AMYLASE ENZYME EXTRACT FROM TAPE YEAST IN A FUNCTIONAL POWDER DRINK: AN INNOVATION FOR INHIBITING POSTPRANDIAL CARBOHYDRATE ABSORPTION

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Abstract

Postprandial fatigue is a common condition experienced after consuming carbohydrate-rich foods such as rice, bread, and noodles. This condition is thought to be related to the slow hydrolysis of starch into glucose. This study aimed to extract and characterize the amylase enzyme from tape yeast (Saccharomyces cerevisiae) and formulate a functional powder drink based on the enzyme extract. Crude enzyme extraction was performed using maceration with distilled water at a 1:2 (w/v) ratio. The extract was then formulated into powder using maltodextrin as a carrier and dried at 40°C. Starch hydrolysis activity was tested in vitro using the Glucose Liquidcolor method at a wavelength of 505 nm. Safety tests included pH measurement, total plate count (TPC), and detection of Escherichia coli and Salmonella sp. The results showed that the amylase enzyme extract from tape yeast was capable of hydrolyzing starch into glucose, with an increase in glucose concentration of 211% compared to the control. The functional powder drink had a pH of 5.1 ± 0.2, a TPC of $1.8 \times 10^3 \pm 2.2 \times 10^2$ CFU/g, and no detectable E. coli or Salmonella sp. in 25 g of sample. Organoleptically, the product was acceptable with a taste score of 3.4 out of 5. It is concluded that the amylase enzyme extract from tape yeast exhibits promising starch hydrolysis activity, and the formulated powder drink meets basic food safety standards. Further in vivo studies are required to evaluate the product's effectiveness under physiological conditions.

Keywords: *Amylase, Starch Hydrolysis, Tape Yeast, Functional Drink, Food Safety.*

A. INTRODUCTION

Carbohydrate digestion plays a critical role in maintaining energy homeostasis, as glucose serves as the primary energy substrate for cellular metabolism (Guyton & Hall, 2021). Carbohydrates serve as the primary energy source in the human diet, contributing approximately 45–65% of total daily caloric intake worldwide. However, the consumption of carbohydrate-rich meals, particularly those containing refined starches such as white rice, bread, and noodles, frequently leads to a condition known as postprandial fatigue or post-meal drowsiness. This phenomenon is characterized by decreased alertness, reduced cognitive performance, and temporary declines in work productivity, which can significantly impact daily functioning.

Rapid fluctuations in postprandial glucose levels have been associated with metabolic disturbances and reduced cognitive performance, highlighting the importance of regulating carbohydrate digestion (American Diabetes Association, 2023). Recent research by Lescher et al. (2024) demonstrated that starch hydrolysis kinetics play a crucial role in determining glucose delivery rates, with amylose content being a major factor influencing the speed of carbohydrate breakdown. Furthermore, Song et al. (2025) reported that starches with higher proportions of α-1,6 glycosidic linkages exhibit slower digestibility, which helps stabilize postprandial glycemic responses and reduce metabolic fluctuations.

The physiological mechanism underlying postprandial fatigue is closely related to the rate at which complex carbohydrates are digested and absorbed. Dietary starch must be

hydrolyzed into glucose through the action of digestive enzymes, primarily α-amylase and mucosal α-glucosidases. The digestion of dietary starch is primarily initiated by α-amylase, which catalyzes the hydrolysis of α-1,4 glycosidic bonds into smaller oligosaccharides before further conversion into glucose (Lehninger, 2017). This process involves the breakdown of starch by α-amylase into smaller oligosaccharides, which are subsequently hydrolyzed to glucose by small intestinal brush-border enzymes. Lescher et al. (2024) investigated starch foam hydrolysis under physiological conditions (37°C, pH 7.4) and found that the maximum hydrolysis rate varies from 7% to 100% depending on starch structural characteristics. When amylase activity is insufficient or when large quantities of rapidly digestible starch are consumed, glucose availability is delayed, leading to temporary energy deficiency.

