Research Article

Microbial Safety, Quality, and Feed Potential of Red-Fleshed Dragon Fruit Peel Fermented with *Saccharomyces cerevisiae*

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Abstract | Red-fleshed dragon fruit (*Hylocereus polyrhizus*) peel is an abundant agricultural byproduct that contains valuable nutrients but is prone to rapid spoilage and microbial contamination, limiting its use in livestock feeding. This study investigated the effects of yeast inoculum density and fermentation duration on the nutritional quality and microbial safety of fermented dragon fruit peel, with the goal of developing a safe feed ingredient for swine. A native *Saccharomyces cerevisiae* strain (CT6b), identified by ITS sequencing, was inoculated at three initial densities (10^5, 10^6, and 10^7 CFU/g) and subjected to solid-state fermentation for 72 h. Samples were collected at 0, 24, 48, and 72 h to evaluate sensory attributes, pH dynamics, yeast population growth, and microbial safety indicators (*Escherichia coli* and Coliforms). Fermentation markedly improved product quality and stability. Optimal sensory characteristics, including deep red pigmentation, uniform texture, and a mild acidic aroma, were observed at 24–48 h, particularly at the 10^6 CFU/g inoculum level. The pH decreased from 4.78 in fresh peel to 4.02–4.14 after 48 h and remained stable thereafter. Yeast growth followed a typical sigmoidal curve, with the highest viable count (7.52 Log_{10} CFU/mL) achieved in the 10^7 CFU/g group at 48 h. Notably, *E. coli* and Coliforms were completely eliminated within 24 h across all treatments, indicating effective suppression of undesirable bacteria. Collectively, these findings highlight that controlled fermentation with *S. cerevisiae* CT6b generates a pathogen-free, stable, and sensorially acceptable product from dragon fruit peel. Fermented peel therefore represents a sustainable feed resource with potential to enhance swine diets, reduce feed costs, and promote circular agriculture through valorization of fruit processing byproducts.

Keywords | Dragon fruit peel, *Saccharomyces cerevisiae*, Fermentation, Microbial safety, Swine feed, Circular agriculture

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INTRODUCTION

Dragon fruit (*Hylocereus polyrhizus*), widely cultivated in tropical and subtropical regions, is increasingly recognized for its nutritional and economic value. While the edible pulp is consumed fresh or processed, the peel representing up to 30-45% of the fruit's weight is typically discarded as waste (Liaotrakoon *et al.*, 2013). This biomass is rich in polysaccharides, dietary fiber, minerals, and bioactive pigments such as anthocyanins, which hold considerable potential for biotechnological valorization (Martins *et al.*, 2023). However, due to its high moisture and sugar content, the peel is highly perishable and prone to microbial spoilage, limiting its direct application in food and feed systems.

Microbial fermentation represents a promising strategy to transform such agro-industrial residues into safe, stable,

and value-added products. *Saccharomyces cerevisiae* (*S. cerevisiae*) is particularly attractive for this purpose due to its well-documented ability to rapidly metabolize simple sugars, lower pH through organic acid production, and outcompete spoilage organisms via competitive exclusion and antimicrobial metabolite synthesis (Fleet, 2007; Bisson, 2019). Previous studies have demonstrated the effectiveness of yeast-driven fermentation in improving the safety and nutritional properties of plant-based substrates, including soybean peel (Hanh and Thu, 2024) and maize silage (Pham *et al*., 2021). Nevertheless, little is known about the fermentation dynamics of dragon fruit peel, particularly regarding the roles of yeast inoculum density and fermentation duration in shaping physicochemical changes, microbial stability, and biosafety outcomes.

Addressing this knowledge gap is essential for both food microbiology and sustainable agriculture. Valorizing dragon fruit peel through controlled fermentation could reduce agricultural waste, enhance feed resources, and contribute to circular bioeconomy strategies. Therefore, this study aimed to evaluate the effect of different inoculum densities of *S. cerevisiae* and fermentation duration on sensory quality, pH dynamics, yeast population growth, and microbial safety, with particular emphasis on *Escherichia coli* and *Coliforms*. Given regional practices and monogastric physiology, fermented fruit by-products can be blended into swine diets at modest inclusion levels to improve hygiene and palatability as the matrix acidifies (~pH 4.0–4.2 by 24–48 h). We therefore selected swine as a pragmatic first target species; evaluation in other livestock remains future work.

