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Synergistic effects of ultrasonication and microbial fermentation on crystallinity and chemical composition of cellulose from palm fruit fiber

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ABSTRACT

The combine use of ultrasonication and microbial fermentation represents a promising strategy for modifying the structural properties of cellulose derived from agricultural residues. This study investigated the synergistic effect of ultrasonication followed by fermentation with *Aspergillus niger* and *Saccharomyces cerevisiae* on the crystallinity index (CrI) and chemical composition of cellulose obtained from palm fruit fiber. Cellulose extracted from palm fruit fiber was subjected to ultrasonication and subsequently fermented using the selected microorganisms. Structural changes were analysed using X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR), while chemical composition was evaluated through one-way ANOVA ($p < 0.05$). The treatments significantly influenced cellulose crystallinity and chemical composition. Ultrasonication followed by *A. niger* fermentation resulted in the highest CrI (55.6%), whereas fermentation with *S. cerevisiae* produced the lowest CrI (39.4%). The results suggest that *A. niger* preferentially degrades amorphous cellulose regions, increasing relative crystallinity, while *S. cerevisiae* promotes greater structural disruption. Both microorganisms increased protein content and reduced dietary fiber levels. These findings demonstrate that combined physical and biological treatments can effectively modify cellulose structure, potentially improving its functionality for food and feed applications.

Keywords: cellulose, combined treatment, crystallinity, fermentation, FTIR, ultrasonication.

INTRODUCTION

Palm fruit fiber is an abundant lignocellulosic by-product generated during palm oil processing and represents a promising raw material for the production of value-added cellulose-based materials. The global palm oil industry generates substantial amounts of lignocellulosic residues each year, creating both environmental challenges and opportunities for biomass valorisation. Cellulose, an ordered polysaccharide composed of β-1,4-linked glucose units, has attached significant interest for applications in food systems, pharmaceuticals, and bioprocessing due to its renewability, biodegradability, and functional properties. The structure of cellulose consists of both crystallinity and amorphous regions, which are commonly quantified by the crystallinity index (CrI). This parameter strongly influences the physicochemical behaviour of cellulose, including its resistance to hydrolysis, enzymatic accessibility, and suitability for applications such as prebiotics or functional biomaterials [Chatterjee et al., 2015]. Consequently, strategies that modify cellulose crystallinity are important for improving its reactivity and functional performance [Dungani et al., 2016]. In this context, combined pretreatment strategies have been proposed to disrupt biomass architecture, enhance accessibility, and alter physicochemical properties such as crystallinity and functional group exposure [Nguyen et al., 2017].

Physical pretreatment methods, particularly ultrasonication, have been widely investigated as environmentally friendly technologies for the modification of lignocellulosic biomass. Ultrasonication generates acoustic cavitation, producing localized high pressure and shear forces that can disrupt the lignin-cellulose matrix, increase surface area, and improve substrate accessibility for further processing steps [Gavrila et al., 2024]. In parallel, biological pretreatment using microbial fermentation offers a selective and mild strategy for modifying cellulose structure through the enzymatic degradation of amorphous regions by cellulases and hemicellulases [Data, 2024]. Such process can alter cellulose crystallinity and modify its chemical composition [Suchova et al., 2022]. Filamentous fungi such as *Aspergillus niger* are well known producers of cellulolytic and hemicellulolytic enzymes capable of degrading structural polysaccharides [Ofongo et al., 2019]. In contrast, *Saccharomyces cerevisiae* primarily metabolizes soluble sugars generated during biomass hydrolysis and can influence fermentation dynamics and biochemical conversion efficiency [Thapa et al., 2020].

Integrating ultrasonication with biological fermentation may provide a complementary strategy in which mechanical disruption by ultrasound enhances cellulose accessibility, thereby facilitating enzymatic degradation during microbial fermentation. Such synergistic interactions could potentially modify cellulose crystallinity more efficiently than individual treatments alone [Junior et al., 2025]. Although combined pretreatment strategies have attached increasing attention in biomass valorisation research, studies specifically examining the interaction between ultrasonication and microbial fermentation for modifying the crystallinity of cellulose derived from palm fruit fiber remain scarce.

