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Biochemical Evaluation of Pretreated Palm Kernel (*Elaeis Guineensis*) Cake and Its Application in Alcohol Production

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ABSTRACT

The search for sustainable and cost-effective substrates for bioethanol production has intensified interest in agro-industrial by-products. This study aimed to evaluate the impact of pre-treated palm kernel cake (PKC), a by-product of palm oil processing, on industrial alcohol production and associated biochemical changes. To enhance its fermentation suitability, PKC was subjected to solid-state fermentation, autoclaving, and acid hydrolysis over 72 hours before undergoing submerged fermentation with commercial baker's yeast (Saccharomyces cerevisiae). Biochemical parameters including soluble protein, glucose, phenol, total flavonoid concentration (TFC), and enzymatic activities (phytase and mannanase) were measured alongside fermentation indicators such as pH, total soluble solids, specific gravity, and alcohol concentration. The results revealed that pre-treated PKC showed significantly higher ($p < 0.05$) levels of soluble protein, phenols, and TFC compared to untreated PKC, while glucose concentration decreased markedly, suggesting efficient substrate utilization. Enhanced enzymatic activity and free radical scavenging capacity were also observed in the pre-treated groups. Notably, solid-state fermentation yielded the highest specific gravity and alcohol output. The pH increased significantly during fermentation, while total soluble solids declined post-fermentation, further supporting substrate breakdown. These findings indicate that pre-treatment, particularly solid-state fermentation, improves the biochemical profile of PKC and enhances its potential for industrial alcohol production, demonstrating its viability as a low-cost, nutrient-rich substrate for bioethanol generation.

***Keywords*:** Palm kernel cake, Fermentation, *Rhizopus oligosporus*, *Saccharomyces cerevisiae*, Alcohol production

Introduction

The Arecaceae, commonly referred to as palms, are a family of monocot plants that resemble stemless trees and hold significant ecological and human value, particularly in tropical regions.[1,2] *Elaeis guineensis*, commonly known as the African oil palm, is considered one of the most agriculturally important palm species worldwide. It is indigenous to West Africa and has served as a semi-wild food resource for traditional communities for over 7,000 years. In its native regions, the oil palm remains crucial for local livelihoods and biodiversity.[3] Initially, cultivation was informal, primarily spread along the West and Central African coastal belt between Guinea/Liberia and Northern Angola.[4] The most productive cultivation zones are typically located in equatorial regions with high rainfall, between latitudes 7° N and 7° S. In recent decades, growing demand for biofuels has accelerated the need for plant-based oils such as palm oil, thereby increasing palm cultivation and oil production in countries like Nigeria.[5]

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Efficient utilization of by-products from palm oil extraction, such as empty fruit bunches, palm press fiber, and palm kernel cake (PKC), is essential for sustainable processing. PKC, a residue from palm kernel oil extraction, contains about 15% protein and is commonly used in animal feed. However, its application is limited by high fiber content and relatively low protein concentration, making it less suitable for high-performance animal diets like broiler feeds.[6,7]

PKC can be more effectively valorized through a biorefinery approach that transforms its carbohydrate content into valuable products such as ethanol.[8] Compared to other oilseed press cakes, PKC contains a high carbohydrate proportion (approximately 50%), with predominant sugars including glucose, galactose, and mannose.[9,10] These fermentable sugars can be utilized by the yeast *Saccharomyces cerevisiae* for ethanol production, with a theoretical yield of 0.51 g ethanol per gram of hexose. This approach offers dual advantages: producing ethanol and improving the nutritional composition of PKC by enhancing its protein content and reducing fiber, thereby increasing its utility as animal feed. Moreover, ethanol has been proposed as a sustainable alternative to methanol in the transesterification process during biodiesel production, reinforcing the economic feasibility of integrating ethanol and biodiesel production within the same biorefinery framework.[11]

Despite its potential, there is limited comparative research on optimizing PKC pre-treatment methods for industrial alcohol production. This study addresses that gap by evaluating the effects of three pre-treatment strategies—solid-state fermentation, autoclaving, and acid hydrolysis—on PKC's biochemical composition and alcohol yield during submerged fermentation with *S. cerevisiae*. The novelty

of this research lies in its integrative assessment of how specific pre-treatment techniques influence both fermentable sugar availability and the biochemical profile of PKC.

The research methods employed are particularly relevant, as they simulate industrially viable, cost-effective processes that can enhance PKC's fermentability and ethanol productivity. These include subjecting PKC to solid-state fermentation with *Rhizopus oligosporus*, acid hydrolysis, and autoclaving for a 72-hour period prior to fermentation with *S. cerevisiae* under submerged conditions. Biochemical parameters such as glucose, soluble protein, phenols, flavonoids, pH, and enzyme activity (mannanase and phytase) were monitored to understand substrate transformation. Nonetheless, further clarity is required to delineate the individual and synergistic effects of each pretreatment step on the observed biochemical modifications and ethanol yield. Moreover, the absence of comparative studies involving untreated or alternatively treated PKC limits the critical evaluation of the process's relative efficacy. Questions also remain concerning the scalability and economic viability of the approach for industrial-level bioethanol production. Addressing these aspects would significantly advance the development of this biotechnological strategy.

Materials and methods

Collection of Material and Starter Organism

The palm kernel cake used in this study was obtained from Songhai Delta Amukpe, Sapele, Nigeria (GPS coordinates: 5.8942° N, 5.6760° E) on 20th July 2024. It was milled before being stored at room temperature until further analysis. The *Rhizopus oligosporus* strains used in this study were originally provided by PT Aneka Fermentasi Industri, Bandung, Indonesia, and were obtained from the culture collection maintained at Harmony Path Ltd., Amukpe, Delta State, Nigeria. The identification and confirmation of the fungal strains were carried out by Professor Daniel A. Ehwarieme, Department of Microbiology, Delta State University, Abraka, Nigeria.