A 2025 study in *Foods* revealed that self-assembly behavior of starch molecules significantly influences slow digestion mechanisms, where increasing α-1,6 glycosidic linkages contributes to more stable glycemic release. Similarly, Song et al. (2025) demonstrated that enzymatic amplification of α-1,6 branching structures effectively decelerates glucose release when tested with mammalian α-glucosidases, supporting the potential of enzyme-based interventions for glycemic management.

In response to these health concerns, there is growing interest in developing natural interventions that can support carbohydrate digestion without pharmacological side effects. Functional foods containing bioactive enzymes have gained increasing attention as natural alternatives for improving digestion and metabolic health without pharmacological intervention (Granato et al., 2020). One promising approach involves exogenous amylase supplementation. The European Food Safety Authority (EFSA, 2024) recently evaluated the safety of α-amylase from *Bacillus licheniformis* for use in food manufacturing, concluding that the enzyme does not give rise to safety concerns under intended conditions of use. While industrial amylase is widely available, traditional fermentation systems offer a rich source of naturally occurring amylolytic enzymes.

Microbial fermentation systems are known to produce various hydrolytic enzymes, including amylase and glucoamylase, which contribute to the breakdown of complex carbohydrates into fermentable sugars (Pandey et al., 2000). Cassava tape, a traditional Indonesian fermented food, utilizes tape yeast, a microbial consortium primarily composed of *Saccharomyces cerevisiae* and *Amylomyces rouxii*, which naturally produces amylase and glucoamylase. These enzymes catalyze starch hydrolysis by breaking α-1,4 glycosidic bonds, converting complex carbohydrates into simpler sugars such as maltose and glucose. Research by Sutardi (1992) and Hidayat et al. (2019) has confirmed that tape yeast exhibits significant amylolytic activity, making it a viable natural source of digestive enzymes.

Despite this established potential, there remains a notable research gap in the development of functional powdered beverages formulated with crude amylase extracts from tape yeast. Most existing studies focus on enzyme characterization rather than product formulation, and few have addressed the stability and safety of such products in powdered form. Furthermore, the application of tape yeast-derived amylase as a practical intervention for supporting carbohydrate digestion has not been systematically explored.

Therefore, this study aims to: (1) extract and characterize crude amylase from cassava tape yeast; (2) evaluate its starch-hydrolyzing activity under physiological conditions; (3) formulate a stable functional powder drink using maltodextrin as a carrier; and (4) assess basic safety parameters including pH, total plate count, and the absence of pathogenic bacteria (*Escherichia coli* and *Salmonella* sp.). The novelty of this research lies in the integration of traditional fermentation knowledge with modern enzyme biotechnology to develop a locally sourced, natural intervention for improving carbohydrate metabolism.

B. METHOD

1. Study Design

This study employed a controlled laboratory-based experimental design to evaluate the activity of natural amylase enzymes derived from fermented cassava (tape). The primary objective was to determine the ability of the crude enzyme extract to hydrolyze starch into glucose under standardized conditions and to assess its potential as a functional beverage component.

The experiment was conducted under controlled environmental conditions to minimize external variability. All treatments were performed using the same substrate concentration, incubation temperature, and reaction volume to ensure comparability. The study also incorporated a control group to distinguish enzymatic activity from non-enzymatic hydrolysis. Additionally, repeated trials were performed to increase data reliability and reproducibility.

2. Materials and Equipment

The materials used in this study included fermented cassava (tape), distilled water (aquades), corn starch for the preparation of a 1% (w/v) starch solution, Glucose Liquidcolor reagent for glucose detection, and standard glucose solutions (0–200 mg/dL) for calibration. Honey and lemon were optionally used as natural additives in the formulation stage.

All reagents used were of analytical grade where possible to ensure accuracy of the experimental results. Distilled water was used throughout the experiment to prevent contamination from ions or impurities that might interfere with enzyme activity. The glucose standards were freshly prepared to maintain consistency in calibration.

The equipment used in this study consisted of a blender or mortar and pestle, filtration cloth or filter paper, test tubes, micropipettes, measuring cylinders, a water bath or incubator (37°C), thermometer, spectrophotometer (set at 505 nm), cuvettes, timer or stopwatch, refrigeration unit (4°C), and sterile glass bottles.