Previous studies have primarily investigated ultrasonication or microbial fermentation as independent treatments for modifying cellulose structure [Chen et al., 2024; Baksi et al., 2023]. In most cases, research has focused on enhancing reducing sugar yields or improving delignification efficiency rather than examining structural changes in cellulose crystallinity [Rizal et al., 2018; Ilyas et al., 2025]. Consequently, the influence of combined physical disruption (ultrasonication) and biological degradation through microbial fermentation on the crystallinity index (CrI) of cellulose remains poorly understood. A deeper understanding of CrI modification is essential because crystallinity strongly influences enzymatic accessibility, hydrolysis efficiency, and the potential functionality of cellulose in food and feed applications.

To address these limitations, the present study investigates the combined effects of ultrasonication followed by microbial fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae* on the crystallinity index and chemical composition of cellulose extracted from palm fruit fiber. Particular attention is given to potential changes in cellulose structure and physicochemical characteristics resulting from the interaction between physical disruption and microbial activity. Structural and compositional modifications are evaluated through analyses of chemical composition, crystallinity, and functional group profiles using analytical techniques such as X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR). This approach aims to provide deeper insight into how combined physical and biological treatments influence cellulose structure in lignocellulosic biomass. Therefore, this study aims to investigate the combined effects of ultrasonication and microbial fermentation on the crystallinity index and chemical characteristics of cellulose derived from palm fruit fiber. We hypothesize that ultrasonication enhances the accessibility of cellulose fibers, thereby promoting microbial enzymatic during fermentation and leading to significant changes in crystallinity and chemical composition. By comparing fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae*, the study seeks to elucidate how different microbial systems influence cellulose structural modification.

MATERIALS AND METHODS

This research was conducted in the Agricultural Product Chemistry Laboratory, Agroindustry Waste Processing Laboratory, Agricultural Microbiology Laboratory, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia, 34145, from June 2025 to December 2025.

Materials

Palm fruit fibre (PFF) used was from PTPN VII (Unit Bekri, Terbanggi Besar, Lampung, Indonesia). The collected PFF was stored under refrigeration prior to use for analysis. The pre-treatment products were shown as follows in Figure 1.

Saccharomyces cerevisiae (FNCC 3210) and *Aspergillus niger* (FNCC 6018) were procured from Biotechnology Lab, Universitas Gadjah Mada-Yogyakarta, Potato Dextrose Agar (Himedia, GM096), Nutrient Broth (Oxoid), Nutrient Agar (Himedia, M001), Malt Extract Agar (Merck, Germany), Malt Extract Broth (Merck, Germany), chemicals for extraction process, chemicals for analysis of lignocellulose, protein, dietary fiber, and reducing sugar. Analytical grade chemicals including sodium hydroxide (NaOH, Sigma-Aldrich, Cat. No. 221465), sulfuric acid (H_2SO_4, Merck,Cat. No. 100731), and hydrogen peroxide (H_2O_2, Merck, Cat. No. 107209) were purchased from Merck/Sigma-Aldrich and used without further purification. All of the chemicals used were analytical standard and purchased in IndoKimia, Bandar Lampung, Indonesia.

Experimental design and statistical analysis

The study involved the preparation of PFF flour, followed by ultrasonication, and fermentation treatments. A complete randomized design (CRD) was applied, with treatment combination and fermentation cultures as the experimental factors. The first factor was ultrasonication treatment, and the second factor was type of microorganism used in fermentation. The cultures used *were Aspergillus niger* and *Saccharomyces cerevisiae*. The treatments consisted of:

- Sample 1: cellulose + ultrasonication + fermentation with *S.cerevisiae.*
- Sample 2: cellulose + ultrasonication
- Sample 3: cellulose + ultrasonication + fermentation *A. niger*.
- Untreated cellulose was as control.

All treatments were performed in four replications. XRD data and functional group analysis (FTIR) were the average of three measurements per sample with standard deviation, and presented descriptively. Other parameters, including cellulose, protein, reducing sugar, and dietary fiber, were analysed using one-way ANOVA, followed by the Least Significant Difference (LSD) test at a 5% significance level. Statistical analyses were conducted using SPSS software (IBM SPSS Statistics version 25.0, IBM Corp, Armonk, NY, USA)