Pretreatment of PKC

To prepare the substrates, six treatment groups were established and labeled as follows based on the pretreatment conditions: A) Untreated PKC – Palm kernel cake (PKC) without any pretreatment, homogenized with 15 mL of 50 mM phosphate buffer (pH 6), serving as the control without microbial inoculation. B) Autoclaved PKC – 5 kg of PKC was homogenized with 15 mL of 50 mM phosphate buffer (pH 6) and autoclaved. C) Autoclaved PKC with 1N Sulfuric Acid – Prepared as in B, but the mixture was autoclaved in 4 L of water containing 80 mL of 1N sulfuric acid to aid acid hydrolysis. D) Solid-State Fermented (SSF) PKC – 5 kg of PKC was inoculated with 1 g of *Rhizopus oligosporus* (obtained from Tonukari Biotechnology Laboratory) and homogenized with 15 mL of 50 mM phosphate buffer (pH 6). E) SSF Autoclaved PKC – SSF PKC (as in D) was further subjected to autoclaving. F) SSF Autoclaved PKC with 1N Sulfuric Acid – SSF PKC (as in D) was autoclaved in 4 L of water containing 80 mL of 1N sulfuric acid. For each treatment, the mixtures were fermented under aerobic conditions for 72 hours at room temperature (~28 ± 2 °C). At the end of fermentation, 6 g aliquots were aseptically collected and homogenized using a sterile mortar and pestle. The homogenates were centrifuged at 5,000 rpm for 10 minutes, and the resulting supernatant was collected as the crude extract. Replicate crude extracts were prepared and stored at 4 °C for subsequent biochemical assays. The methodology employed for substrate preparation, solid-state fermentation, and crude extract recovery was validated and slightly modified from previously described methods.[12,13]

Enzyme Extraction Procedure

At the end of the 72-hour solid-state fermentation period, 20 mL of sterile distilled water was added to each flask containing the fermented PKC substrate. The contents were gently swirled to ensure uniform mixing, followed by agitation on a rotary shaker at 200 rpm for 30 minutes to enhance enzyme release. The mixtures were then filtered through Whatman No. 1 filter paper to remove solid residues. The filtrates obtained were used as crude enzyme extracts for enzymatic assays. This procedure was consistently applied to all treated PKC samples. Mannanase activity was determined using a modified protocol from Araujo and Ward[14]. Phytase activity was evaluated by quantifying the release of inorganic phosphate from sodium phytate, as described by Bogar et al.[15]

Preparation of pretreated substrate for alcohol production

Following pretreatment, the pretreated PKC underwent yeast fermentation using the technique outlined in Ogodo et al.[16] Specifically, 0.8 g/l of commercial baker's yeast (*S. cerevisiae*) was combined with 200 ml of the fermentation broth and stirred. The mixture was left to ferment for three days. Post-fermentation, 6 grams of pretreated PKC in yeast were gathered for further analysis. The aliquots were homogenized with a mortar and pestle, and 10 mL of distilled water was added to the resulting mixture. The mixture was then centrifuged for 10 min, and the supernatant was collected as crude extract. Replicate samples of crude extracts were prepared for subsequent assays.

Biochemical procedure for pretreated PKC in yeast fermentation for alcohol production

The total soluble protein (TSP) content of each sample was estimated at an absorbance reading of 540 nm using a UV-Visible spectrophotometer (Model: UV-1800, Shimadzu Corporation, Japan), with bovine serum albumin (BSA) as a reference standard, following the method of Gornall et al.[17]. Glucose concentration (expressed in mg/dL) was determined using a diagnostic glucose assay kit (Randox Laboratories Ltd., Crumlin, UK), based on glucose oxidase-peroxidase enzymatic reactions, as per the manufacturer's instructions. The pH of the test samples was measured using a Mettler Toledo FiveEasy™ pH meter (Mettler-Toledo GmbH, Switzerland). Total reducing sugar (TRS) was estimated using the dinitrosalicylic acid (DNS) method at 540 nm, as described by Miller[18]. Alpha-amylase activity was assayed at 540 nm using maltose as a standard reference, following the protocol of Nouadri et al.[19]

The antioxidant potential of each sample was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, in which absorbance was measured at 517 nm using a spectrophotometer (Model: UV-1800, Shimadzu Corporation, Japan), according to the method of Hatano et al.[20] Total phenolic content (TPC) was determined at 760 nm using the Folin–Ciocalteu method as described by Singleton and Rossi[21]. Total flavonoid content (TFC) was analyzed at 510 nm, following the colorimetric aluminum chloride method of Jia et al.[22] In addition, the ferric reducing antioxidant power (FRAP) assay was conducted to assess antioxidant potential based on the reduction of Fe^{3+} to Fe^{2+} at low pH, forming a blue-colored ferrous-tripyridyltriazine complex measured at 593 nm, following the protocol of Benzie and Strain[23]

Determination of total soluble solids

An Atago Master series handheld refractometer from Japan was utilized in this experiment. To standardize the refractometer, a single drop of distilled water was applied onto the prism. The refractometer was then positioned in such a way that sunlight could enter the prism, and the coarse and fine adjustments were properly adjusted. The eye-piece was used to observe the standardization process, as described in the AOAC[24] guidelines.

Specific Gravity

The percentage alcohol content was calculated using the following formula based on the specific gravity (SG) obtained (equation 1): $\% \text{ ABV} = \frac{(\text{Initial SG} - \text{Final SG})}{7.36 \times 1000}$ where the initial and final SG values were obtained by measuring 50 mL of the sample into a measuring cylinder at 20 °C and dipping a hydrometer into it to determine the specific gravity (with appropriate temperature correction factor), in accordance with the method described by Balogu and Towobola[25]

Results and Discussion

Figure 1A illustrates the outcomes of the analysis of the soluble protein concentration of various pre-treated palm kernel cake (PKC) samples during yeast fermentation. The findings indicate that the pre-treated PKC had significantly higher ($p < 0.05$) levels of soluble protein concentration compared to the untreated sample. Among the

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different pre-treatments, solid-state fermented PKC in yeast fermentation demonstrated the highest concentration of soluble protein.The increase in soluble protein concentration observed in the SSF-treated PKC during yeast fermentation can be attributed to the breakdown of the complex protein structures into simpler, more soluble forms.[26] This breakdown is carried out by the action of enzymes produced by the microorganisms during fermentation. These enzymes include proteases, which break down the peptide bonds between amino acids in proteins,

