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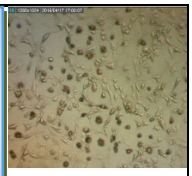


# Effect of Pineapple Peel on Enzyme Production and Antioxidant Potential in Scoby Fermentation

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**Abstract**

The study demonstrated that pineapple peel supplementation enhances microbial activity and enzyme production in kombucha fermentation, with the Symbiotic Culture of Bacteria and Yeast (SCOBY) brewing solution exhibiting a rapid pH decline to 2.96 within 7 days due to organic acid production from acetic acid bacteria and yeasts, ensuring a safe fermentation environment (pH ≤ 4.6). Pineapple peels sustained microbial populations such as yeast, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) at 37 °C, but at 45 °C it caused severe inhibition due to thermal stress, while acting as nutrient sources to support higher cell counts (AAB: 2,620 CFU/mL) compared to controls. This study evaluated protease, cellulase, and lipase activities at 37 °C and 45 °C using agar plate hydrolysis assays. Protease exhibited activity, with hydrolysis indices of 2.2-2.3 at 37 °C and 3.0 at 45 °C, demonstrating temperature-enhanced performance consistent with bromelain's thermal optima. Cellulase showed peak activity at 37 °C (index 2.4, day 3), declining thereafter, while 45 °C sustained higher indices (2.1-3.3), indicating thermal stability. Lipase displayed moderate activity at 37 °C (index 2.5, day 6) but improved at 45 °C (index 2.7, day 3), aligning with prior reports on thermophilic activation. The results highlight pineapple peels as an effective substrate for thermostable protease and cellulase production, with potential applications in bioconversion processes.

## INTRODUCTION

Pineapple (*Ananas comosus* L. Merr.) is a globally important tropical fruit, widely consumed fresh and processed into various products such as juices, canned goods, and flavoring agents [1]. Global production exceeded 28 million metric tonnes in 2023, with major contributions from Costa Rica, the Philippines, and Thailand [2,3]. However, this substantial production generates agricultural waste, particularly pineapple peels, which constitute 30-40% of the fruit's total weight [4]. Pineapple peel waste (PPW) represents approximately 15-40% of the processed fruit mass, depending on extraction methods [5]. For instance, Malaysia's pineapple production in 2020 yielded approximately 335,488 tonnes of fruit, resulting in over 137,550 tonnes of peel waste [6]. Conventional disposal methods, including landfilling, contribute to environmental pollution through greenhouse gas emissions and elevated biochemical oxygen demand (BOD) due to the peel's high organic content [7]. Despite being classified as waste, PPW contains valuable bioactive compounds, including natural sugars (sucrose, glucose, fructose), dietary fiber, phenolic compounds, and enzymes such as bromelain [8].

Bromelain, a proteolytic enzyme with demonstrated antioxidant and antimicrobial properties, along with other enzymes such as cellulase and lipase, enhances the functional potential of pineapple peel [9]. Additionally, PPW is rich in

phenolic compounds, including ferulic acid, epicatechin, and gallic acid, as well as some antioxidants (vitamin A and C) with strong activity [10,11]. These properties make PPW a promising substrate for value-added bioproduct development through microbial fermentation. However, the full potential of PPW remains largely untapped due to insufficient valorization strategies.

A Symbiotic Culture of Bacteria and Yeast (SCOBY) is a complex microbial consortium comprising yeasts, acetic acid bacteria (AAB), and lactic acid bacteria (LAB), which plays a central role in fermentation processes, particularly in kombucha production [12]. Kombucha, a traditional fermented tea beverage, is typically prepared using sweetened black, green, or oolong tea and a SCOBY culture [13]. Fermentation occurs aerobically over 7-20 days, during which the SCOBY forms a floating cellulosic pellicle [14]. The SCOBY microbiome includes genera such as Saccharomyces, Acetobacter, Gluconobacter, Zygosaccharomyces, and Schizosaccharomyces, which synergistically metabolize sugars into organic acids, ethanol, and bioactive compounds while producing a cellulose-based biofilm [15,16].

