individual fermentable sugars were not quantified, and ethanol was not chromatographically confirmed. More intensively controlled studies have demonstrated conversion of pineapple-derived soluble sugars and cell-wall carbohydrates by *Saccharomyces cerevisiae* (Phong *et al.*, 2022; Salafia *et al.*, 2022; Mgeni *et al.*, 2024), but those yields are not directly comparable with the present densimetric screening response.

The lower apparent alcoholic response of passion fruit may reflect matrix-related differences in fermentable-sugar profile, pectin, minerals, organic acids or hydrolysis-derived inhibitors. These mechanisms remain plausible but untested in the present experiment, because individual fermentable sugars, pectin, furfural, 5-hydroxymethylfurfural, organic acids and final pH were not quantified. Megawati *et al.* (2020) obtained higher glucose and ethanol after enzymatic hydrolysis following pectin and essential-oil extraction, whereas Borah and Goud (2025) used dilute acid, detoxification and *Pichia stipitis* to exploit a xylose-rich fraction. Those process routes differ from the present non-detoxified, fixed-dose *Saccharomyces cerevisiae* system. The cellulose, lignin and ash fractions reported by Silva *et al.* (2021), together with the biorefinery potential described by Borges *et al.* (2021), support the broader potential of passion fruit peel as a fermentation feedstock. The present data, however, establish a difference in apparent response under the tested conditions, rather than the specific mechanism responsible for that difference.

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Table 8. Individual fermentation observations used in the statistical analyses

Raw material	Time (h)	Replicate	B_i (°Brix)	Bf (°Brix)	ΔB	ARRSS (%)	Aapp (% v/v)	Papp (% v/v h^{-1})	Initial pH	Final pH	Incubation (°C)
Pineapple (*Ananas comosus*)	24	1	10.0	5.6	4.4	44.00	2.1	0.0875	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	24	2	9.8	5.5	4.3	43.88	2.6	0.1083	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	24	3	9.5	5.4	4.1	43.16	2.7	0.1125	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	36	1	10.0	4.3	5.7	57.00	3.5	0.0972	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	36	2	9.8	4.2	5.6	57.14	3.7	0.1028	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	36	3	9.5	4.1	5.4	56.84	3.9	0.1083	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	72	1	10.0	1.4	8.6	86.00	4.9	0.0681	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	72	2	9.8	1.3	8.5	86.73	4.9	0.0681	NR[1]	NR[2]	34 ± 2[3]
Pineapple (*Ananas comosus*)	72	3	9.5	1.2	8.3	87.37	5.0	0.0694	NR[1]	NR[2]	34 ± 2[3]
Passion fruit (*Passiflora edulis*)	24	1	8.3	4.8	3.5	42.17	1.8	0.0750	NR[1]	NR[2]	34 ± 2[3]
Passion fruit (*Passiflora edulis*)	24	2	8.2	4.7	3.5	42.68	2.0	0.0833	NR[1]	NR[2]	34 ± 2[3]
Passion fruit (*Passiflora edulis*)	24	3	7.8	4.6	3.2	41.03	2.1	0.0875	NR[1]	NR[2]	34 ± 2[3]
Passion fruit (*Passiflora edulis*)	36	1	8.3	3.7	4.6	55.42	2.5	0.0694	NR[1]	NR[2]	34 ± 2[3]
Passion fruit (*Passiflora edulis*)	36	2	8.2	3.6	4.6	56.10	2.9	0.0806	NR[1]	NR[2]	34 ± 2[3]
Passion fruit (*Passiflora edulis*)	36	3	7.8	3.5	4.3	55.13	3.0	0.0833	NR[1]	NR2	34 ± 23
Passion fruit (*Passiflora edulis*)	72	1	8.3	2.3	6.0	72.29	3.3	0.0458	NR[1]	NR2	34 ± 23
Passion fruit (*Passiflora edulis*)	72	2	8.2	2.2	6.0	73.17	3.4	0.0472	NR[1]	NR2	34 ± 23
Passion fruit (*Passiflora edulis*)	72	3	7.8	2.1	5.7	73.08	3.8	0.0528	NR[1]	NR2	34 ± 23

Note. [1]Initial pH was adjusted batchwise to 4.5 ± 0.1 before aliquoting and was not recorded for each flask. [2]Final pH was not recorded. [3]Temperature is the operational incubation range, not an independent reading for each flask. NR = not recorded. The three replicates were technical fermentation replicates from one hydrolysate batch per raw material.

The °Brix decline provides evidence of a changing soluble-solids matrix, rather than of a direct mass balance of sugars. Ethanol, acids, salts, pectins and other solutes affect refractive behavior, and therefore ARRSS was used only for within-protocol comparison. The stronger decline in pineapple coincided with its higher apparent alcoholic percentage, but exact sugar conversion cannot be calculated without glucose, fructose, sucrose and pentose measurements. The same caution should be applied to apparent productivity, which is based on the densimetric response rather than on confirmed ethanol.