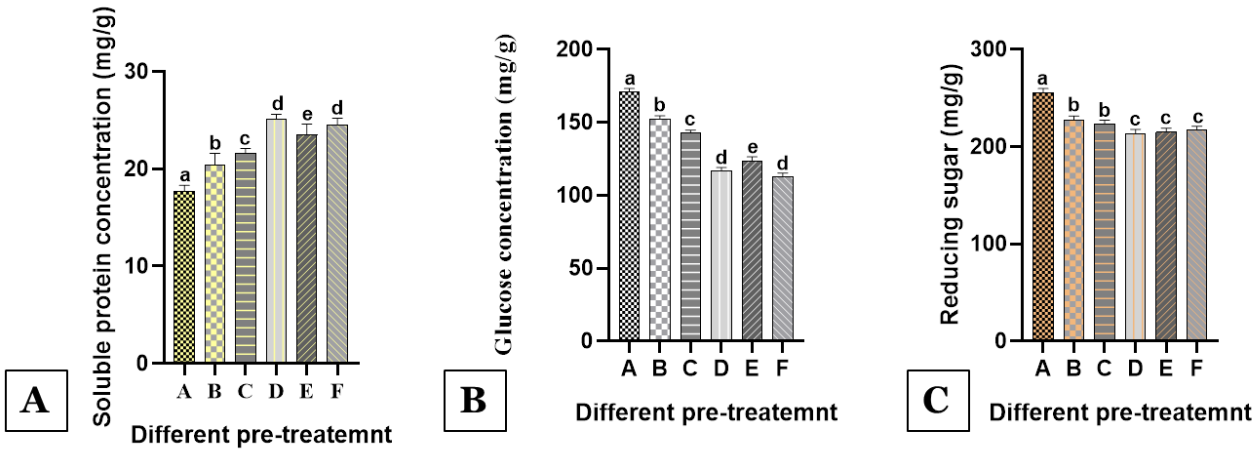

Figure 1: (A) Levels of soluble proteins, (B) Glucose concentration, and (C) Reducing sugar concentration at different pretreatments of PKC in submerged fermentation with *S. cerevisiae* for alcohol production. Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$). A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

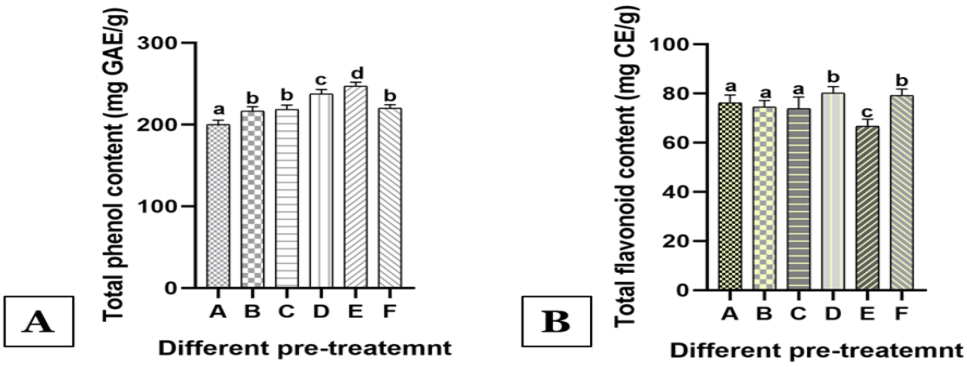

Figure 2: (A) Total phenolic content (TPC) and (B) Total flavonoid content (TFC) at different pretreatments of PKC in submerged fermentation with *S cerevisiae* for alcohol production. Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$). A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

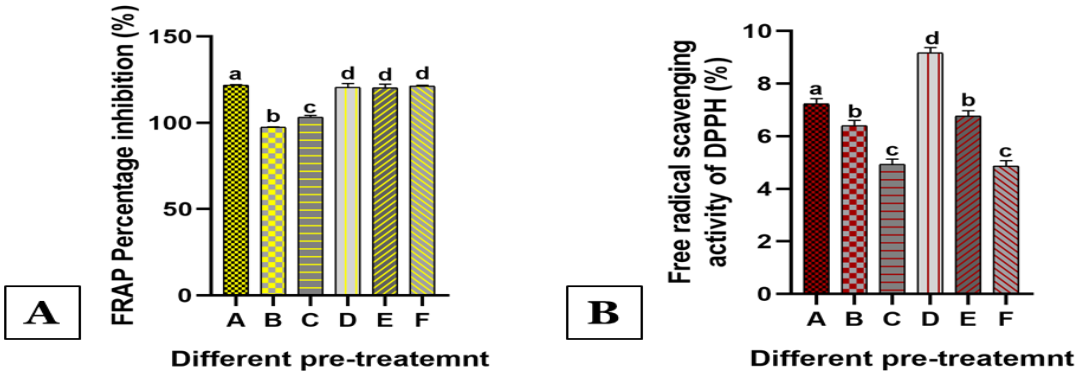

Figure 3: (A) FRAP percentage inhibition and (B) Free radical scavenging activity of DPPH (%) at different pretreatments of PKC in submerged fermentation with S cerevisiae for alcohol production. Values with superscripted letter a, b, c, d are significantly different

from the control ($p < 0.05$). A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

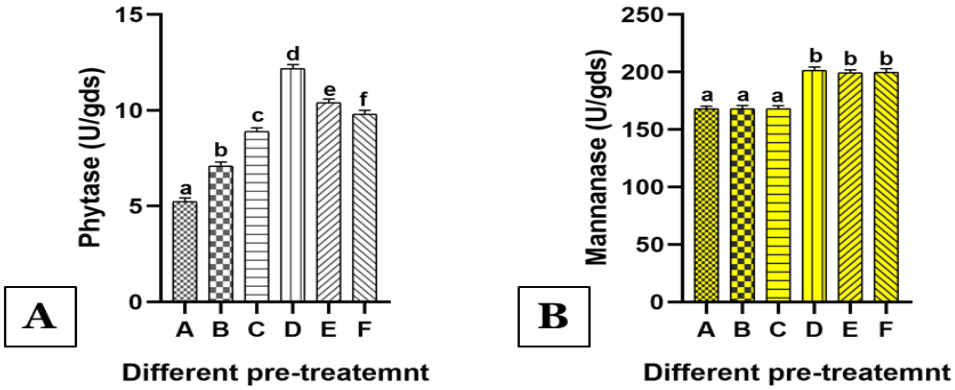

Figure 4: (A) Phytase and (B) Mannanase activity at different pretreatments of PKC in submerged fermentation with S cerevisiae for alcohol production. Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$). A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

leading to the release of soluble protein fragments.[27,28] Additionally, the microorganisms themselves can contribute to the increase in soluble protein concentration. Yeasts, for example, have been shown to synthesize and secrete proteins during fermentation, some of which are soluble and can contribute to the overall soluble protein concentration of the PKC.[29]

Figure 1B displays the outcomes of measuring glucose concentration for various pre-treatments of PKC during submerged fermentation with *S. cerevisiae* for producing alcohol. The results indicate a notable reduction ($p < 0.05$) in glucose concentration for the pre-treated PKC compared to the control group.