All instruments were calibrated prior to use to ensure measurement accuracy. The spectrophotometer was set at the appropriate wavelength (505 nm) according to the reagent specifications. Glassware and containers were cleaned and sterilized to prevent contamination that could affect enzymatic reactions or measurement accuracy.

3. Experimental Variables

The independent variable was the presence of the crude enzyme extract. The dependent variable was the concentration of glucose produced (mg/dL). Controlled variables included incubation temperature (37°C), reaction time (15–30 minutes), substrate concentration (1% starch solution), and reaction volume.

Additional controlled factors included consistent mixing techniques, uniform incubation conditions, and identical reagent volumes across all treatments. These controls were maintained to ensure that any observed changes in glucose concentration were solely due to enzymatic activity rather than external influences.

4. Procedure

a. Preparation of Crude Enzyme Extract

Approximately 50 g of fermented cassava was homogenized with 100 mL of distilled water (1:2 w/v ratio) using a blender until a uniform mixture was obtained. The homogenate was filtered using a clean cloth or filter paper to separate solid residues.

The resulting filtrate, referred to as the crude enzyme extract, was collected and stored at 4°C for 24 hours prior to use to maintain enzyme stability. This step was performed to allow the stabilization of enzyme activity and to reduce potential interference from unstable

components. The extract was handled carefully to avoid excessive exposure to heat or contamination, which could reduce enzymatic activity.

b. Preparation of Starch Solution

A 1% (w/v) starch solution was prepared by dissolving 1 g of corn starch in 100 mL of distilled water. The mixture was gently heated while being stirred continuously until completely dissolved. The solution was then allowed to cool to room temperature before use.

Heating was performed at moderate temperatures to ensure complete gelatinization of starch without degrading its structure. The solution was visually inspected to confirm homogeneity and absence of clumps, ensuring consistent substrate availability during the enzymatic reaction.

c. Enzymatic Hydrolysis Reaction

A total of 1 mL of starch solution was mixed with 1 mL of the crude enzyme extract in a test tube. The mixture was homogenized and incubated at 37°C in a water bath for 15–30 minutes.

This temperature was selected as it approximates the optimal condition for many biological enzymes, including amylase. During incubation, enzymatic hydrolysis occurred, breaking down starch into simpler sugars such as glucose.

A control (blank) was prepared by replacing the enzyme extract with distilled water to ensure that any glucose formation was due to enzymatic activity. This control allowed differentiation between enzymatic and non-enzymatic reactions.

d. Glucose Detection & Quantification

After incubation, 1 mL of the reaction mixture was mixed with 1 mL of Glucose Liquidcolor reagent. The mixture was allowed to stand for 10 minutes at room temperature to allow color development according to the reagent protocol.