MATERIALS AND METHODS

Study area and experimental period

This study was conducted from October 2024 to March 2025 at the Faculty of Agriculture and Natural Resources, An Giang University – VNUHCM, located in Long Xuyen City, An Giang Province, Vietnam (Latitude 10.37204°N, Longitude 105.43231°E). An Giang is a key agricultural province in the Mekong Delta, with a tropical monsoon climate and rich availability of dragon fruit peel as a post-harvest byproduct.

Yeast strain identification and inoculum preparation

The yeast strain designated CT6b was previously isolated from naturally fermented tropical fruit and preserved in the Microbial Biotechnology Laboratory at An Giang University. Identification of the strain was performed by amplification and sequencing of the internal transcribed spacer (ITS) (Ciardo *et al*., 2006). The PCR product was purified and sequenced using Sanger sequencing. The obtained sequence was deposited in NCBI GenBank under accession number PX112498. BLAST analysis showed >99% similarity to reference *Saccharomyces cerevisiae* strains, confirming its taxonomic identity. This method was chosen due to its superior accuracy compared to conventional biochemical profiling (Ciardo *et al*., 2006; Pham, 2009; Kurtzman and Fell, 1998).

For inoculum propagation, five colonies of CT6b were inoculated into 500 mL of YPD broth (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) and incubated at 30 °C with shaking at 200 rpm for 48 h. Then, 100 mL of this culture was transferred to five fresh 500 mL YPD flasks and incubated under the same conditions for another 48 h to achieve a final concentration of ~10^8 CFU/mL, verified by serial dilution and plating on YPDA agar.

Experimental design and fermentation conditions

A completely randomized design (CRD) was used to evaluate the effect of yeast inoculum density with four treatments and four replications, totaling 16 experimental units (Table 1). The experiment evaluated the effect of yeast inoculum density on the fermentation of red-fleshed dragon fruit (*Hylocereus polyrhizus*) peel.

Table 1: Treatments and inoculum densities of *S. cerevisiae* strain CT6b.

Treatment	Inoculum density (CFU/g)	Inoculum preparation method
NT0	0 (control)	No yeast added
NT1	1×10^5	1 mL of 10^8 CFU/mL in 999 g peel
NT2	1×10^6	10 mL of 10^8 CFU/mL in 990 g peel
NT3	1×10^7	100 mL of 10^8 CFU/mL in 900 g peel

Pre-treatment of peel: Fresh peel was washed under running water, drained 10 min, and chopped to ~1–2 cm. Initial moisture content was measured by oven drying at 105°C to constant weight.

Each unit consisted of 1 kg of fresh chopped peel, mixed manually with the appropriate volume of yeast suspension under aseptic conditions. The mixture was packed loosely into heavy-duty polyethylene bags. Bags were left partially open (2–3 cm gap secured with clips) for 5 h to allow brief aerobic adaptation and CO_2 venting, then tightly sealed. Headspace oxygen was not monitored. Fermentation was carried out at ambient temperature (30±2°C) in a shaded, ventilated room. Fermentation was monitored at 0, 24, 48, and 72 hours. At each time point, triplicate samples were collected to assess microbial and physicochemical characteristics. The overall fermentation workflow and microbial interaction are shown in Figure 1.

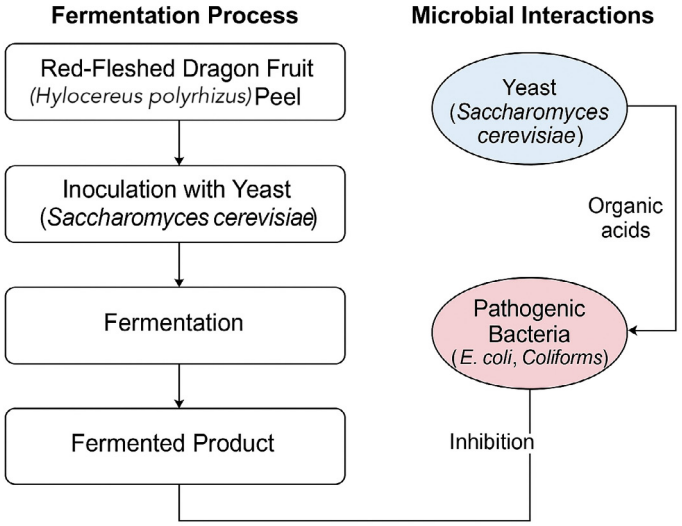

Figure 1: Schematic overview of the experimental design for the fermentation of red-fleshed dragon fruit peel with *S. cerevisiae* CT6b.