The pre-treatment of PKC likely caused the breakdown of complex carbohydrates into simpler forms, including glucose, which can be easily utilized by *S. cerevisiae* during submerged fermentation for alcohol production. This breakdown may have been caused by various mechanisms such as enzymatic hydrolysis or acid hydrolysis.[30] Furthermore, it is possible that the pre-treatment of PKC improved the accessibility of enzymes to the complex carbohydrates, thus enhancing the efficiency of enzymatic hydrolysis.[31] This could lead to a reduction in the glucose concentration of the pre-treated PKC compared to the control group. In addition, the use of *S. cerevisiae* during submerged fermentation can also contribute to the reduction of glucose concentration. This is because *S. cerevisiae* is known to efficiently metabolize glucose, consuming it as a carbon source for energy and alcohol production.[32] Overall, the observed reduction in glucose concentration in the pre-treated PKC during submerged fermentation using *S. cerevisiae* for alcohol production is likely due to a combination of enzymatic and microbial activity.

Figure 1C illustrates the findings of measuring the concentration of reducing sugar for various pre-treatments of PKC during submerged fermentation with *S. cerevisiae* for producing alcohol. The results demonstrate a noteworthy decrease ($p < 0.05$) in the concentration of reducing sugar for the pre-treated PKC in comparison to the untreated PKC group during submerged fermentation using *S. cerevisiae* for alcohol production.

The pre-treatment of PKC may have facilitated the breakdown of complex carbohydrates into simpler forms such as reducing sugars, which are readily utilized by *S. cerevisiae* during submerged fermentation for alcohol production. The reduction in the concentration of reducing sugars observed in the pre-treated PKC compared to the untreated PKC could be attributed to various mechanisms such as enzymatic hydrolysis or acid hydrolysis.[33]

The pre-treatment of PKC may have also enhanced the accessibility of enzymes to the complex carbohydrates, resulting in more efficient enzymatic hydrolysis and a decrease in the concentration of reducing sugars.[34] Additionally, during submerged fermentation, *S. cerevisiae* is known to metabolize reducing sugars, consuming them as a source of energy and producing alcohol as a byproduct.[35] Therefore, the observed reduction in the concentration of reducing sugars for the pre-treated PKC during submerged fermentation using *S. cerevisiae* for alcohol production may be due to both the pre-treatment method and the metabolism of reducing sugars by the yeast.

Figure 2A presents the outcomes of the assessment of total phenol concentration (TPC) for pre-treated PKC during submerged fermentation, where *S. cerevisiae* was utilized for alcohol production. The results indicate a noteworthy elevation ($p < 0.05$) in the phenol concentration of pre-treated PKC in the process of submerged fermentation with *S. cerevisiae* compared to the untreated PKC.

During the process of submerged fermentation, *S. cerevisiae* utilizes various compounds present in the pre-treated PKC for the production of alcohol.[36] These compounds may include phenolic compounds that are naturally present in the PKC, such as hydroxycinnamic acids and flavonoids.[36] These phenolic compounds may undergo various transformations during the fermentation process, leading to the formation of new phenolic compounds or the release of previously bound phenolic compounds.[37] These transformations are likely to have contributed to the observed increase in total phenol concentration (TPC) of the pre-treated PKC during submerged fermentation with *S. cerevisiae*.

Phenolic compounds are known to have antioxidant properties and can contribute to the flavor, color, and health benefits of fermented foods and beverages.[38] However, their presence in PKC may also have negative effects on the fermentation process, as they can inhibit the growth and activity of yeast cells.[39] Therefore, the increase in TPC observed in the pre-treated PKC during submerged fermentation with *S. cerevisiae* may have both positive and negative effects on the overall process of alcohol production.

The findings from figure 2B indicate a noteworthy elevation [statistically significant at $p<0.05$) in the total flavonoid concentration [TFC] of pre-treated PKC during submerged fermentation with *S. cerevisiae* for alcohol production when compared to the control.Flavonoids are a class of naturally occurring compounds found in various plants that have a wide range of biological activities, including antioxidant, anti-inflammatory, and anti-cancer effects.[40]

They are synthesized through the flavonoid biosynthesis pathway, which involves the conversion of phenylalanine into flavanones and then into flavones, flavonols, and other classes of flavonoids.[41] In the context of fermentation, flavonoids have been shown to have a positive impact on yeast growth and metabolism, possibly by acting as antioxidants and modulating signaling pathways involved in stress response.[42] Flavonoid-rich plant extracts have been used as supplements to enhance the productivity of various fermentation processes, including alcohol production.[43]

In the case of pre-treated PKC, it is possible that the pre-treatment method used in the study (specifically SSF) resulted in the release or activation of flavonoids or other compounds that could have promoted yeast growth and metabolism during fermentation, leading to the observed increase in TFC.[38] It is also possible that the pre-treatment method affected the expression of genes involved in flavonoid biosynthesis in the PKC, leading to a higher concentration of flavonoids in the starting material for fermentation.[44]

The findings displayed in figure 3A demonstrate that the percentage inhibition activity of FRAP in pre-treated PKC during submerged fermentation with *S. cerevisiae* for alcohol production exhibited a significant increase ($p < 0.05$) when compared to the control. On the other hand, Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid treatments resulted in a noteworthy decrease.