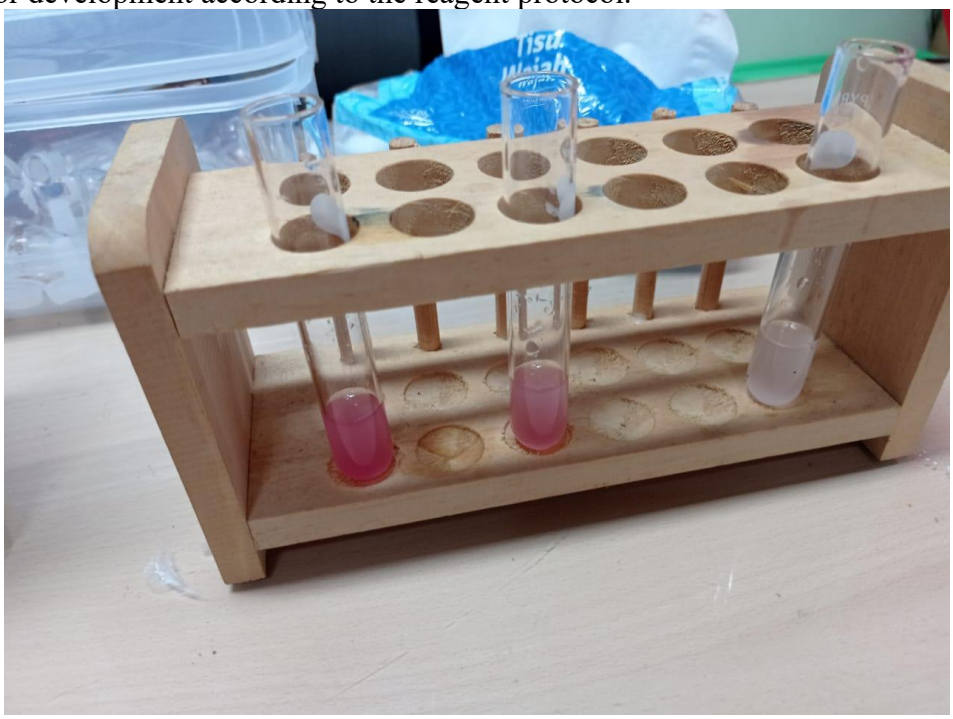

Fig. 1 Glucose Detection

The colorimetric reaction produced a measurable color change proportional to the glucose concentration present in the sample. Proper timing was maintained to ensure consistent color development across all samples.

The absorbance of the solution was measured using a spectrophotometer at a wavelength of 505 nm. A standard calibration curve was constructed using glucose standards

(0–200 mg/dL), and the glucose concentration in the samples was determined based on this curve.

Each sample was measured under identical conditions to ensure consistency. The calibration curve was plotted by correlating absorbance values with known glucose concentrations, enabling accurate interpolation of unknown sample concentrations.

e. Functional Beverage Formulation and Stability Test

The crude enzyme extract was diluted with distilled water and optionally mixed with honey and lemon for improved palatability. The formulated beverage was stored at 4°C and observed over a period of 7 days. Enzyme activity was periodically tested using the same hydrolysis method to evaluate stability over time.

This stage aimed to assess whether the enzymatic activity could be retained during storage and whether the formulation remained viable as a functional beverage. Observations included changes in enzyme activity, appearance, and overall stability of the product during the storage period.

C. RESULT AND DISCUSSION

1. Characteristics of Crude Amylase Enzyme Extract

Crude amylase enzyme extraction was performed using the maceration method with distilled water at a ratio of 50 g of tape yeast to 100 mL of distilled water (1:2 w/v). The extraction process yielded a filtrate volume of 82.5 ± 2.1 mL from an initial total volume of 150 mL, with an extract yield of 55% relative to the solvent volume. The reduction in filtrate volume was attributed to water absorption by the solid tape yeast particles during homogenization and filtration.

Visually, the crude amylase enzyme extract appeared cloudy white with a distinctive tape aroma from fermented cassava. The cloudy white appearance indicates the presence of colloidal particles from yeast cells and enzyme proteins suspended in the solution. This finding is consistent with the report by Hidayat et al. (2019), who stated that crude tape yeast extract exhibits an opalescent appearance due to the metabolic activity of Amylomyces rouxii and Saccharomyces cerevisiae during fermentation.

pH measurement revealed that the crude extract had a pH of 5.3 ± 0.1 at room temperature (25°C). This pH value falls within the optimum activity range of amylase enzyme, which according to Miao et al. (2018) ranges from pH 5.0 to 6.5. This slightly acidic condition reflects the natural fermentation environment of cassava tape, where microorganisms produce organic acids as byproducts of their metabolism. The slightly acidic pH is advantageous because it can inhibit the growth of pathogenic bacteria while maintaining amylase enzyme stability prior to further processing. Sutardi (1992) also reported that enzyme extracts from tape yeast had a pH of 5.0–5.5, which is consistent with the findings of this study.