Physicochemical measurements

pH Determination: Ten grams of fermented material was homogenized in 90 mL sterile distilled water using a vortex mixer. pH was measured using a Hanna Instruments HI5221 Research Grade pH/ORP Bench Meter (Hanna Instruments, Woonsocket, RI, USA), calibrated with standard buffer solutions at pH 4.0 and 7.0 prior to each measurement. Measurements followed AOAC method 981.12.

Sensory characteristics: A five-member internal panel evaluated color, aroma, mold-free appearance, and overall acceptability on an anchored 1–5 scale (1 = pale/flat/visible mold/poor; 3 = moderate; 5 = deep red/pleasant mild acidic aroma/no visible mold/excellent). Samples were 3-digit coded, serving order randomized, and duplicate cups per treatment × time were assessed individually under standardized lighting. Visual inspection and photographs documented color and aroma changes; mold or deterioration were recorded.

Microbiological analysis

Sample preparation: Fermented peel (1.00 g) was aseptically transferred into 9.00 mL sterile buffered peptone water and homogenized (stomacher, 60 s) to obtain a 1:10 primary homogenate. Ten-fold serial dilutions were prepared. Plates with 30–300 colonies were counted.

Yeast enumeration: Aliquots (0.10 mL) were surface-plated in duplicate on YPDA agar supplemented with chloramphenicol (100 µg/mL) and incubated at 30°C for 48 h. Counts were recalculated and expressed as CFU/g of original material.

E. coli and total Coliforms: Aliquots (0.10 mL) of serial dilutions were plated in duplicate on Chromocult® Coliform Agar (Merck) and incubated at 37 °C for 24 h. Characteristic colonies were enumerated and reported as CFU/mL of the 1:10 primary homogenate to keep a constant analytical matrix in this solid-state system. With 0.10 mL plated from the 10^{-1} dilution, the limit of detection (LOD) = 1×10^2 CFU/mL. For visualization, values < LOD were plotted at 0.50×10^2 CFU/mL. For mass-based units, CFU/g = CFU/mL × 10 (1.00 g → 9.00 mL).

Statistical analysis

All data were initially organized, cleaned, and plotted using Microsoft Excel 2019. Statistical analyses were performed using Minitab version 16.0 (Minitab Inc., State College, PA, USA). The design was CRD (4 inoculum levels × 4 bags) with destructive sub-sampling at 0, 24, 48, 72 h, creating within-bag correlation. Responses were fitted using GLM with bag specified as a random effect and fixed effects inoculum, time, and inoculum×time; Tukey adjustments were used for pairwise comparisons ($\alpha = 0.05$). pH was analyzed untransformed; yeast counts as $\log_{10}$(CFU/g). *E. coli*/ Coliforms were handled by a two-part approach: (i) detection (≥ LOD) compared by Fisher's exact/chi-square; (ii) positive counts analyzed as $\log_{10}$(CFU/mL) with the same GLM (bag random). Results are mean ± SEM; figures display temporal trends in pH, yeast counts, and hygiene indicators.

RESULTS AND DISCUSSION

Sensory evaluation of fermented red-fleshed dragon fruit peel

Sensory evaluation was conducted at four fermentation time points (0h, 24h, 48h, and 72h) across four treatments (NT0–NT3), focusing on four attributes: Color, aroma, mold presence, and overall acceptability (Figures 2 and 3).

At 0 hours, all treatments exhibited a vibrant bright red color, characteristic of fresh dragon fruit peel. During fermentation, particularly between 24 and 48 hours, the peel color shifted to deep red or reddish-brown, most notably in NT2 and NT3. This transformation is attributable to the acidification of the matrix caused by yeast metabolism, affecting anthocyanin pigments the main colorant compounds in dragon fruit peel. These observations align with Liaotrakoon (2013), who reported that anthocyanins in dragon fruit are highly sensitive to acidic conditions and undergo structural modification, resulting in deeper color during fermentation.