The fermentation process involves a dynamic interaction between yeasts and bacteria; yeasts hydrolyze sucrose into glucose and fructose via invertase, followed by glycolysis to produce ethanol and $CO_2$, while AAB oxidize glucose to gluconic acid and, ethanol to acetic acid [17]. This metabolic activity imparts kombucha with its characteristic tangy flavor and effervescence. Concurrently, bacterial cellulose production forms new SCOBY layers, enabling continuous propagation [16]. Recent studies suggest that SCOBY can utilize alternative substrates beyond traditional sweetened tea, including fruit peels and agricultural residues [18]. Pineapple peel, with its high sugar content, phenolic compounds, and indigenous microbiota, presents an ideal growth medium for SCOBY fermentation. The synergistic interaction between PPW components and SCOBY microorganisms may enhance the production of bioactive metabolites, including enzymes and antioxidants, offering a sustainable solution for waste valorization. The increasing global demand for pineapples has led to a proportional rise in peel waste, which accounts for 30-40% of the fruit's weight [4].

Current disposal methods exacerbate environmental challenges, including greenhouse gas emissions and resource wastage [19]. Despite its rich bioactive composition, PPW remains underutilized, representing a missed opportunity for sustainable resource recovery. SCOBY fermentation offers a promising approach to valorize PPW by converting it into high value bioproducts. However, the influence of PPW composition on SCOBY fermentation dynamics and the biochemical property of the resulting extract remains poorly understood. This research aims to assess pineapple peel efficacy as a SCOBY growth medium and investigate how PPW-derived nutrients and indigenous microbes influence enzyme production, and overall fermentation efficiency. The findings will contribute to sustainable waste management strategies while generating valuable bioactive compounds for nutraceutical, pharmaceutical, and industrial applications.

## MATERIALS AND METHODS

### Materials

Josapine variety pineapples (*Ananas comosus* L.; 1.0-1.5 kg) were sourced from Whole Foods Express (Sri Serdang), with peels manually excised using sterile knives. SCOBY cultures (105-118 g) were obtained from KombuCharm (Sungai Buloh), while fermentation substrates included brown sugar, distilled water, and commercial tea bags. Microbial identification utilized selective media: yeast extract peptone dextrose (YPD) agar, potato dextrose agar (PDA), de Man, Rogosa and Sharpe (MRS) agar and, YGCE agar (comprising yeast extract, glucose, $CaCO_3$, ethanol, and agar). Biochemical analyses employed a calibrated pH meter, DNS reagent, HCl/NaOH for hydrolysis, and spectrophotometric assays. Enzyme screening involved casein agar (for protease), carboxymethyl cellulose (CMC) agar (for cellulase) and tributyrin agar (for lipase), with Congo red, phenol red, and iodine as indicators. All chemicals were analytical grade, and equipment included autoclaves, centrifuges, and incubators maintained at 37 °C/45 °C.

### Sample Preparation

Pineapple Peel Sample Preparation: Fresh Josapine variety pineapples (*Ananas comosus* L.), weighing 1.0-1.5 kg, were obtained from a local market. The peels (~1 cm thickness) were manually excised using a sterile knife, cut into uniform pieces, and dried at 40 °C for 24 h in a convection oven. Dried samples were stored in a desiccator for proximate analysis [20].

Culture Medium and SCOBY Inoculum: Preparation Four SCOBY cultures were used. A nutrient medium was prepared by boiling 1 L distilled water with 150 g brown sugar and two tea bags for 5 min. After cooling to ambient temperature, the SCOBY and 100 mL starter tea were aseptically transferred into the medium and brewed for 7 days at 25 ± 2 °C. Daily pH monitoring ensured optimal conditions prior to pineapple peel fermentation.