The consequences of acid treatment are dependent upon the substrate and treatment conditions. Saleem *et al*. (2022) and Chaudhary *et al*. (2024) evaluated acid concentration, temperature and duration for sugar release from pomegranate and melon peels. In pineapple waste treated with 0.5% v/v H_2SO_4 at 121°C for 15 min, Phong *et al*. (2022) measured sugars, organic acids and furfural. Borah and Goud (2025) coupled acid hydrolysis of spent passion fruit rind with detoxification. These studies cannot confirm inhibitor formation during the milder, nominally 24 ± 2°C treatment applied here. Because individual sugars, furfural, 5-hydroxymethylfurfural and organic acids were not quantified as part of the present experiment, neither sugar release nor inhibitor formation was demonstrated directly; consequently, the mechanism behind the substrate difference remains inferential. Furthermore, the absence of independent hydrolysate batches means that raw-material effects cannot be generalized beyond the prepared batches without further replication.

The significant time × raw material interaction shows that fermentation duration affected the two hydrolysates differently. Apparent productivity, calculated as cumulative apparent alcoholic percentage divided by total fermentation time, was lower at 72 h, but this arithmetical decrease does not independently demonstrate kinetic slowing, substrate depletion or product inhibition. Using the treatment means, pineapple apparent alcoholic percentage increased by 1.23% v/v from 24 to 36 h and by a further 1.23% v/v from 36 to 72 h, corresponding to interval-normalized mean increments of approximately 0.103 and 0.034% v/v h^{-1}, respectively. For passion fruit, the corresponding increases were 0.83 and 0.70% v/v, equivalent to approximately 0.069 and 0.019% v/v h^{-1}. These between-time mean contrasts are consistent with slower incremental accumulation after 36 h under the tested protocol, but they do not constitute instantaneous production rates because separate flasks were harvested at each endpoint. Substrate depletion,

changes in pH, inhibitor effects and product stress therefore remain mechanistic hypotheses because residual sugars, final pH, inhibitor concentrations, chromatographically confirmed ethanol and physiological stress indicators were not measured. Varize *et al*. (2022) investigated ethanol tolerance in modified industrial yeast strains, while Shirvanyan *et al*. (2025) tested pH and oxygen conditions in fruit-peel hydrolysates. The present findings identify factors for subsequent experiments, but do not demonstrate the cause of the temporal response. The modeled optimum near 69 h lies close to the 72 h observation, but three time levels are insufficient to claim a confirmed optimum, and independent runs around the predicted region will be required (NIST/SEMATECH, 2012, Section 5.4.6).

Distillation followed by alcoholometry and densimetry standardized the comparison, but did not identify ethanol specifically. OIV and OIML alcoholometric procedures are based on density relationships in hydroalcoholic mixtures at 20°C (OIML, 1975; OIV, 2021), and other volatile compounds can contribute to the measured response. HPLC or gas chromatography is therefore required to confirm ethanol, calculate mass yield and distinguish it from other volatiles. The current study supports preliminary residue screening and future process development, but it does not demonstrate environmental sustainability, economic viability or industrial readiness. Subsequent development would require defined system boundaries, mass and energy balances, water use, emissions and cost data. Duarte *et al*. (2021) provide an example of economic, environmental and social assessment for a different bioethanol system based on coffee residues.

Wastewater management is a separate consideration for future process development. Hoyos-Alayo and Leiva-Piedra (2026) evaluated organic and inorganic coagulants for vinasse clarification, with BOD_5 and turbidity as responses, while Hoyos-Alayo (2025b) reviewed frugal innovation in industrial water treatment in emerging economies. These sources identify treatment options and implementation considerations for subsequent assessment; the composition and treatability of the effluent from the present process were not evaluated.

Cross-sector studies by Hoyos-Alayo (2026c) on construction circularity, Hoyos-Alayo (2026b) on urbanization and ecosystem services, and Hoyos-Alayo (2026a) on energy poverty and thermal comfort provide only a broader assessment context; they do not offer evidence of fermentation performance or process-specific environmental or social benefits.

5. Conclusions

Both peel hydrolysates showed an apparent alcoholic response under the tested laboratory conditions. Pineapple reached the highest observed value, at 72 h, 4.93 ± 0.06% v/v, and the greatest apparent relative reduction in soluble solids, 86.70 ± 0.68%. Fermentation time, raw material and their interaction significantly influenced the response. The quadratic model predicted an exploratory optimum near 69 h for pineapple at approximately 4.96% v/v, but this condition was not independently validated and was not demonstrated to be superior to 72 h. Ethanol was not confirmed by HPLC or gas chromatography, and °Brix was not an exact sugar measurement. Independent hydrolysate batches and direct analyses of sugars, ethanol and inhibitors will be required prior to scale-up. This study supports the potential valorization of fruit residues and provides preliminary comparative evidence for subsequent process development.

Authors' contributions

Carlos Gabriel Martinez Mendoza: Conceptualization, methodology, investigation, data curation and writing (original draft).

Maricarmen Baldera Montalvan: Investigation, methodology, data curation, visualization and writing (revision and editing).

Walter Manuel Hoyos-Alayo: Supervision, validation, formal analysis, project administration and writing (revision and editing).

All the authors revised and approved the final manuscript.

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Potential conflicts of interest

The authors declare that they have no potential conflicts of interest.

Ethical considerations

The study did not involve human participants, animals, protected species or intervention in protected natural areas. The laboratory work was conducted in accordance with safety, biosafety and waste management criteria for the handling of acids, bases, yeast cultures and organic residues.

Data availability

The 18 individual observations used in the statistical analyses are provided in Table 8, in Section 3.5. The source spreadsheet and statistical output are available from the corresponding author upon reasonable request.

Artificial Intelligence use statement

The authors declare that they did not use artificial intelligence (AI) tools to write or prepare this manuscript.

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