A mild, pleasant acidic aroma indicative of proper fermentation was clearly perceived in treatments NT2 and NT3 after 24–48 hours. In contrast, NT0 and NT1

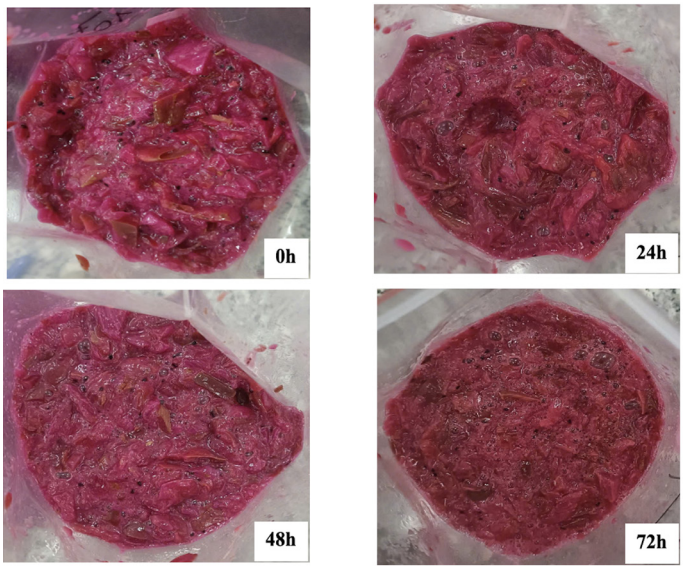

Figure 2: Sensory evaluation of fermented red-fleshed dragon fruit peel at different fermentation times (0, 24, 48, and 72h). Radar axes display anchored 1–5 scores for Color, Aroma, Mold-free appearance (higher = no visible mold), and Overall acceptability. Anchors: 1 = pale/flat/visible mold/poor; 3 = moderate; 5 = deep vivid red/pleasant mild acidic aroma/no visible mold/excellent.

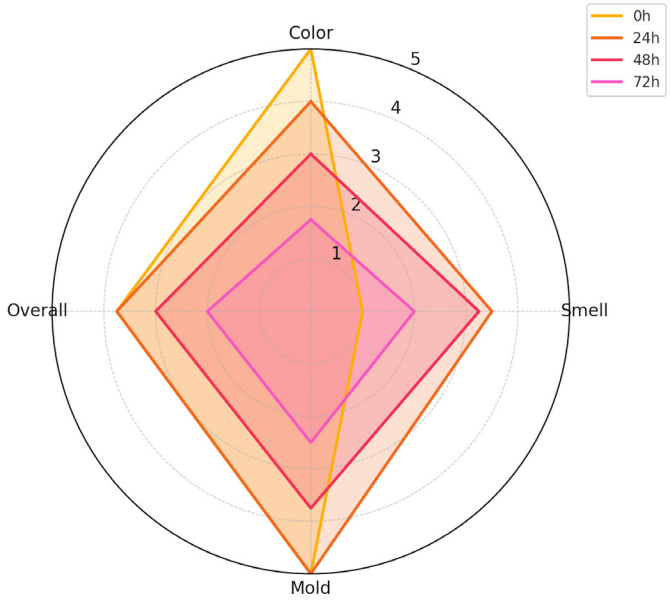

Figure 3: Color changes of red-fleshed dragon fruit peel during fermentation with *S. Cerevisiae* strain CT6b at 0, 24, 48, and 72h. Representative photographs illustrating the visual transformation of dragon fruit peel during fermentation. At 0h, the peel displayed a bright red color typical of fresh material. After 24h, the color became deeper, accompanied by slight textural softening. At 48h, the peel exhibited a reddish-brown tone, reflecting progressive acidification and anthocyanin modification. By 72h, the color further darkened, indicating the completion of fermentation and stabilization of pigments in the acidic environment.

exhibited weak or no aromatic development. Notably, NT2 (10^6 CFU/g) yielded the most balanced aroma, while NT3 (10^7 CFU/g) developed a stronger, sometimes sharp acidic odor, likely due to excessive metabolite accumulation from high yeast activity. This suggests that yeast inoculum density directly influences the intensity and quality of aroma during fermentation.

Mold growth was first observed in NT0 (no yeast added) after 48 hours, with visible proliferation by 72 hours. In contrast, yeast-inoculated treatments (NT1–NT3) exhibited no mold growth within 48 hours. NT2 remained mold-free throughout the 72-hour fermentation, highlighting the antimicrobial effect of *S. cerevisiae*, likely through acid production and competitive exclusion of spoilage organisms.