Extraction of cellulose from palm fruit fiber

Palm fruit fiber (PFF) extraction commenced with the preparation of PFF flour in accordance with the procedure carried out Kustyawati et al. [2024] as followed: 500 grams of palm fruit fiber were washed, drained for 30 min, cut into 5 cm pieces, and oven-dried at 105 °C for 3 h. The dried sample was then grounded to 80 mesh. The flour sample was then extracted through the delignification, bleaching, and acid hydrolysis. Delignification was done by heating the flour in 10% w/v NaOH (2M) at 80–85 °C for 1 hour, with solid-to-liquid ratio of 1:10. After that, it was sieved and washed with water to neutralize the pH from 9.0 to 6.5–7.0, and then was sun dried. The next step was bleaching carried out by heating the flour in a 10% b/v H_2O_2 at 85–90 °C for 1.5 hours, with solid-to liquid ratio of 1:15. After bleaching, the flour was repeatedly washed with distilled water until neutral pH was achieved (from 3.0 to 6.5–7.0), and then it was sun dried again. Following step was hydrolysis done by heating the dried flour in 2% H_2SO_4 at 115 °C for 2 hours. The suspension was mixed periodically every 10 min to ensure uniform contact between the acid solution with the fiber substrate. Following hydrolysis, the sample was separated and neutralized through repeated washing with distilled water to obtain the cellulose, which was then dried and stored in an airtight container at -5 °C until further use.

Ultrasonication treatment

The extracted cellulose was dispersed in distilled water at ratio of 1:20 (w/v) prior to sonication. Ultrasonication was performed using a probe-type

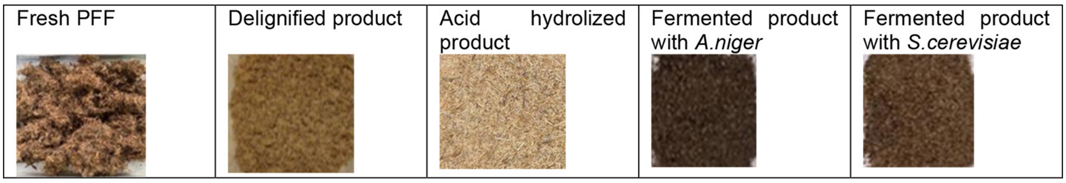

Figure 1. The pre-treatment products

ultrasonic processor equipped with a CL-18 converter (QSonica, USA), operating at a frequency of 20 kHz with a 13 mm titanium probe. The treatment was conducted at 45% amplitude with a maximum power output of 750 W, and using a pulse mode of 8 s ON and 5 s OFF to prevent excessive heating during the process. Each treatment was processed for 60 min at room temperature, following conditions established in preliminary studies. After treatment, the suspension was filtered, and the sample was oven-dried in a laboratory drying oven (Memmert UN55 Universal Oven, Germany) at 50 °C for 24 h or until constant weight was achieved. The samples were stored in an airtight container at -5 °C until further use.

Activation of *Aspergillus niger* and *Saccharomyces cerevisiae*

S. cerevisiae and *A. niger* were activated prior to their use in the fermentation experiment. Activation of *A. niger* was performed by streaking one inoculation loop onto a Petri dish containing 17 mL of potato dextrose agar (PDA), followed by incubation at 30 °C for 5 days. The culture was considered active when fungal growth and sporulation covered the entire surface of the agar plate, with the density of 10^8 cell/mL, quantifying through the pour plate counting technique.

Similarly, *S. cerevisiae* was inoculated into a test tube containing malt extract (ME) broth and incubated at 27 °C until visible growth occurred; a cloudy appearance of the medium, with the density of 10^8 cfu/mL quantifying through the pour plate counting method, indicated successful activation. All activated cultures were stored at 4 °C in a refrigerator until further use. Prior to the main fermentation process, one loop of each activated culture was transferred into separate test tubes containing ME broth for *S. cerevisiae* and potato dextrose (PD) broth for *A. niger*, each supplemented with 1 g of fermented lignin-free PFF product. This pre-culturing step was conducted to acclimate the microorganisms to the fermentation substrate. The inoculated test tubes were incubated at 30 °C for 24 h before being used as inoculum in the Erlenmeyer flask fermentation.