The increase in FRAP inhibition activity in pre-treated PKC may be attributed to the activation of enzymes and the release of bioactive compounds during the pre-treatment process. For example, it has been reported that pre-treatment of PKC with enzymes such as cellulase, xylanase, and protease can result in the release of phenolic compounds, which have been shown to have antioxidant properties.[45] Additionally, pre-treatment with acid can cause the breakdown of complex molecules, resulting in the release of bioactive compounds.[46]

On the other hand, the decrease in FRAP inhibition activity in Autoclaved PKC and Autoclaved PKC with 1N Sulfuric acid treatments may be due to the denaturation of enzymes and the breakdown of bioactive compounds during the treatments. High-temperature autoclaving can denature proteins and enzymes, rendering them inactive.[47] Similarly, treatment with sulfuric acid can break down bioactive compounds, rendering them ineffective.[48]

In figure 3B, the outcomes of the analysis conducted to determine the scavenging activity of free radicals using 1,1-diphenyl-2-picrylhydrazyl (DPPH) in pre-treated PKC during submerged fermentation with *S. cerevisiae* for alcohol production have been presented. The results indicate that the scavenging activity of free radicals in SSF PKC, SSF autoclaved PKC, and SSF Autoclaved PKC with 1N Sulfuric acid in submerged fermentation has improved when compared to the control. However, autoclaved PKC with 1N Sulfuric acid exhibited a significant decrease in scavenging activity.

The DPPH assay is a widely used method to assess the antioxidant activity of various samples, as it can determine the ability of antioxidants to scavenge free radicals.[49] The outcomes shown in figure 3B demonstrate that pre-treated PKC during submerged fermentation with *S. cerevisiae* for alcohol production had enhanced free radical scavenging activity in SSF PKC, SSF autoclaved PKC, and SSF Autoclaved PKC with 1N Sulfuric acid in comparison to the control. The improvement in scavenging activity can be attributed to the increased release of antioxidant compounds due to pre-treatment of PKC.[50] Pre-treatment of PKC may result in the release of more phenolic compounds, which are recognized to have powerful antioxidant activities.[51] In contrast, autoclaved PKC with 1N Sulfuric acid resulted in a significant reduction in scavenging activity. The acidic conditions and the high temperature during autoclaving may have led to the destruction of these antioxidant compounds.[52]

The results presented in Figure 4A indicate that the phytase activity of PKC during the submerged fermentation process with *S. cerevisiae* for alcohol production was significantly higher ($p < 0.05$) compared to the phytase activity of untreated PKC. The use of phytase enzymes to degrade phytic acid has been shown to improve the bioavailability of these essential minerals.[53] During submerged fermentation with *S. cerevisiae* for alcohol production, the microorganisms present in the fermentation broth can also produce phytase, which can further contribute to the hydrolysis of phytic acid. The increase in phytase activity observed in pre-treated PKC can be attributed to the increased bioavailability of phytase and the potential synergistic effect of phytase produced by the microorganisms.[54] Studies have consistently demonstrated that pre-treating palm kernel cake (PKC) through autoclaving can result in a greater availability of phosphorus and other crucial minerals, which are vital for the growth and development of animals.[55] This effect is attributed to the heightened activity of phytase in pre-treated PKC, as previously reported in research.[56] Autoclaving is believed to enhance the bioavailability of phytase in PKC by breaking down its cell wall and protein matrix, leading to an increase in enzymatic activity.[57]In the study, it was observed that the mannanase activity of pre-treated PKC increased significantly ($p < 0.05$) during the submerged fermentation process with *S. cerevisiae* for alcohol production (Figure 4B). The increase in mannanase activity was found to be significant when compared to the mannanase activity of untreated PKC. Mannanases are enzymes that hydrolyze the beta-1,4 linkage in the backbone of mannan polysaccharides, liberating mannan oligosaccharides as well as mannose as a monosaccharide unit.[58] In the case of PKC, it is known that mannan is one of the principal hemicellulose components of the cell wall. Submerged fermentation process with *S. cerevisiae*, a type of yeast, has been shown to be an effective method for pre-treating PKC.[59] During this process, enzymes such as mannanases are released from the yeast and can potentially act on the cell wall of PKC, leading to an increase in mannanase activity. This could be due to the fact that mannanases are also produced by yeast during fermentation and may act on the cell wall of PKC, thereby increasing the release of mannan oligosaccharides.[60]

Previous studies have reported an increase in mannanase activity when PKC is pre-treated with different microorganisms, including bacteria and fungi, through solid-state fermentation or submerged fermentation processes.[61,62] The increased mannanase activity could potentially increase the release of mannan oligosaccharides, which can have beneficial effects on animal health and nutrition.[63]

The changes in physicochemical parameters of pre-treated PKC during submerged fermentation with *S. cerevisiae* for alcohol production were presented in Table 1.0. The pre-treated PKC samples exhibited a significant increase in pH throughout the fermentation period (24-72

Table 1: Physiochemical changes pre-treated PKC in submerged fermentation with *S. cerevisiae* for alcohol production for 72 hours

Various pre-treatments	pH (Initial)	pH (24 hrs)	pH (48 hrs)	pH (72 hrs)	Total soluble solids (Initial)	Total soluble solids (24 hrs)	Total soluble solids (48 hrs)	Total soluble solids (72hrs)	Specific gravity (Initial)	Specific gravity (24 hrs)	Specific gravity (48 hrs)	Specific gravity (72 hrs)
A	6.70	7.30	7.80	8.40	2.10	1.20	1.10	1.20	1.19	1.03	1.03	1.03
B	7.70	8.30	8.60	8.90	2.40	1.70	1.60	1.40	1.25	1.04	1.03	1.01
C	4.10	7.20	7.80	8.80	1.90	1.10	1.10	1.10	1.06	1.03	1.03	1.03

D	4.60	7.20	7.90	8.90	2.90	1.20	1.20	1.20	4.16	4.01	3.92	3.74
E	3.10	6.70	6.80	8.80	1.10	1.30	1.00	1.30	2.04	1.93	1.91	1.87
F	3.10	6.50	7.30	8.30	1.60	1.10	1.10	1.10	1.15	1.03	1.01	1.00

A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

hours). Moreover, a notable reduction in total soluble solids was observed post fermentation. Of note, the specific gravity of the SSF PKC in yeast fermentation was found to be the highest (Figure 5). Additionally, a considerable improvement in the percentage of alcohol in the SSF PKC in yeast fermentation was observed.