The radar chart (Figure 2) illustrates that sensory quality peaked at 24–48 hours, particularly in NT2. However, by 72 hours, both NT0 and NT3 showed marked declines-NT0 due to mold and NT3 due to over-acidification and color darkening. These findings suggest that prolonged fermentation or excessively high yeast inoculum may compromise sensory attributes. Our findings are consistent with prior reports. Hanh and Thu (2024) observed that *S. cerevisiae* fermentation of soybean peel resulted in enhanced preservation and aroma due to organic acid and ester production. Similarly, Pham *et al.* (2021) reported a progressive color change and mild acidic aroma during lactic fermentation of maize, reinforcing the shared biochemical mechanisms between yeast and lactic acid fermentation of plant substrates.

Table 2: pH values of fermented red-fleshed dragon fruit peel with *S. cerevisiae* strain CT6b over time.

Treatment	pH 0h	pH 24h	pH 48h	pH 72h
NT0 (Control)	4.78^a	4.68^a	4.53^a	4.40^a
NT1 (10^5 CFU/g)	4.78^a	4.64^b	4.45^b	4.29^b
NT2 (10^6 CFU/g)	4.77^a	4.62^b	4.40^c	4.23^c
NT3 (10^7 CFU/g)	4.75^b	4.57^c	4.28^d	4.17^d
SEM	0.0036	0.0062	0.0067	0.0073
P-value	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Note: Means within the same column with different superscript letters ($^{a, b, c, d}$) different significantly at P<0.05; NT0 (Control): peel without yeast inoculation; NT1: inoculated at 10^5 CFU/g; NT2: inoculated at 10^6 CFU/g; NT3: inoculated at 10^7 CFU/g.

Effect of yeast inoculum density on pH of red-fleshed dragon fruit peel during fermentation

The dynamics of pH across treatments are presented in Table 2. A clear and consistent trend was observed in which the pH decreased progressively from 0 h to 72 h in all treatments, indicating the effective acidification of the substrate by yeast metabolism. This acidification

reflects the conversion of fermentable carbohydrates in dragon fruit peel into organic acids, thereby confirming the fermentative activity of *S. cerevisiae* strain CT6b. The stabilization of pH values after 48–72 h suggests that the fermentation reached a stationary phase, likely due to substrate depletion or the establishment of a new equilibrium between acid production and the buffering capacity of the medium.

At 0 h, the control treatment (NT0) exhibited a pH of 4.78, while the yeast-inoculated treatments were 4.78 (NT1), 4.77 (NT2), and 4.75 (NT3); NT3 was slightly but significantly lower than the others. After 24 h, the divergence among treatments became more pronounced, with pH decreasing in the order NT0 (4.68) > NT1 (4.64) ≈ NT2 (4.62) > NT3 (4.57). By 48 and 72 h, the separation was clear and stepwise (NT0 > NT1 > NT2 > NT3): 4.53, 4.45, 4.40, 4.28 at 48 h and 4.40, 4.29, 4.23, 4.17 at 72 h. This pattern demonstrates that higher yeast inoculum densities (10^6–10^7 CFU/g) accelerated acid production, leading to stronger and faster acidification compared with the lower inoculum density (10^5 CFU/g). At 24 h, inoculated groups reached lower pH than the control (NT0= 4.68 vs NT2= 4.62), and visible mold appeared in NT0 by 48–72 h but not in NT2, indicating better spoilage control with inoculation.

These findings are consistent with reports on plant-based fermentations where yeast activity reduces pH to an acidic range conducive for preservation. For instance, Hanh and Thu (2024) showed that *S. cerevisiae* fermentation of soybean peel supplemented with rice bran and molasses decreased pH significantly, enhancing both shelf life and nutritional value. Similarly, Aleman *et al.* (2024) reported that inoculation density directly influenced the rate and extent of acid production during fruit waste fermentation, highlighting the critical role of inoculum size in determining fermentation kinetics.

The final pH values of 4.17–4.40 observed in this study are within the safe acidic range that inhibits the growth of most spoilage microorganisms and pathogens, consistent with the antimicrobial role of acidification described by Ray *et al.* (2025) in fruit peel fermentations. Moreover, the acidification observed here can be explained not only by ethanol fermentation but also by the production of secondary organic acids such as acetic, lactic, and succinic acids either directly synthesized by yeast or through potential microbial co-metabolism in non-sterile environments.