Fermentation process

Following sonication, the treated material was sterilized (at 121 °C, 15 min, 1 atm) and subjected to solid state fermentation under limited moisture conditions. Fermentation was performed aerobically in 250 mL Erlenmeyer flasks (Pyrex) plugged with sterile cotton to allow air exchange. Eight Erlenmeyer flasks were prepared, each containing 15 g of sonicated cellulose, 50 mL distilled water, and supplemented with nutrient consisting of 0.3 g of yeast extract, 0.05 g of $MgSO_4$, and 0.05 g of KH_2PO_4. The flasks were then sterilized at 121 °C, 15 min and 1 atm. After cooling, a total of 2.5 ml of pre-culture inoculum of *A. niger* and *S.cerevisiae* with the density of 10^8 cell/g and 10^8 cfu/mL respectively (as described previously) was added to each flask following the treatments. The flasks were incubated in a shaking incubator at 150 rpm, at 30 °C for 7 days and at 27 °C for 48 h for *A. niger* and *S. cerevisiae* respectively.

The fermented sonicated cellulose was subsequently analysed for cellulose, hemicellulose, lignin, protein, reducing sugar, dietary fibre, functional group (FTIR), and crystallinity index (XRD). All treatments were conducted in triplicate.

Chemical analysis

Protein content was determined using the Kjeldahl method, which consists of three stages: oxidation, distillation, and titration described by Association of Official Analytical Chemist Official method 984.13. Approximately 0.5–1.0 g of dried sample was weighed into a Kjeldahl digestion tube. Then 10 mL of concentrated H_2SO_4 and a catalyst mixture consisting of K_2SO_4 and $CUSO_4$ were added to accelerate digestion. The mixture was digested at 380–420 °C until a clear solution was obtained, indicating the conversion of organic nitrogen into ammonium sulfate. After cooling, the digest was diluted with distilled water (about 50 mL) and transferred to a distillation unit. Subsequently, 40% NaOH was added to liberate ammonia from the ammonium sulfate. The released ammonia was distilled and captured in 4% boric acid solution containing mixed indicator (methyl red and bromocresol green). The absorbed ammonia was titrated with standard HCl solution (0.1 N) until the endpoint color changed from green to pink. All analyses were performed in triplicate, including reagent blanks. Nitrogen content was calculated using the following equation:

$$\% N = \frac{(V_s - Vb) \times N \times 14.007 \times 100}{W \times 1000} \tag{1}$$

where: Vs – volume of HCl used for sample titration (mL), Vb – volume of HCl used for blank titration (mL), N – normality of HCl, 14.007 – atomic weight of nitrogen, W – weight of sample (g).

The crude protein was calculated using the nitrogen conversion factor:

$$Protein\ (\%) = \%\ N \times 6.25 \tag{2}$$

where: 6.25 is the commonly used nitrogen-to-protein conversion factor for food and biomass samples.

Analysis of total dietary fiber was conducted using the gravimetric method according to AOAC 992.16. [Ferreira et al., 2018] with slight modification. The sample was subjected to sequential enzymatic digestion using α-amylase, protease, and amyloglucosidase, followed by filtration and gravimetric determination of the residue. Briefly, approximately 1 g of dried sample was accurately weighed and placed into a digestion flask. The sample was first treated with heat-stable α-amylase in phosphate buffer (pH 6.0) and incubated at 95–100 °C for 30 min with continuous agitation to hydrolyse starch. After cooling to 60 °C, protease enzyme was added and the mixture was incubated for 30 min to digest proteins. Subsequently, the pH was adjusted to 4.5, and amyloglucosidase was added, followed by incubation at 60 °C for 30 min to convert remaining starch into glucose. The enzymatically digested mixture was then filtered through a pre-weighed crucible containing filter aid (Celite). The residue was washed sequentially with hot distilled water, 95% ethanol, and acetone to remove soluble components. The crucible containing the residue was dried in an oven at 105 °C until constant weight was achieved, cooled in a desiccator, and weighed. Total Dietary Fiber was calculated gravimetrically using the following equation:

$$Total\ Dietary\ Fiber\ (\%) = \frac{(Wr - Wb)}{Ws} \times 100 \tag{3}$$

where: Wr – weight of crucial + dried residue (g),
Wb – weight of blank residue (g),
Ws – weight of sample (g).