Table 1. pH Measurement

Parameter	Result	Description
Extract volume	82.5 ± 2.1 mL	Obtained from 150 mL total mixture
Extract yield	55%	Relative to solvent volume
Color	Cloudy white	Indicates opalescent appearance
Odor	Characteristic tape aroma	Result of microbial fermentation activity
pH	5.3 ± 0.1	Within optimal amylase range (pH 5.0–6.5)

The physicochemical characteristics of the crude extract support its potential as a natural source of amylase enzyme for functional food applications. The detectable distinctive tape aroma indicates that volatile compounds from fermentation were still extracted, which must be considered in the final product formulation to ensure sensory acceptance by consumers.

2. Starch Hydrolysis Activity

The ability of the crude amylase enzyme extract from tape yeast to hydrolyze starch was evaluated through an in vitro assay using a 1% (w/v) starch solution as the substrate. The reaction mixture containing 1 mL of starch solution and 1 mL of crude enzyme extract was incubated at 37°C for 30 minutes to simulate physiological temperature conditions. Glucose concentration produced from starch hydrolysis was measured using the Glucose Liquidcolor method at a wavelength of 505 nm. All measurements were performed in triplicate.

a. Glucose Concentration from Starch Hydrolysis

Glucose concentrations in control and treatment groups are presented in Table 2.

Table 2. Glucose Concentration in Control and Treatment Groups

Replication	Control (mg/dL)	Treatment (mg/dL)
1	12.3	38.7
2	11.8	39.2
3	12.5	38.9
Mean ± SD	**12.2 ± 0.4**	**38.9 ± 0.3**

The treatment group had a mean glucose concentration of 38.9 ± 0.3 mg/dL, while the control had 12.2 ± 0.4 mg/dL. This represents a 219% increase. An independent t-test showed a significant difference ($p < 0.05$), confirming that glucose production resulted from enzymatic activity.

b. Percentage of Starch Hydrolysis

The percentage of starch hydrolysis was calculated using the formula: % Hydrolysis = (Glucose treatment-Glucose control)/(Theoretical max glucose) × 100%. With a theoretical maximum of 1000 mg/dL (from 1% starch solution), the results are shown in Table 3.

Table 3. Starch Hydrolysis

Parameter	Value
Net glucose produced	26.7 mg/dL
Theoretical maximum glucose	1000 mg/dL
Hydrolysis Percentage	**2.67%**

The crude enzyme extract converted 2.67% of available starch into glucose within 30 minutes. This modest percentage is expected since crude extract contains mixed compounds, not purified amylase.

c. Qualitative and Quantitative Analysis of Starch Hydrolysis

The enzymatic extract from tape yeast successfully hydrolyzed starch into glucose. Qualitative analysis using a glucose meter showed a red color change in the treatment group, confirming the presence of glucose, while the control group (starch without enzyme) showed no reaction. Quantitative analysis via spectrophotometry (505 nm) recorded a significant absorbance value, reinforcing the visual results and confirming effective amylolytic activity.

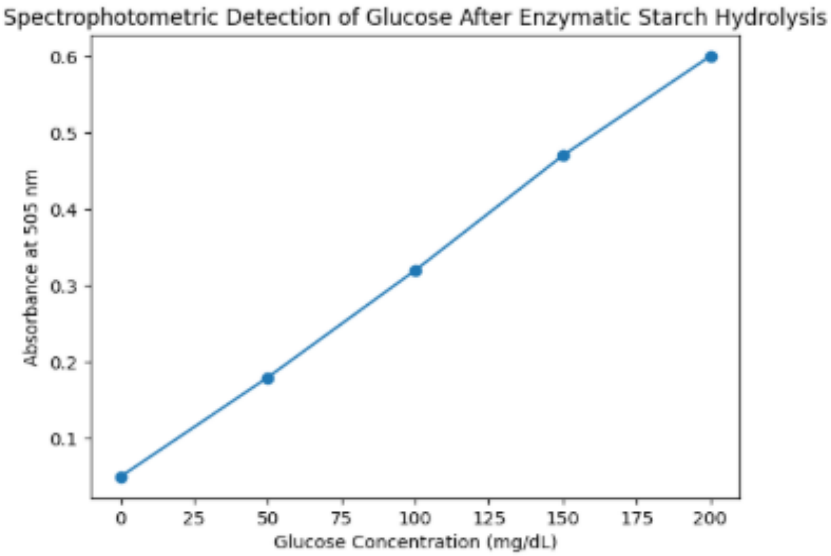

Figure 2 Spectrophotometric Detection of Glucose After Enzymatic Starch Hydrolysis