Interestingly, the stabilization of pH after 48 h aligns with results from Liaotrakoon (2013), who highlighted the buffering effect of natural components in dragon fruit peel, including minerals and organic compounds, which may moderate further pH decline. This stabilization indicates that the main fermentation phase was completed, and the system reached a balance between acid generation and the intrinsic buffering properties of the peel matrix.

Yeast inoculum density had a significant effect on the acidification kinetics of red-fleshed dragon fruit peel fermentation. Treatments NT2 and NT3 achieved the most pronounced pH reduction, demonstrating that higher inoculum densities (10^6–10^7 CFU/g) promote faster and more extensive fermentation. The final pH values (4.17–4.40) were both microbiologically safe and favorable for product preservation. These results emphasize the potential of *S. cerevisiae* strain CT6b to valorize dragon fruit peel into stable, fermented products with extended shelf life and improved safety profiles.

Changes in the population dynamics of *S. cerevisiae* during fermentation

The average viable counts of *S. cerevisiae* strain CT6b in red-fleshed dragon fruit peel across treatments and fermentation times are presented in Table 3. The results clearly demonstrate the distinct phases of microbial growth in a constrained substrate environment. In all inoculated treatments (NT1, NT2, NT3), yeast populations increased substantially from the initial point, peaked at either 24 or 48 h, and then declined slightly by 72 h. This trend reflects the classical microbial growth curve, encompassing the lag, exponential, stationary, and decline phases.

Table 3: Yeast counts (Log_{10} CFU/mL) of *S. cerevisiae* strain CT6b during fermentation of red-fleshed dragon fruit peel at different time points.

Treatment	0h	24h	48h	72h
NT0 (Control)	4.30^{c}	5.65^{d}	5.55^{b}	5.12^{d}
NT1 (10^5 CFU/g)	5.06^{c}	6.40^{c}	6.43^{b}	6.01^{c}
NT2 (10^6 CFU/g)	6.11^{b}	6.57^{b}	6.47^{b}	6.19^{b}
NT3 (10^7 CFU/g)	7.07^{a}	7.13^{a}	7.52^{a}	6.33^{a}
SEM	0.07	0.03	0.05	0.04
P-value	P < 0.001	P < 0.001	P < 0.001	P < 0.001

Note: Means within the same column with different superscript letters ($^{a, b, c, d}$) different significantly at P<0.05; NT0 (Control): peel without yeast inoculation; NT1: inoculated at 10^5 CFU/g; NT2: inoculated at 10^6 CFU/g; NT3: inoculated at 10^7 CFU/g.

At 0 h, NT3 (10^7 CFU/g) exhibited the highest initial cell count (7.07 Log_{10} CFU/mL), which was significantly greater than NT2 (6.11 Log_{10} CFU/mL), NT1 (5.06 Log_{10} CFU/mL), and the control NT0 (4.30 Log_{10} CFU/mL) (P < 0.001). This pattern persisted throughout the experiment, with NT3 consistently maintaining the highest yeast populations. At 48 h, NT3 reached the maximum density recorded in the study (7.52 Log_{10} CFU/mL), marking the most active exponential phase of growth.

By 72 h, although all treatments exhibited a decline in yeast counts compared to their peaks, the differences among treatments remained statistically significant, confirming the sustained effect of initial inoculum density on population dynamics.

Interestingly, the control group (NT0) also showed an increase in yeast counts between 0 and 24 h, followed by a gradual decline. This suggests the presence of indigenous yeasts naturally associated with dragon fruit peel, as previously noted in fruit-based substrates (Liaotrakoon, 2013). Similarly, Nguyen *et al.* (2012) reported diverse natural yeast populations in palm sap, highlighting the ubiquitous presence of wild yeasts in plant-derived substrates. Nevertheless, the significantly higher populations in inoculated treatments confirm that supplementation with a selected, high-performing strain such as strain CT6b is essential to drive efficient and predictable fermentation outcomes.

The strong population growth in NT2 and NT3 can be attributed to the abundant availability of simple sugars (glucose and fructose) in dragon fruit peel, which facilitated rapid biomass accumulation during the first 24–48h. The maximum yeast density achieved in NT3 (7.52 Log_{10} CFU/mL at 48h) underscores the high adaptability of strain CT6b to this substrate. Comparable results were reported by Ho (2017), who optimized *S. cerevisiae* propagation in pomelo juice and achieved densities up to 9.09 Log_{10} CFU/mL after 24 h, suggesting that nutrient optimization and inoculum size are critical determinants of maximum yeast growth.