Reducing sugar content was analyzed using the colorimetric method with 3,5-dinitrosalicylic acid (DNS) reagent. Absorbance was measured using a UV-Vis Spectrophotometer (Thermo Fisher SCIENTIFIC, Model G10S) at a wavelength (λ) of 540 nm following the standard procedure implemented at the Agriculture Product Chemistry Laboratory, Faculty of Agriculture. Briefly, one mL of sample was mixed with 1 mL of DNS reagent in a test tube. The mixture was vortexed and heated in a boiling water bath (95–100 °C) for 10 min to allow the reduction of DNS by reducing sugars, forming a reddish-orange complex. After heating, the solution was cooled to room temperature and diluted with 8 mL of distilled water. The absorbance was measured at 540 nm using a UV-Vis spectrophotometer against a reagent blank. A glucose standard solution (0–1 mg mL^{-1}) was used to construct a calibration curve. Reducing sugar concentration was calculated from a glucose standard curve:

$$Y = aX + b \tag{4}$$

where: Y – absorbance at 540 nm, X – concentration of reducing sugar (mg mL^{-1}),
a – slope of calibration curve, b – intercept

Thus, the reducing sugar concentration was calculated as:

$$X = \frac{(Y - b)}{a} \tag{5}$$

If dilution was applied, the final concentration was calculated as:

$$Reducing\ sugar\ (mg/mL) = \frac{(Y - b)}{a} \times DF \tag{6}$$

where: DF is the dilution factor.

The method showed good linearity within the range of 0-1 mg mL^{-1} glucose with a correlation coefficient (R^2) > 0.99. The limit of detection (LOD) and limit of quantification (LOQ) were estimated based on the standard deviation of the response and the slope of the calibration curve:

$$LOD = \frac{3.3 \times SD}{a} \tag{7}$$

$$LOQ = \frac{10 \times SD}{a} \tag{8}$$

where: SD is the standard deviation of the blank response and a is the slope of the calibration curve.

Lignocellulosic components (NDF, ADF, and ADL) were determined using a fiber analyzer ANKOM 200 Fiber Analyzer, ANKOM Technology, USA). Approximately 0.5–1.0 g of dried sample was placed in filter bags and digested at 100 °C with controlled agitation (65 rpm). The instrument automatically performed digestion, rinsing, and filtration steps according to the Van Soest detergent fiber method (Van Soest, 1963), using the following equations:

$$Lignin\ (\%) = \\ = \frac{d - e}{sample\ weight\ (a)} \times 100\% \tag{9}$$

$$Cellulose\ (\%) = \%\ ADF - \\ - \%\ insoluble\ ash - \%\ lignin \tag{10}$$

$$Hemicellulose = \%\ NDF - \%\ ADF \tag{11}$$

where: NDF – neutral detergent fiber (cellulose + hemicellulose + lignin), ADF – acid detergent fiber (cellulose + lignin), ADL – acid detergent lignin (lignin).

X-ray diffraction analysis

The crystalline structure of the cellulose was analysed using X-ray diffraction (XRD) (Bruker D8 Advance X-ray Diffractometer, Germany). Diffraction patterns were recorded using X-ray diffractometer with Cu Kα radiation (λ= 1.54060 Å), operated at 40 kV and 30 mA. The data were collected in the 2θ range of 5–100° with a step size of 0.01° under continuous scanning mode. The scan step time was set at 10.16 s per step. Measurements were performed at room temperature (25 °C) using a fixed divergence slit with a divergence slit size of 1°. The goniometer radius was 240 mm, and the specimen length was 10 mm. Pressed powder was used as sample preparation. The crystallinity index (CrI) was calculated using the Segal method based on the intensity of the crystalline (002) peak at 2θ≈22–23° and the minimum intensity of the amorphous region at 2θ≈18°.

FTIR analysis

Fourier transform infrared (FTIR) spectroscopy (PerkinElmer Spectrum IR Version 10.6.1) was employed to evaluate functional group structural changes in cellulose induced by sonication and fermentation. FTIR spectra were using an ATR-FTIR spectrometer in the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. Prior to analysis, cellulose samples were dried in a hot air oven at 50–60 °C for 24 h to remove moisture. The dried samples were then ground using a laboratory grinder and sieved to obtain a uniform particle size of approximately 60–100 mesh (150–250 µm) to ensure homogenous spectral acquisition. The prepared powder was directly analysed using the ATR-FTIR technique, and spectra were recorded in the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans. The acquired spectra were baseline-corrected and normalized before further interpretation.