### Fermentation Protocol

Peels were homogenized with distilled water (1:3:10 w/v ratio of brown sugar:peels:water) in 1 L sterile bottles. An 8 cm diameter SCOBY disk was inoculated into each bottle, while controls omitted peels. Fermentation proceeded for 7 days at 37 °C and 45 °C, with loose caps permitting aerobic conditions. Daily samples were centrifuged (5,000 rpm, 10 min) for supernatant analysis [21].

### Microbial Identification

Selective media quantified yeast, lactic acid bacteria (LAB), and acetic acid bacteria (AAB). Colony-forming units (CFU/mL) were enumerated, with >300 and <30 colonies classified as Too-Many-to-Count (TMTC) and Too-Few-To-Count (TFTC), respectively [22].

### Yeast Isolation

Yeast extract peptone dextrose (YPD; 13 g/200 mL) and potato dextrose agar (PDA; 7.8 g/200 mL) were autoclaved (121 °C, 15 min), streaked with 0.1 mL inoculum, and incubated at 37 °C [23].

### LAB and AAB Isolation

De Man, Rogosa and Sharpe (MRS) agar (12.4 g/200 mL) and YGCE agar (1% yeast extract, 5% glucose, 2.5% $CaCO_3$, 4% ethanol, 2% agar) were sterilized, plated, and incubated at 37 °C for LAB and AAB detection [24]. Ethanol was added post-autoclaving to YGCE to prevent volatilization.

### Reducing Sugar Quantification (DNS Assay)

Samples (1 mL) mixed with DNS reagent (1 mL) and 1N NaOH (2 drops) were boiled (5 min), cooled, diluted to 10 mL, and analyzed spectrophotometrically at 540 nm [25]. Glucose standard curves (0.1-1.0 g/L) enabled quantification.

**Sucrose Hydrolysis**
Acid hydrolysis (10% HCl, 100 °C, 10 min) converted sucrose to glucose/fructose, neutralized with 10% NaOH, and quantified via DNS. Sucrose concentration was derived from the difference in glucose pre- and post-hydrolysis [26].

**Invertase Activity**
Samples incubated with sucrose (0.1 M acetate buffer, 3 min) released reducing sugars, measured via DNS. Activity (U/mL) was calculated as reported by Ridzuan et al. and Zhou et al. [27,28].

**Protease Activity**
Casein agar plates (10 g/L casein, 1 g/L glucose, 20 g/L agar) were used and microorganisms were inoculated, incubated (37 °C, 24 h), and clear zones were measured [29].

**Cellulase Activity**
CMC agar (5 g/L CMC, pH 7.0) was stained with Congo Red (2 g/L), destained with 1N NaCl, and hydrolysis zones were quantified [30].

**Lipase Activity**
Tributyrin agar (25 g/L, 0.01% phenol red) was used to show activity via pH-dependent color change (red to orange) and clear zones [31].

**Amylase Activity**
Starch agar (20 g/L) was flooded with iodine post-incubation (37 °C, 48 h). Clear zones against a blue-brown background indicate starch hydrolysis [32].

## RESULT AND DISCUSSION

**Effect of SCOBY Brewing on pH**
The pH measurement of the SCOBY brewing solution (kombucha tea) during inoculum preparation showed that samples fermented at room temperature for one week started at an average pH of 3.17, with minor variations observed across samples [33]. A pH drop occurred from day 0 to day 2 (**Fig. 1**) due to rapid organic acid production by SCOBY microorganisms such as acetic acid bacteria (AAB) by *Acetobacter xylinum* and acid-tolerant yeast, which convert glucose to gluconic acid and ethanol to acetic acid [34,35]. These processes involve releasing protons and lowering pH [36]. The pH stabilized after day 2, reaching 2.96 by day 7, indicating equilibrium in acid production and nutrient consumption, consistent with studies by Chou et al. [35] and Jayabalan [33], who reported a similar pH decline within 5-7 days. This acidic environment (pH ≤ 4.6 within a week) prevents harmful bacterial growth, ensuring safe fermentation condition. Stabilization suggests a slowdown in acid production, possibly due to nutrient limitations or microbial adaptation, as organic acids accumulated [37].