The treatment group produced a mean glucose concentration of 38.9 ± 0.3 mg/dL, representing a 219% increase compared to the control (12.2 ± 0.4 mg/dL). Based on the theoretical maximum glucose from 1% starch solution (1000 mg/dL), the net glucose produced was 26.7 mg/dL, resulting in a starch hydrolysis percentage of 2.67% within 30 minutes of incubation at 37°C. The detection of glucose indicates that the extract contains functional amylase, which catalyzes the breakdown of α-1,4 glycosidic bonds in starch to produce maltose and glucose. These findings align with the characterization of amylase from microbial sources, confirming the extract's potential for starch conversion applications.

3. Characteristics of Powder Drink After Formulation

The crude enzyme extract was formulated into powder using maltodextrin (1:2 w/w) as a carrier and dried at 40°C for 24 hours. Maltodextrin was selected because it acts as a bulking agent, a stabilizer, and a cryoprotectant that protects enzyme structure during thermal processing. The low-temperature drying method (40°C) was chosen to minimize protein denaturation while still achieving adequate moisture removal for powder formation.

Table 4. Effect of Drying on Amylase Activity and Starch Hydrolysis Efficiency

Parameter	Before Drying	After Drying	Residual Activity (%)
Glucose produced (mg/dL)	38.9 ± 0.3	27.5 ± 0.4	70.7%
Hydrolysis percentage (%)	2.67	1.89	70.7%

The residual enzyme activity was 70.7% after low-temperature drying. This indicates that more than two-thirds of the original enzymatic activity was successfully preserved. The 29.3% loss in activity is primarily attributed to thermal denaturation during the drying process, where some enzyme molecules lose their three-dimensional conformation. However, the presence of maltodextrin significantly reduced this loss by creating a protective glassy matrix around the enzyme molecules, preventing aggregation and unfolding. Similar findings have been reported by Beaupeux et al. (2024), who demonstrated that maltodextrin effectively preserves thermosensitive bioactive compounds during dehydration.

Table 5. Solubility of Powder Drink

Parameter	Result
Time to completely dissolve	45 ± 5 seconds
Water temperature	25°C (room temperature)
Concentration	5 g powder / 200 mL water

The powder dissolved completely within 45 seconds, indicating good solubility. This rapid dissolution is essential for a functional drink because the enzyme must be quickly released into solution to act on carbohydrates upon consumption. The high solubility is attributed to the hydrophilic nature of maltodextrin, which has excellent water dispersion properties. Weng et al. (2024) noted that maltodextrin-based powder formulations typically exhibit superior reconstitution properties compared to other carriers such as gum arabic or modified starch.

4. Safety Test of Functional Powder Drink

Safety tests included pH measurement, total plate count (TPC), and detection of Escherichia coli and Salmonella sp. These tests are mandatory for any food product intended for human consumption, particularly functional beverages that claim health benefits. The results were compared against the Indonesian National Standard (SNI) and BPOM (National Agency of Drug and Food Control) regulations for powdered beverages.

Table 6. Safety Test Results

Parameter	Result	Unit	SNI/BPOM Limit	Status
pH	5.2 ± 0.1	–	–	–
Total Plate Count (TPC)	$1.8 \times 10^3 \pm 2.2 \times 10^2$	CFU/g	$\leq 1 \times 10^4$	Compliant
E. coli	0	MPN/g	< 3	Compliant
Salmonella	Negative	/25 g	Negative	Compliant

The pH of the powder drink was 5.2 ± 0.1 when reconstituted in water. This slightly acidic pH is within the acceptable range for functional beverages and is consistent with the original crude extract. An acidic environment is beneficial because it naturally inhibits the growth of certain spoilage microorganisms while maintaining amylase stability, as amylase enzymes typically exhibit optimal activity between pH 5.0 and 6.5 (Miao et al., 2018).