RESULTS AND DISCUSSIONS

Effect of the treatments on XRD analysis

The X-ray diffraction pattern of all samples exhibited characteristic diffraction peaks at 2θ≈22–23° and 2θ≈18° corresponding to the crystalline structure and amorphous structure, respectively. Table 1 showed the crystallinity index (CrI), calculated using Segal method, decreased progressively from sample 3 (55.6%), sample 2 (44.4%) to sample1 (39.4%), indicating an increase in the amorphous fraction of cellulose, and decrease in crystallinity index. This structural modification suggests that the applied treatments effectively disrupted the ordered crystalline domains, which may enhance enzymatic accessibility and improve the functional properties of cellulose as food or feed materials [Stanciu et al., 2024]. The higher CrI observed in cellulose subjected to combined sonication and fermentation with *A. niger* can be attributed to the selective degradation of amorphous regions. Sonication increased cellulose accessibility by disrupting the fiber structure, thereby facilitating enzymatic attack. During fermentation, cellulolytic enzymes preferentially hydrolyse the amorphous domains, resulting in a relative enrichment of crystalline regions and consequently a higher CrI value. In contrast, sonication alone caused non-selective structural disruption, while fermentation with *S. cerevisiae*, a non-cellulolytic microorganism, mainly induced structural disorder without significant removal of amorphous components, leading to lower CrI value [Fang and Catchmark, 2014].

These finding demonstrated that changes in CrI are governed not only by structural disruption but also by the selective removal of

Table 1. XRD parameters and crystallinity index (CrI) of cellulose under various treatments

Treatment	Peak (2θ, °)	I_{002} (counts)	I_{am} (counts)	Method	CrI (%)
Sample 1: cellulose+sonication+fermentation *S.cerevisiae*	22-23	~3550	~2150	Segal	39.4
Sample 2: cellulose+sonication	22-23	~4050	~2250	Segal	44.4
Sample 3: cellulose+sonication+fermentation *A.niger*	22-23	~5400	~2400	Segal	55.6

amorphous cellulose during enzymatic fermentation. In contrast to our findings, the crystallinity index of cellulose chemically extracted from empty palm fruit bunches reached 72%, indicating degradation of the amorphous structure and transformation into a crystalline structure [Al-Rajabi and Haan, 2022].

Effect of the treatments on FTIR profiles

FTIR spectra of all cellulose samples exhibited characteristic absorption bands corresponding to cellulose functional groups, including the broad O-H stretching vibration at 3330–3410 cm^{-1} and the strong C-O stretching band at 1050–1060 cm^{-1}. Differences in band intensity and sharpness among treatments indicate structural modifications induced by sonication and fermentation (Table 2).

The cellulose fermented with *S. cerevisiae* (Sample 1, blue line, Figure 2) exhibited the lowest crystallinity index (CrI =39.4%, Table 1), indicating a highly disordered structure. The FTIR spectrum showed weakened crystalline-related bands at 1425 and 1322 cm^{-1}, accompanied by a broad O-H stretching band at 3334 cm^{-1} and a noticeable absorption at approximately 1600 cm^{-1} associated with bound water in amorphous regions. As *S. cerevisiae* is non-cellulolytic microorganisms [Kustyawati, 2018], the observed decrease in CrI is mainly attributed to structural rearrangements, fiber sweeling, and hydrogen-bond weakening rather than selective removal of amorphous cellulose. These effects promote increased amorphicity, leading to a lower overall crystallinity. In another study, fermentation using *S. cerevisiae* in simultaneous saccharification fermentation (SSF) preceded by alkali pretreatment was shown to increase the conversion efficiency of coffee husk lignocellulose into bioethanol [Azmi et al., 2025].

The FTIR spectrum of sonicated cellulose (Sample 2, red line, Figure 2) is consistent with the reduced crystallinity index (CrI = 44.4%) obtained from XRD analysis. The relatively strong absorption band at 898 cm^{-1}, associated with amorphous β-glycosidic linkages, together with the moderate intensity of the crystalline band at 1424 cm^{-1}, indicates an increase in amorphous regions. Sonication induces acoustic cavitation that disrupts intermolecular hydrogen bonding and distorts the ordered cellulose structure in a non-selective manner, leading to partial transformation of crystalline domains into amorphous ones.