**Microbial Identification of SCOBY and Pineapple Peel**
Microbial identification of SCOBY and pineapple peel was conducted to analyze the primary microbial populations involved in fermentation, using selective media (YPD, PDA, MRS, and YGCE agar) to identify and quantify yeast, lactic acid bacteria (LAB), and AAB. These microorganisms can influence enzymatic activity and organic acid production [22]. The kombucha SCOBY primarily contains AAB and yeasts, with microbial diversity varying based on sources, cultivation medium, fermentation conditions, and raw materials [38].

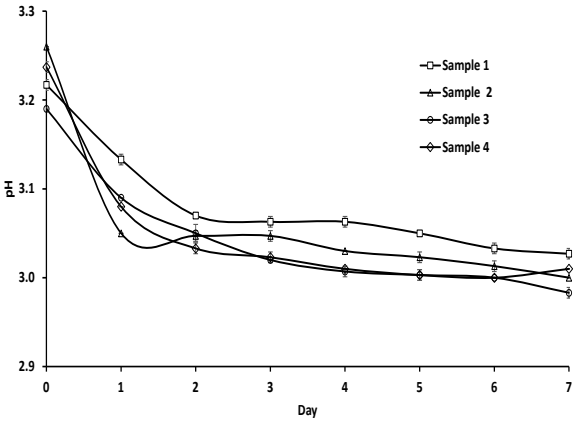



**Fig. 1**. pH measurement of SCOBY during inoculum preparation process at room temperature.

Microorganisms metabolize glucose from the fermentation medium and pineapple peel, increasing total microbial counts while producing metabolites like alcohol and organic acids [39]. Microbial cell counts before and after 7-day fermentation revealed that yeast counts in the control groups (kombucha without pineapple peel) were 400 CFU/mL at 37 °C pre-fermentation, reaching Too-Many-To-Count (TMTC) at post-fermentation. The sample group (kombucha with fermented pineapple peel extract, FPPE) started higher (760 CFU/mL) and reached TMTC as well, supporting the idea that pineapple peel supported yeast growth (37 °C). However, at 45 °C, yeast counts in the control were initially at TMTC level but dropped to Too-Few-to-Count (TFTC) at post-fermentation. The sample groups show a similar decline (480 CFU/mL to TFTC), indicating temperature sensitivity.

The yeast cell counts on YPD and PDA agar for control and sample groups at 37 °C and 45 °C were presented in **Tables 1** and **2**. For PDA, before fermentation (37 °C), yeast growth was substantial, with 3120 CFU/mL in the control groups and TMTC in the sample groups. After fermentation, yeast counts decreased to 330 CFU/mL in the control and 1210 CFU/mL in the sample, pointing to the fact that nutrient depletion. At 45 °C, both groups (control and samples) exhibited TMTC before fermentation, however, similar pattern was absent for yeast to indicate poor yeast survival at high temperature [40].

LAB cell counts on MRS agar for control and sample groups at 37 °C and 45 °C before and after 7-day fermentation were reported in **Table 3**. At 37 °C, before fermentation, the LAB counts were higher in the sample groups (2270 CFU/mL) compared to the control groups (1860 CFU/mL). After fermentation, LAB counts decreased in the control groups (TFTC), while the sample groups exhibited TMTC, suggesting active microbial proliferation in the presence of pineapple peel. At 45 °C, LAB counts before fermentation were 2040 CFU/mL in the control groups and 1380 CFU/mL in the sample groups. After fermentation, the LAB in the control groups reduced to TFTC, and LAB in the sample groups were absent, confirming that high temperatures inhibited LAB survival during fermentation [41].