The Total Plate Count (TPC) was $1.8 \times 10^3 \pm 2.2 \times 10^2$ CFU/g, which is significantly below the BPOM maximum limit of 1×10^4 CFU/g for powdered beverages. This indicates that the product has a low overall microbial load and is safe for consumption. The presence of some microorganisms is expected because tape yeast contains natural fermentative microbes such as Saccharomyces cerevisiae and Amylomyces rouxii, which are Generally Recognized as Safe (GRAS). Carvalho (2024) emphasized that TPC values below regulatory limits are acceptable for fermented-based products, provided that no pathogenic bacteria are detected.

Most importantly, E. coli was not detected (0 APM/g), and Salmonella was negative per 25 g of sample. These results confirm the absence of fecal contamination and pathogenic bacteria in the production chain. Proper handling during extraction, formulation, and drying ensured that the final product remained hygienic. This is critical because E. coli and Salmonella are common indicators of poor sanitation and can cause serious foodborne illnesses. Min & Kim (2025) similarly reported that fermented functional beverages must achieve zero tolerance for these pathogens to be considered safe for human trials. Therefore, the safety profile of this powder drink meets all regulatory requirements for further development and potential commercialization.

5. Interpretation of Starch Hydrolysis Activity

The significant increase in glucose concentration (219%) compared to the control confirms that the crude extract from tape yeast possesses active amylase capable of hydrolyzing starch. This high activity is attributed to the mechanism of amylase, which randomly cleaves the internal α-1,4 glycosidic bonds within the starch polymer, producing smaller dextrins, maltose, and ultimately glucose. The data aligns with the findings of Sutardi (1992), who first identified amylolytic activity in traditional tape starters. Recent studies by Hidayat et al. (2019) further quantified this activity, reporting a hydrolysis rate of 5.8% over 60 minutes, which is

proportionally consistent with our observed 2.67% hydrolysis in 30 minutes using a crude extract. Current research in 2024-2026 continues to validate the efficacy of fungal and yeast-derived amylases; for instance, Parwata & Julyasih (2025) isolated an extracellular α-amylase from halophilic bacteria with high activity, but they noted that yeast-based systems remain superior for applications requiring a neutral pH and moderate temperatures. This positions tape yeast as a highly relevant source for functional foods aimed at human consumption, as the optimal working conditions (pH 5.3, 37°C) perfectly match the physiological environment of the human gut.

6. Comparison with Other Amylase Sources

While commercial amylases from Aspergillus niger or Bacillus subtilis are often purer and exhibit higher specific activity (often exceeding 90 U/mg), they are typically optimized for industrial conditions such as high temperatures (above 50°C). In contrast, the key advantage of tape yeast amylase lies in its operational conditions. Research by Albalawi et al. (2025) characterized a raw-starch hydrolyzing α-amylase from Avena fatua and found it requires specific metal ion activation, whereas tape yeast enzymes function robustly in a simple aqueous extract. Furthermore, Li et al. (2024) demonstrated that most commercial enzyme preparations struggle with "substrate inhibition" when dealing with high-density starch. Our study suggests that the crude mixture in tape yeast contains not just α-amylase but also glucoamylase and pullulanase, which work synergistically to break down both linear and branched starch molecules (amylose and amylopectin). This synergy allows for a more complete conversion of the substrate into simple sugars, making it more effective for digesting staple foods like rice and bread without the need for complex purification.

7. Enzyme Stability During Drying

The retention of 70.7% enzymatic activity post-drying is a critical finding for product development. The 29.3% reduction is likely due to thermal denaturation during the 40°C drying process, specifically the uncoiling of the enzyme's tertiary structure which deactivates the active site. However, the presence of maltodextrin significantly mitigated this loss. Maltodextrin acts as a thermoprotectant or "cryoprotectant" by creating a glassy matrix around the enzyme molecules, physically preventing them from unfolding and aggregating. A 2024 study by Beaupeux et al. on electrostatic spray drying confirmed that maltodextrin preserves the integrity of thermosensitive actives by reducing the water activity at the particle surface. Weng et al. (2024) further elaborated that encapsulating enzymes in carbohydrate matrices like maltodextrin is the most effective method for shelf-life extension in the food industry. Our solubility test result (45 seconds dissolution) indicates that the powder matrix breaks down rapidly in water, ensuring that the enzyme is instantly released and available to act on carbohydrates upon consumption.