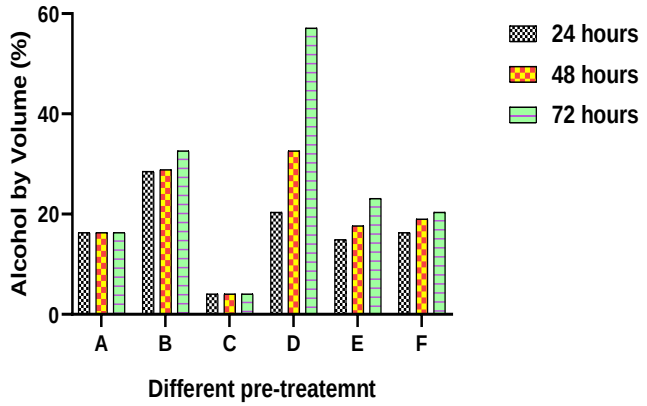

Figure 5: Alcohol percentage by volume at different pretreatments of PKC in submerged fermentation with *S. cerevisiae* for alcohol production. Values with superscripted letter a, b, c, d are significantly different from the control ($p < 0.05$). A) Untreated PKC, B) Autoclaved PKC, C) Autoclaved PKC with 1N Sulfuric acid, D) SSF PKC, E) SSF Autoclaved PKC, F) SSF Autoclaved PKC with 1N Sulfuric acid

During the process of submerged fermentation, *S. cerevisiae* metabolizes the sugars present in PKC to produce alcohol. As a result of this process, there are several physicochemical changes that occur. The increase in pH observed in pre-treated PKC during fermentation can be attributed to the production of ammonia and other alkaline metabolites by yeast, which act as buffering agents and increase the pH of the medium.[64] The reduction in total soluble solids can be attributed to the consumption of sugars by yeast during fermentation. The specific gravity of the SSF PKC in yeast fermentation is a measure of the density of the medium and can be affected by factors such as the concentration of dissolved solids, the amount of yeast biomass, and the production of ethanol.[65,66]

The considerable improvement in the percentage of alcohol in the SSF PKC in yeast fermentation observed in this study can be attributed to the higher concentration of fermentable sugars present in pre-treated PKC, which can be utilized by yeast to produce more alcohol.[67,68] The increase in mannanase and phytase activity observed in pre-treated PKC during fermentation can also play a role in improving the availability of sugars for yeast to ferment, by breaking down complex carbohydrates present in PKC to simpler sugars.[69]

Conclusion

Pre-treatment of PKC with SSF using yeast fermentation can increase soluble protein concentration through the breakdown of complex protein structures and synthesis of soluble proteins. During submerged fermentation with *Saccharomyces cerevisiae* for alcohol production, there is a reduction in glucose concentration due to enzymatic and microbial activity, resulting in a decrease in the concentration of reducing sugars. The increase in mannanase and phytase activity observed in pre-treated PKC during fermentation can improve the availability of sugars for yeast by breaking down complex carbohydrates into simpler sugars. This research opens new avenues for the development of value-added feed ingredients from agro-industrial by-products. Further studies may focus on optimizing fermentation parameters for large-scale application, exploring additional microbial strains for enhanced enzymatic activity, and evaluating the bioavailability and performance effects of the treated PKC in animal feeding trials.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Cosiaux A, Gardiner LM, Stauffer FW, Bachman SP, Sonké B, Baker WJ. Low extinction risk for an important plant resource: conservation assessments of continental African palms (Arecaceae/Palmae). Biol. Conserv. 2018;221:323–333.
2. Murphy DJ, Goggin K, Paterson RRM. Oil palm in the 2020s and beyond: challenges and solutions. CABI Agric. Biosci. 2021;2(1):1–22.
3. Runtuboi YY, Permadi DB, Sahide MAK, Maryudi A. Oil palm plantations, forest conservation and indigenous peoples in West Papua Province: what lies ahead? For. Soc. 2021;5(1):23–31.
4. Ejigbo A, Adejo E, Omada MO, Akoh TA, Tukura TP, Abdullahi S. The Potentials of the Alloma Oil Palm Project in Improving the Socioeconomic Fortunes of Kogi State. Int. J. Glob. Aff. Res. Dev. 2024;2(1):72–83.
5. Lam MK, Tan KT, Lee KT, Mohamed AR. Malaysian palm oil: Surviving the food versus fuel dispute for a sustainable future. Renew. Sustain. Energy Rev. 2009;13(6–7):1456–1464.
6. Egbune EO, Tonukari NJ. Fermented mixture of cassava roots and palm kernel cake can substitute for maize in poultry feed formulation. Afr. J. Bioch. Res., 2023;*17*(1), 1-8.
7. Hakim AH, Zulkifli I, Farjam AS, Awad EA. Feeding fermented palm kernel cake with higher levels of dietary fat improved gut bacterial population and blood lipid concentration but not the growth performance in broiler chickens. Ital. J. Anim. Sci. 2021;20(1):1671–1680.
8. Rahim NA, Indera LAA, Abdul PM, Jahim JM, Bukhari NA. Towards Sustainable Production of Bio-based Lactic Acid via a Bio-based Technical Route: Recent Developments and the Use of Palm Kernel Cakes in the Bioconversion. BioResour. 2022;17(2).
9. Abedini A, Alizadeh AM, Mahdavi A, Golzan SA, Salimi M, Tajdar-Oranj B, Hosseini H. Oilseed cakes in the food industry; a review on applications, challenges, and future perspectives. Curr. Nutr. Food Sci. 2022;18(4):345–362.
10. Egbune EO, Egbune OU, Ezedom T, Dennis-Eboh U, Eraga LI, Alexander IB, Tonukari NJ. Exploring Biochemical Parameters and Enzyme Profiles in Yeast-Assisted Fermentation of Biofortified Palm Kernel Cake. Ind. Biotech., 2024;20(5), 204-211.
11. Batlle EAO, Palacio JCE, Lora EES, Bortoni EDC, Nogueira LAH, Caballero GEC, Escorcia YC. Energy, economic, and environmental assessment of the integrated production of palm oil biodiesel and sugarcane ethanol. J. Clean. Prod. 2021;311:127638.