**Table 1**. Yeast cells count on YPD agar for control and sample groups at 37 °C and 45 °C.

| Temperature | Before fermentation | | After fermentation | |
|---|---|---|---|---|
| | Observation | Cell count (CFU/mL) | Observation | Cell count (CFU/mL) |
| 37 °C | | | | |
| Control |  | 400 |  | TMTC |
| Sample |  | 760 |  | TMTC |
| 45 °C | | | | |
| Control |  | TMTC |  | TFTC |
| Sample |  | 480 |  | TFTC |

**Table 2**. Yeast cells count on PDA agar for control and sample groups at 37 °C and 45 °C.

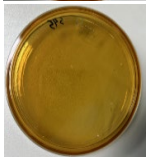
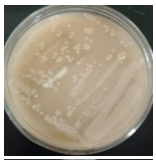
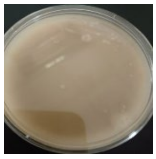
| Temperature | Before fermentation | | After fermentation | |
|---|---|---|---|---|
| | Observation | Cell count (CFU/mL) | Observation | Cell count (CFU/mL) |
| 37 °C | | | | |
| Control |  | 3120 |  | 330 |
| Sample |  | TMTC |  | 1210 |
| 45 °C | | | | |
| Control |  | TMTC |  | None |
| Sample |  | TMTC |  | None |

**Table 3**. Lactic acid bacteria cells count (MRS agar, 37 °C and 45 °C).

| Temperature | Before fermentation | | After fermentation | |
|---|---|---|---|---|
| | Observation | Cell count (CFU/mL) | Observation | Cell count (CFU/mL) |
| 37 °C | | | | |
| Control |  | 1860 |  | TFTC |
| Sample |  | 2270 |  | TMTC |
| 45 °C | | | | |
| Control |  | 2040 |  | TFTC |
| Sample |  | 1380 |  | None |

**Table 4**. Acetic acid bacteria cell counts (YGCE agar, 37 °C and 45 °C).

| Temperature | Before fermentation | | After fermentation | |
|---|---|---|---|---|
| | Observation | Cell count (CFU/mL) | Observation | Cell count (CFU/mL) |
| 37 °C | | | | |
| Control |  | TMTC |  | TFTC |
| Sample |  | TMTC |  | 2620 |
| 45 °C | | | | |
| Control |  | 390 |  | None |
| Sample |  | TMTC |  | TFTC |

The cell counts of AAB on YGCE agar for control and sample groups at 37 °C and 45 °C before and after 7-day fermentation were shown in **Table 4**. At 37 °C, before fermentation, the AAB population in the control groups was TMTC, while after fermentation the level reduced to TFTC, reflecting low AAB activity in the control groups. AAB counts were also TMTC in the sample groups before fermentation. After fermentation, a measurable count of 2620 CFU/mL was observed, revealing that the pineapple peel in the sample groups promoted AAB activity and sustained their population by providing carbon source for bacterial biomass production [42]. At 45 °C, before fermentation, a relatively low count of 390 CFU/mL was recorded (control groups), and after fermentation, AAB colony were failed to be detected. This finding indicates that higher temperatures might hinder AAB survival in the control groups. Before fermentation, the AAB population in the sample groups was TMTC. However, after fermentation, the count dropped to TFTC, reflecting temperature stress or resource depletion during fermentation. Most AAB have their growth and fermentation activity severely inhibited when the fermentation temperature rises above 34 °C, however thermotolerant strains can survive at 37 °C [43].

The experimental results demonstrate that nutrient availability, particularly carbon sources, influenced microbial cell counts, with most bacterial populations declining as sugars were depleted during fermentation (**Tables 1** to **4**). However, pineapple peel supplementation enhanced and sustained microbial viability. This finding aligns with the work of Sornkayasit et al. [44], who reported the beneficial effects of pineapple by-products in kombucha production, as they provide essential sugars, organic acids, vitamins, and minerals [40]. Temperature exerted a critical influence on microbial dynamics, as evidenced by the sustained activity at 37 °C versus complete inhibition at 45 °C. The latter resulting from thermal stress exceeding mesophilic tolerance thresholds [45] and ethanol accumulation [46]. Additional factors affecting microbial populations included fermentation duration and pH, with prolonged fermentation leading to inhibitory organic acid accumulation.