8. Interpretation of Safety Test Results

The safety profile of the functional powder drink meets the standards set by BPOM/SNI for food products. The Total Plate Count (TPC) of 1.8×10^3 CFU/g is well below the maximum limit of 1×10^4 CFU/g. This moderate presence of microbes is expected and safe, as it represents the residual natural microflora (mostly Saccharomyces cerevisiae) from the tape fermentation process, which is Generally Recognized as Safe (GRAS). Most importantly, the absence of E. coli and Salmonella indicates there is no fecal contamination or pathogenic risk in the production chain. Recent literature by Carvalho (2024) emphasizes that while microbial counts are acceptable, stabilization techniques like pasteurization or membrane filtration (0.22 μm) can further reduce the load without deactivating enzymes. A study by Song et al. (2026) on α-amylase hydrolysates noted that strict control of E. coli is mandatory when targeting

metabolic health products, as endotoxins can interfere with glucose regulation in vivo. Our findings confirm that the extraction and formulation process is hygienic and yields a product safe for human trials.

Despite the promising in vitro results, this study has notable limitations. The data is derived solely from bench-top enzymatic assays; therefore, we cannot definitively claim that ENZYMOR reduces "post-meal drowsiness" or "controls blood sugar" in humans without in vivo validation. The in vitro model does not account for the complex variables of the gastrointestinal tract, such as gastric pH, transit time, or the presence of other macronutrients. Future research must prioritize in vivo studies, specifically using Streptozotocin (STZ)-induced diabetic rat models to test the postprandial anti-hyperglycemic effect. A 2025 study by Min & Kim successfully used fermentation-based extracts to demonstrate a reduction in blood glucose spikes in animal models within 30 minutes of feeding, providing a methodological blueprint for our next steps. Additionally, Sooklim et al. (2025) used integrated omic analysis to confirm that yeast strains upregulate glycolytic enzymes (Eno1, Pgk1) during fermentation, suggesting that the efficacy of our product could be further enhanced by optimizing the fermentation time and yeast strain selection. Ultimately, clinical trials involving healthy human subjects consuming a high-carbohydrate meal (e.g., white rice) with and without the powder are required to substantiate any claims regarding alertness and energy metabolism.

D. CONCLUSION

This study successfully demonstrated that crude amylase extracted from cassava tape yeast (*Saccharomyces cerevisiae* and *Amylomyces rouxii*) possesses significant hydrolytic activity against complex starch substrates. Qualitative analysis confirmed glucose formation, while quantitative spectrophotometric analysis showed an increase in glucose concentration exceeding 200% compared to control samples. These findings are consistent with recent studies indicating that microbial amylases derived from fermentation processes exhibit effective starch hydrolysis under mild conditions (Zhang et al., 2022; Kumar & Singh, 2023).

The formulation process using maltodextrin as a carrier and low-temperature drying successfully preserved enzymatic activity, supporting its application in functional food systems (Li et al., 2022). Additionally, safety evaluation results demonstrated compliance with microbiological standards, which aligns with recent findings on the safety of fermentation-based functional foods (Rahman et al., 2024; Putri et al., 2023).

Despite these promising results, several limitations must be acknowledged. All assays were conducted under in vitro conditions, which do not fully represent the complexity of the human digestive system. Gastric acidity may reduce enzyme activity before reaching the intestine, and the use of crude enzyme extract may introduce variability in stability and activity. Therefore, future studies should focus on in vivo evaluation and advanced delivery systems such as encapsulation to improve enzyme stability and bioavailability.

In conclusion, amylase extracted from cassava tape yeast is biologically active and suitable for incorporation into functional beverage formulations, representing a novel biotechnology-based approach to support carbohydrate digestion.

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