12. Ughe FO, Egbune EO, Anigboro AA, Tonukari NJ. Assessment of biochemical potential of Rhizopus oligosporus as starter culture to enhance the nutritional value of palm kernel cake for animal feed formulation. J. Appl. Sci. Environ. Manage., 2024;28(9), 2887-2903.
13. Egbune EO, Tonukari NJ. Fermented mixture of cassava roots and palm kernel cake can substitute for maize in poultry feed formulation. Afr. J. Biochem. Res., 2023;17(1), 1-8.
14. Araujo A, Ward OP. Extracellular mannanases and galactanases from selected fungi. J. Ind. Microbiol., 1990;6, 171-178.
15. Bogar B, Szakaes G, Linden JC, Pandey A, Tengerdy RP. Optimization of phytase production by solid-state fermentation. J. Microbiol. Biotechnol., 2003;30(3), 183-189.
16. Ogodo AC, Ugbogu OC, Agwaranze DI, Ezeonu NG. Production and Evaluation of Fruit Wine from *Mangifera indica* (cv Peter). Appl. Microbiol. 2018;4:144.
17. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 1949;177(2):751–756.
18. Miller GL. Use of the Dinitrosalicylic Acid Reagent for the Determination of Reducing Sugar. Anal. Chem. 1959;31:426–428.
19. Nouadri T, Meraihi Z, Shahrazes DD, Leila B. Purification and characterization of amylase isolated from *Penicillium camemberti* PL21. Afr. J. Biochem. Res. 2010;4(6):155–162.
20. Hatano T, Kagawa H, Yasuhara T, Okuda T. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging affects. Chem. Pharm. Bull. 1988;36:2090–97.
21. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Vitic. 1965;16:144–158.
22. Jia Z, Tang M, Wu J. The determination of flavonoid contents of mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64:555–559.
23. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem., 1996;239(1), 70-76.
24. AOAC. Off. Methods Anal. 15th ed. Arlington, VA, 1990.
25. Balogu TV, Towobola O. Production and quality analysis of wine from honey and coconut milk blend using *Saccharomyces cerevisiae*. Fermentation 2017;3(2):16.
26. Wu D, Tu M, Wang Z, Wu C, Yu C, Battino M, Du M. Biological and conventional food processing modifications on food proteins: Structure, functionality, and bioactivity. Biotechnol. Adv. 2020;40:107491.
27. Anigboro AA, Aganbi E, Tonukari NJ. Solid state fermentation of maize (Zea mays) offal by *Rhizopus oligosporus* under acidic and basic conditions. J. Sci. Res., 2020;12(4), 751-756.
28. Egbune EO, Avwioroko OJ, Orororo OC, Egbune OU, Aganbi E, Anigboro AA, Tonukari NJ. *Rhizopus oligosporus* alkaline protease in cassava fermentation: Characterization and detergent potential. Biocat. Agri. Biotech., 2023;*54*, 102954.
29. Tan Y, Chen L, Li K, Lou B, Liu Y, Liu Z. Yeast as carrier for drug delivery and vaccine construction. J. Control. Release 2022;346:358–379.
30. Ohwode JO, Egbune EO, Dennis-Eboh U, Ezedom T, Orororo OC, Anigboro AA, Tonukari NJ Biochemical evaluation of pre-treated unpeeled cassava roots (Manihot esculenta Crantz) and its application in alcohol production. Niger. J. Sci. Env., 2023;21(2).
31. Verardi A, Lopresto CG, Blasi A, Chakraborty S, Calabrò V. Bioconversion of lignocellulosic biomass to bioethanol and biobutanol. In: Lignocellul. Biomass Liq. Biofuels, 2020;67–125.
32. Rusli ND, Ghani AAA, Mat K, Yusof MT, Zamri-Saad M, Hassim HA. The potential of pretreated oil palm frond in enhancing rumen degradability and growth performance: A review. Adv. Anim. Vet. Sci., 2021.
33. Sun L, Jin YS. Xylose assimilation for the efficient production of biofuels and chemicals by engineered *Saccharomyces cerevisiae*. Biotechnol. J., 2021;16(4), 2000142.
34. Zhang R, Li XY, Cen XL, Gao QH, Zhang M, Li KY, Huang ZX. Enzymatic preparation of manno-oligosaccharides from locust bean gum and palm kernel cake, and investigations into its prebiotic activity. Electron. J. Biotechnol., 2021;49, 64–71.
35. Constantino A, Rodrigues B, Leon R, Barros R, Raposo S. Alternative chemo-enzymatic hydrolysis strategy applied to different microalgae species for bioethanol production. Algal Res., 2021;56, 102329.
36. Martiniano SE, Philippini RR, Franco-Marcelino PR, da Silva SS. Effect of selenium uptake on growth metabolism in yeasts for the production of enriched single-cell protein using agro-industrial by-products. Biomass Convers. Biorefin., 2020;1–9.
37. Egbune EO, Ezedom T, Odeghe OB, Orororo OC, Egbune OU, Ehwarieme AD, Tonukari NJ. Solid-state fermentation production of L-lysine by *Corynebacterium glutamicum* (ATCC 13032) using agricultural by-products as substrate. World Journal of Microbiol. Biotechnol., 2024;40(1), 20.
38. Rocchetti G, Gregorio RP, Lorenzo JM, Barba FJ, Oliveira PG, Prieto MA, Lucini L. Functional implications of bound phenolic compounds and phenolics–food interaction: A review. Compr. Rev. Food Sci. Food Saf., 2022;21(2), 811–842.
39. Adebo OA, Gabriela MI. Impact of fermentation on the phenolic compounds and antioxidant activity of whole cereal grains: A mini review. *Molecules*, 2020;25(4), 927.
40. Asri NM, Muhialdin BJ, Zarei M, Saari N. Low molecular weight peptides generated from palm kernel cake via solid state lacto-fermentation extend the shelf life of bread. LWT, 2020;134, 110206.
41. Roy A, Khan A, Ahmad I, Alghamdi S, Rajab BS, Babalghith AO, Islam M. Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. Biomed. Res. Int., 2022;5445291.
42. Ferreyra MLF, Serra P, Casati P. Recent advances on the roles of flavonoids as plant protective molecules after UV and high light exposure. Physiol. Plant., 2021;173(3), 736–749.
43. Aganbi E, Egbune EO, Orororo OC, Ezedom T, Egbune OU, Tonukari NJ. Effect of microbial cell-size on solid state fermentation of cowpea (Vigna unguiculata L. Walp) and groundnut (Arachis hypogaea L.) by *Rhizopus oligosporus*. J. App. Sci. Environm. Manag., 2023;27(6), 1093-1103.
44. Li Y, Fang L, Xue F, Mao S, Xiong B, Ma Z, Jiang L. Effects of bamboo leaf extract on the production performance, rumen fermentation parameters, and rumen bacterial communities of heat-stressed dairy cows. Anim. Biosci., 2021;34(11), 1784.
45. Ożarowski M, Karpiński TM. Extracts and flavonoids of *Passiflora* species as promising anti-inflammatory and antioxidant substances. Curr. Pharm. Des., 2021;27(22), 2582–2604.
46. Cebrián M, Ibarruri J. Filamentous fungi processing by solid-state fermentation. In: Curr. Dev. Biotechnol. Bioeng., 2023;251–292.
47. Krakowska-Sieprawska A, Kiełbasa A, Rafińska K, Ligor M, Buszewski B. Modern Methods of Pre-Treatment of Plant material for the extraction of bioactive compounds. Molecules, 2022;27(3), 730.
48. Rasane P, Sharma N, Fatma S, Kaur S, Jha A, Kaur D, Singh J. Ultra-high temperature (UHT) processing: Technological significance and updates. Curr. Nutr. Food Sci., 2020;16(8), 1183–1195.
49. Lahiri D, Dash S, Dutta R, Nag M. Elucidating the effect of anti-biofilm activity of bioactive compounds extracted from plants. J. Biosci., 2019;44(2), 52.
50. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative