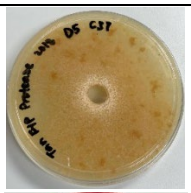
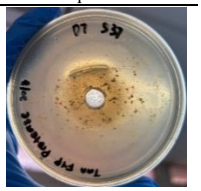
Acidic conditions and $CO_2$-mediated nutrient transport limitations further suppressed growth [47]. Microbial characterization of pineapple peel solutions revealed indigenous microorganisms (IMO) including yeasts, AAB, and LAB, in agreement with Selvanathan & Masngut [48], with pineapple peels exhibiting superior AAB content relative to other fruit residues [49] and serving as a viable LAB source [50]. Particularly, while pineapple peels harbored diverse IMOs, their microbial counts were substantially lower than in fermented extracts, underscoring SCOBY's dominant role in shaping the final microbial profile.

These findings collectively highlight the complex interplay between substrate composition, environmental parameters, and microbial ecology in fermentation systems.

## Enzyme Evaluation

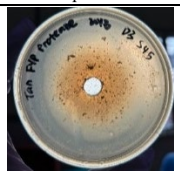
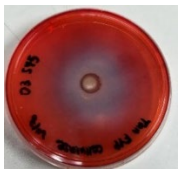
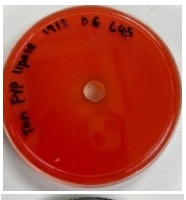
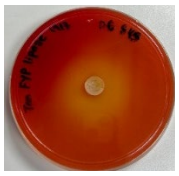
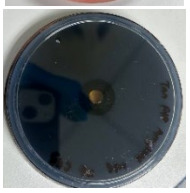
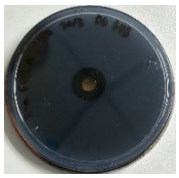
### Enzyme Screening

**Table 5**. Observation of clear zone formation in the control and sample groups at 37 °C.

| Enzyme | Control 37 °C | Sample 37 °C |
|---|---|---|
| Protease |  |  |
| Cellulase |  |  |
| Lipase |  |  |
| Amylase |  |  |

The enzyme evaluation tests conducted on the control groups and the sample groups presented distinct enzymatic activities at 37 °C and 45 °C. The diameter of the clear zone and the hydrolysis index indicated the production and the efficiency of the enzymes, including protease, cellulase, and lipase (**Tables 5** and **6**), in using the specific agar medium. The presence or absence of clear zones around substrates on agar plates reflecting the enzymatic activity for those enzymes at 37 °C and 45 °C. The formation of clear zones qualitatively indicated enzyme activity and reflected substrate hydrolysis.

**Table 6**. Observation of clear zone formation in the control and sample groups at 45 °C.

| Enzyme | Control 45 °C | Sample 45 °C |
|---|---|---|
| Protease |  |  |
| Cellulase |  |  |
| Lipase |  |  |
| Amylase |  |  |

Protease activity was quantitatively assessed through hydrolysis zone measurements on casein agar plates, revealing enzymatic activity in the sample groups compared to controls (**Figs. 2** and **3**). At 37 °C, the sample groups exhibited substantial proteolytic capacity, with hydrolysis index 2.2 by day 3, maintaining high activity through day 5 (index 2.3) and day 7 (index 2.2). Particularly, elevated temperatures enhanced protease performance, as demonstrated at 45 °C where hydrolysis index, 3.0 and zones expanded (**Table 6**). The activity sustained throughout the experimental period, consistent with established temperature optima for bromelain activity at 50 °C [51]. This temperature-dependent activity enhancement, coupled with the sample groups' superior performance, can be attributed to the liberation of natural proteolytic enzymes-particularly bromelain-from pineapple peels during microbial degradation [52]. These results collectively establish pineapple peels as a valuable natural source of proteolytic enzymes with temperature-modulated activity profiles suitable for various biotechnological applications.