The subsequent decline in yeast counts at 72 h reflects the depletion of readily available nutrients and/or the accumulation of inhibitory metabolites such as ethanol and organic acids. Ethanol toxicity and acid stress are well-known factors limiting yeast survival during extended fermentation (Gibson *et al.*, 2007; Bisson, 2019). This decline phase, although expected, highlights the importance of controlling fermentation duration to avoid loss of viable biomass and potential spoilage risks.

Overall, the population dynamics observed here not only confirm the growth potential of *S. cerevisiae* strain CT6b on dragon fruit peel but also illustrate the typical life cycle of yeast populations in a closed fermentation system, where nutrient availability and metabolic by-products dictate population stability. These findings reinforce the value of controlled inoculation strategies and optimized fermentation duration for valorizing fruit byproducts into stable, fermented products.

Dynamics of *E. coli* and *Coliforms* in fermented red-fleshed dragon fruit peel

Changes in *E. coli* population

The dynamics of *E. coli* during fermentation are summarized in Figure 4. At 0 h, *E. coli* was present in all treatments, with populations ranging from 0.50×10^2 to 204×10^2 CFU/mL. Significant differences were observed ($P < 0.001$), with NT3 showing an anomalously high initial load, likely due to random contamination during sample preparation. Despite this variation, a remarkable finding was that *E. coli* counts in all treatments including the control dropped below the detection limit (<1 × 10^2 CFU/mL) after just 24h and remained undetectable through 72h.

This rapid decline highlights the strong hygienic effect of fermentation. The primary mechanism is the rapid acidification of the environment, as demonstrated in Table 2, where pH values dropped below 4.6 after 24 h. *E. coli* is highly sensitive to acidic stress, and survival is markedly reduced at pH < 4.5 (Segura and Sourjik, 2025). In addition, competitive exclusion by *S. cerevisiae* strain CT6b played a key role. Yeasts rapidly consumed simple

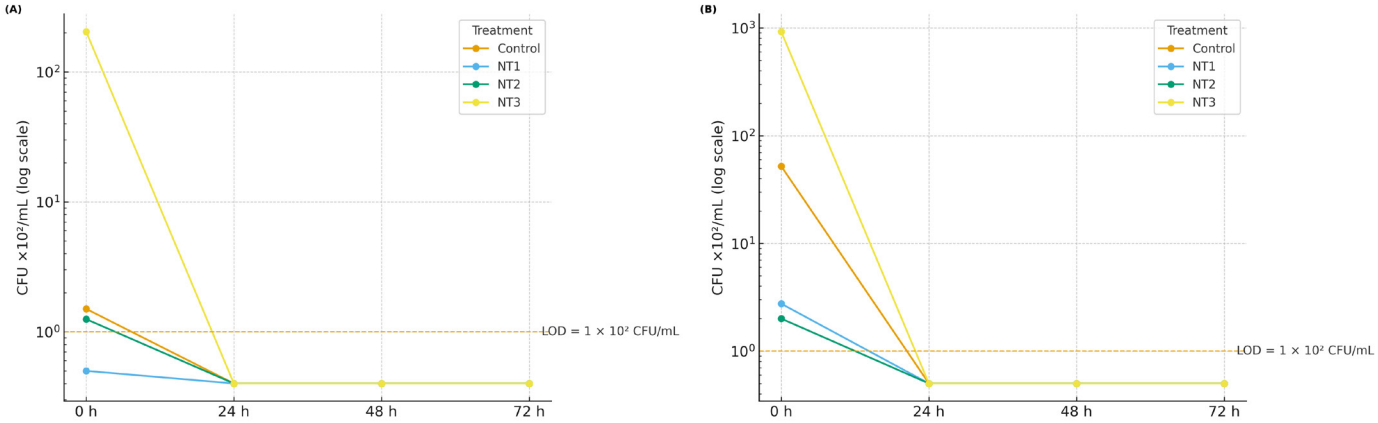

Figure 4: Dynamics of *Escherichia coli* (A) and total Coliforms (B) during fermentation of red-fleshed dragon fruit peel with *S. cerevisiae* CT6b. Y-axis shows CFU×10^2/mL (log_{10} scale); the dashed line marks 1 ×10^2 CFU/mL, and values < LOD are plotted at 0.50 ×10^2 CFU/mL.

sugars, depriving *E. coli* of essential nutrients, and their dominance in the microbial ecosystem restricted the growth of competing bacteria. Previous studies have shown similar effects, with *S. cerevisiae* supplementation in poultry reducing *E. coli* loads in feces (Nguyen and Ho, 2015), supporting the role of yeast as a probiotic-like competitor in complex microbial environments.