phytochemical analysis of *Ficus religiosa*. Molecules, 2022;27(4), 1326.
51. Barteková M, Adameová A, Görbe A, Ferenczyová K, Pecháňová O, Lazou A, Giricz Z. Natural and synthetic antioxidants targeting cardiac oxidative stress and redox signaling in cardiometabolic diseases. Free Radic. Biol. Med., 2021;169, 446–477.
52. Saji N, Francis N, Schwarz LJ, Blanchard CL, Santhakumar AB. The antioxidant and anti-inflammatory properties of rice bran phenolic extracts. Foods, 2020;9(6), 829.
53. Kataria A, Sharma S, Dar BN. Changes in phenolic compounds, antioxidant potential and antinutritional factors of Teff (*Eragrostis tef*) during different thermal processing methods. Int. J. Food Sci. Technol., 2022;57(11), 6893–6902.
54. Kumar A, Singh B, Raigond P, Sahu C, Mishra UN, Sharma S, Lal MK. Phytic acid: Blessing in disguise, a prime compound required for both plant and human nutrition. Food Res. Int., 2021;142, 110193.
55. Dailin DJ, Hanapi SZ, Elsayed EA, Sukmawati D, Azelee NIW, Eyahmalay J, El Enshasy H. Fungal Phytases: Biotechnological applications in food and feed industries. Recent Adv. White Biotechnol. Fungi, 2019;2:65–99.
56. Tamreihao K, Mukherjee S, Khunjamayum R, Devi LJ, Asem RS, Ningthoujam DS. Feather degradation by keratinolytic bacteria and biofertilizing potential for sustainable agricultural production. J. Basic Microbiol., 2019;59(1):4–13.
57. Saji N, Francis N, Schwarz LJ, Blanchard CL, Santhakumar AB. Rice bran derived bioactive compounds modulate risk factors of cardiovascular disease and type 2 diabetes mellitus: An updated review. Nutrients, 2019;11(11):2736.
58. Wiesinger JA, Marsolais F, Glahn RP. Health implications and nutrient bioavailability of bioactive compounds in dry beans and other pulses. Dry Beans Pulses: Prod. Process. Nutr., 2022;505–529.
59. Lindstad LJ, Lo G, Leivers S, Lu Z, Michalak L, Pereira GV, La Rosa SL. Human gut *Faecalibacterium prausnitzii* deploys a highly efficient conserved system to cross-feed on β-mannan-derived oligosaccharides. mBio, 2021;12(3):e03628-20.
60. Yaashikaa PR, Kumar PS, Varjani S. Valorization of agro-industrial wastes for biorefinery process and circular bioeconomy: A critical review. Bioresour. Technol., 2022;343:126126.
61. Gomez-Osorio LM, Nielsen JU, Martens HJ, Wimmer R. Upgrading the Nutritional Value of PKC Using a Bacillus subtilis Derived Monocomponent β-Mannanase. Molecules, 2022;27(2):563.
62. Akula S, Golla N. Significance of process parameters on fungal cellulase production. Biofuel Prod. Technol.: Crit. Anal. Sustain., 2020;299–324.
63. Hlalukana N, Magengelele M, Malgas S, Pletschke BI. Enzymatic conversion of mannan-rich plant waste biomass into prebiotic mannooligosaccharides. Foods, 2021;10(9):2010.
64. Sathitkowitchai W, Sathapondecha P, Angthong P, Srimarut Y, Malila Y, Nakkongkam W, Rungrassamee W. Isolation and Characterization of Mannanase-Producing Bacteria for Potential Synbiotic Application in Shrimp Farming. Animals, 2022;12(19):2583.
65. Van Leemputte F, Vanthienen W, Wijnants S, Van Zeebroeck G, Thevelein JM. Aberrant intracellular pH regulation limiting glyceraldehyde-3-phosphate dehydrogenase activity in the glucose-sensitive yeast tps1Δ mutant. mBio, 2020;11(5):e02199-20.
66. Tse TJ, Wiens DJ, Reaney MJ. Production of bioethanol—A review of factors affecting ethanol yield. Fermentation, 2021;7(4):268.
67. López-Sandin I, Zavala-García F, Levin L, Ruiz HA, Hernández-Luna CE, Gutiérrez-Soto G. Evaluation of bioethanol production from sweet sorghum variety Roger under different tillage and fertilizer treatments. BioEnergy Res., 2021;14(4):1058–1069.
68. Diyanilla R, Hamidon TS, Suryanegara L, Hussin MH. Overview of pretreatment methods employed on oil palm biomass in producing value-added products: A review. BioResources, 2020;15(4):9935.
69. Verma N, Kumar V, Bansal MC. Valorization of waste biomass in fermentative production of cellulases: A review. *Waste Biomass Valor.*, 2021;12:613–640. Abdullah JJ, Greetham D, Du C, Tucker GA. Viability of municipal solid waste as a source for bioenergy products production. Int. J. Environ. Agric. Biotechnol., 2020;5(2).