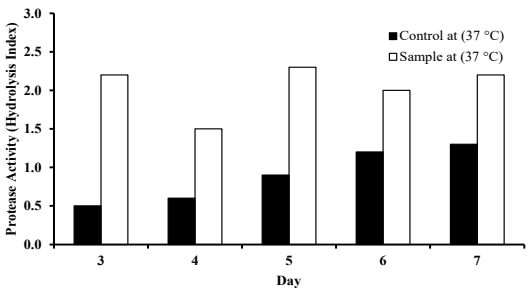



**Fig. 2**. Hydrolysis index of protease activity in the control and sample groups at 37 °C.

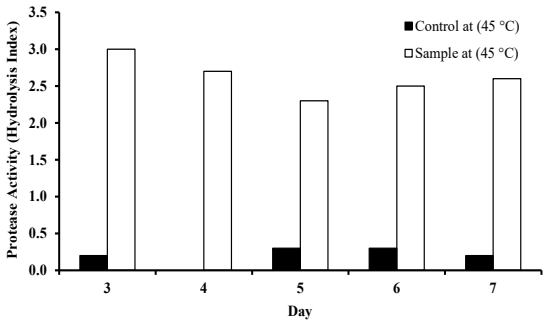



**Fig. 3**. Hydrolysis index of protease activity in the control and sample groups at 45 °C.

Cellulase activity, mediated by exoglucanase and endoglucanase enzymes, was evaluated through hydrolysis zone measurements on CMC agar medium, demonstrating the enzymatic conversion of cellulose into glucose via intermediate oligomers [53]. At 37 °C, the sample groups exhibited moderate to high cellulase activity, with peak hydrolysis zones observed on day 3 with cellulase activity index, 2.4 (**Fig. 4** and **5**), consistent with findings by Ridzuan et al. [27]. However, the activity declined subsequently (days 5-7: index 1.0-1.2), perhaps due to nutrient depletion and metabolic byproduct accumulation. In contrast, 45 °C promoted sustained cellulase production, yielding maximal hydrolysis activity on day 3 (index 3.3) with consistently high indices (2.1-3.3).

These results validate the thermal enhancement effect previously observed in *Saccharomyces cerevisiae* cultures [54]. The superior performance of pineapple peel samples compared to controls highlights their dual function as both a substrate and an inducer of microbial cellulase secretion. The peel-derived cellulose stimulates SCOBY microbiota and native cellulolytic bacteria, enhancing the enzymatic degradation of structural polysaccharides. These temperature-dependent activity profiles highlight pineapple peels as effective substrates for optimized cellulase production in bioconversion processes. Lipase activity, reflecting lipid hydrolysis, was evaluated at 37 °C and 45 °C (**Fig. 6** and **7**). At 37 °C, the sample group demonstrated moderate lipase activity and an increase on day 6 (index 2.5), with variable daily fluctuations demonstrating inconsistent enzyme production.