Changes in coliform population

The changes in Coliforms populations are shown in Figure 4. At 0h, Coliforms were detected in all treatments, ranging from 2.00×10^2 CFU/mL (NT2) to 923×10^2 CFU/mL (NT3). This wide variation reflects natural contamination from soil, water, and handling during harvesting, which is common in raw agricultural byproducts. However, similar to *E. coli*, all Coliforms dropped below detectable levels ($<1\times10^2$ CFU/mL) after 24 h and remained undetectable at 48 h and 72 h across all treatments, with no statistical differences at later time points.

The elimination of Coliforms within 24 h underscores the synergistic antimicrobial mechanisms of fermentation. Beyond acidification, yeasts may produce antimicrobial metabolites such as ethanol and small peptides (Fleet, 2007), which act alongside nutrient competition to create an inhospitable environment for enteric bacteria. This outcome aligns with reports from agro-waste fermentation studies, where yeast-driven fermentations improved microbiological safety by eliminating fecal indicator bacteria (Ray *et al*., 2025).

Implications and comparative analysis

The combined results for *E. coli* and Coliforms demonstrate that fermentation with *S. cerevisiae* strain CT6b creates a microbiologically safe product, regardless of initial contamination levels. Even the control treatment (without added yeast) showed pathogen suppression, likely due to the contribution of natural acidification and indigenous microbiota, though at a slower rate compared with inoculated treatments.

These findings are in line with broader evidence that fermentation is a reliable biopreservation method, improving food and feed safety by inhibiting pathogenic bacteria through a multifactorial mechanism: Acidification, competitive exclusion, ethanol accumulation, and potential antimicrobial metabolite production (Gibson *et al*., 2007; Bisson, 2019). We did not quantify ethanol or individual organic acids in this study; targeted metabolite profiling will strengthen the mechanistic interpretation.

Fermentation of red-fleshed dragon fruit peel with *S. cerevisiae* strain CT6b not only improved sensory and physicochemical qualities but also completely eliminated *E. coli* and Coliforms within 24 h. This rapid microbial safety enhancement positions strain CT6b-fermented dragon fruit peel as a promising raw material for applications in food, feed, and agricultural biotechnology, offering both value addition and biosafety assurance.

CONCLUSION

This study demonstrates that fermentation of red-fleshed dragon fruit peel with *Saccharomyces cerevisiae* strain CT6b improves both product quality and microbial safety. An inoculum of 10^6 CFU/g and a 24–48 h fermentation provide a practical balance of rapid microbial safety, attractive color/aroma, and mold control for scale-up. Importantly, *E. coli* and Coliforms were completely eliminated within 24 h, underscoring the strong biosafety potential of this process. Beyond its value as a safe, stable, and sensorially acceptable byproduct, the fermented peel shows high promise as an alternative ingredient for livestock feed, particularly for swine, supporting sustainable agriculture and circular bioeconomy strategies.

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NOVELTY STATEMENT

This study is the first to demonstrate that controlled fermentation of red-fleshed dragon fruit peel using a native *Saccharomyces cerevisiae* strain (CT6b), deposited in the NCBI GenBank under accession number PX112498 and isolated from An Giang Province, Vietnam, ensures rapid microbial safety. The complete elimination of *E. coli* and Coliforms within 24 hours highlights a novel and practical approach for valorizing fruit waste into safe swine feed.

AUTHORS' CONTRIBUTION

NTHC, PDT and TTT jointly conceptualized and designed the study. NTH Chi performed the literature review, methodology development, experimental investigation, and prepared the original draft of the manuscript. TT Tuan and PD Tho interpreted the data, supervised the work, and critically revised the manuscript. All authors contributed intellectually and approved the

final version of the manuscript.

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Generative AI and AI-assisted technology statement

The authors declare that no Generative AI or AI-assisted technology was used in the creation of this manuscript.

Conflict of interests

The authors have declared no conflict of interest.

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