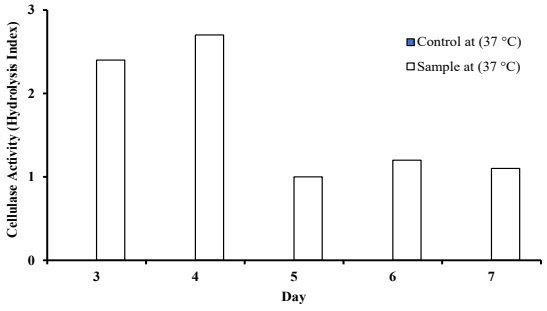



**Fig. 4**. Hydrolysis index of cellulase activity in the control and sample groups at 37 °C.

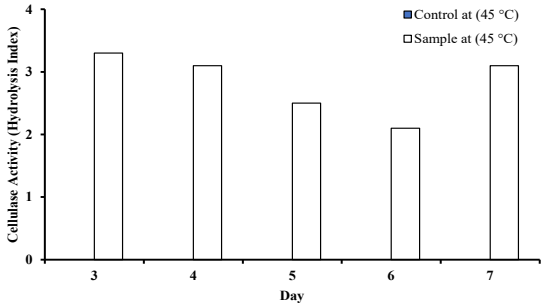



**Fig. 5**. Hydrolysis index of cellulase activity in the control and sample groups at 45 °C.

Elevated temperature (45 °C) enhanced lipase activity, particularly on days 3 (index 2.7) and 6 (index 2.5), revealing thermal activation of lipases within this range. This aligns with Sitepu et al. [55], who reported optimal conversion (82.7%) at 46.5 °C using pineapple-derived lipases. The sample groups' superior performance versus controls likely stem from pineapple peel's lipid content acting as enzyme inducers [20], supported by similar findings in garbage enzyme [56] and pineapple peel fermentation research [57]. Conversely, amylase activity was undetectable in both groups at both temperatures, with null hydrolysis indices contradicting reports of successful amylase production from pineapple peels [58] and *Aspergillus niger* cultures [59]. This difference may indicate either methodological limitations in detecting amylase-producing microbes or suboptimal conditions for enzyme expression/ activity in the current experimental setup.

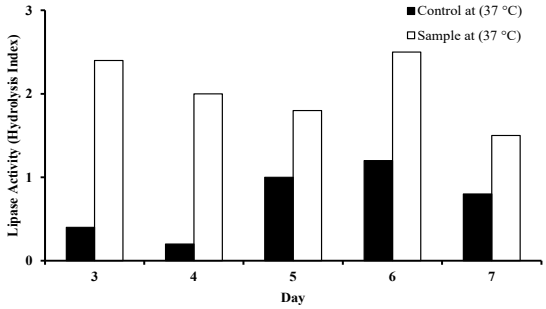



**Fig. 6**. Hydrolysis index of lipase activity in the control and sample groups at 37 °C.

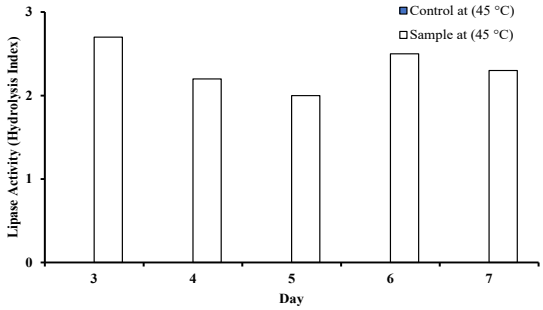



**Fig. 7**. Hydrolysis index of lipase activity in the control and sample groups at 45 °C.

## CONCLUSION

The study found that adding pineapple peels to kombucha fermentation boosted microbial growth and enzyme activity. The fermentation process quickly turned more acidic, creating an ideal environment for safe fermentation. Pineapple peels helped maintain healthy populations of beneficial bacteria and yeast at moderate temperatures, though higher temperatures proved harmful. The peels were particularly effective at enhancing key enzymes that break down proteins, fibers, and fats. These enzymes work best at warmer temperatures. The natural nutrients in pineapple peels helped sustain the fermentation process, showing their potential for improving kombucha production and other food technology applications. Pineapple peels contribute to the production of key enzymes, including proteases, cellulases, and lipases, improving fermentation efficiency. This approach also promotes sustainability by utilizing agricultural byproducts. Further investigation is required to address the limited detection of some enzyme activity. By implementing these practices, kombucha fermentation can be improved in both quality and effectiveness.

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## CONFLICT OF INTEREST

The author declares that there are no conflicts of interest related to this publication.

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