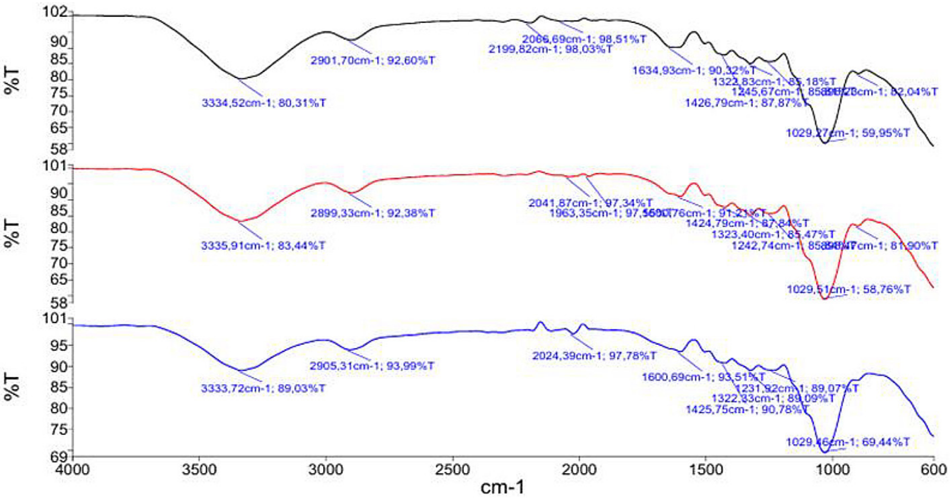

Figure 2. FTIR performance of cellulose+sonication+fermentation with *A.niger* (sample 3/black), cellulose+sonication (sample 2/red), cellulose+ sonication+fermentation *S.cerevisiae* (sample 1/blue)

Table 2. FTIR absorption bands of cellulose samples and the structural interpretation

Wavenumber (cm^{-1})	Transmittance	Structural assignment	Sample-1	Sample-2	Sample-3
3330–3410	Broad, medium-high	O-H stretching (intra-and inter,olecular hydrogen bonding in cellulose	Broad	Less broad	Sharper
2890–2920	Medium	C-H stretching of aliphatic-CH and -CH2 groups	Present	Present	Present
1730–1740	Weak/absent	C=O stretching of hemicellulose or residual lignin	Weak	Very weak	Absent
1640–1650	Weak	O-H bending of absorbed water	present	reduced	Minimal
1430–1445	Medium	CH2 symmetric bending (crystallinity-related band)	Low	moderate	Higher
1370–1380	Medium	C-H bending vibration of cellulose	Present	Present	Present
1315–1325	Weak-medium	O-H in-plane bending (associated with crystalline cellulose i)	Weak	Weak	Clearer
1160–1165	Medium	Asymetric C-O-C stretching (-1,4-glycosidic linkage)	Present	Present	Strong
1050–1060	Strong	C-O stretching of pyranose ring	Strong	Strong	Very strong
897–900	Medium	-glycosidic linkage (amorphous cellulose indicator)	Clear	Increased	Reduced

Note: Sources [Oh et al., 2005; Sun et al. 2021]. Sample 1 is cellulose+fermentation with *S.cerevisiae*, Sampel 2 is cellulose+ sonication, Sampel 3 is cellulose+sonication+fermentation with *A.niger*.

The broad O-H stretching band at 3336 cm^{-1}, further reflects weakened hydrogen bonding, supporting the observed decrease in crystallinity. These results confirm that sonication alone primarily increases cellulose amorphicity, resulting in a moderate decrease in crystallinity compared to enzymatic fermentation-assisted treatments.

The FTIR spectrum of cellulose subjected to combined sonication and *A. niger* fermentation is in consistent with the high crystallinity index (CrI 55.6%, Table 1, Figure 2, Sample 3, black line) supports the XRD findings. The presence of a distinct absorption band at 1426 cm^{-1}, associated with CH$_2$ bending vibrations of crystallinity cellulose, together with a moderate intensity of the amorphous-related band at 896 cm^{-1}, indicate a relative enrichment of crystalline regions. The O-H stretching band at 3334 cm^{-1}, suggests a more ordered hydrogen-bonding network, while the moderate absorption at 1634 cm^{-1}, reflects residual bound water associated with amorphous domains. These results confirm that the high CrI is primarily due to selective degradation of amorphous regions during combined sonication and *A. niger* fermentation, as *A. niger* is cellulolytic microorganisms.

Functional group analysis of cellulose by Yuniar et al. [2025] revealed that delignification treatment produced prominent absorption peak only at 1508 and 1415 cm^{-1}, associated with hydroxyl (OH) groups which suggest that lignin removal exposed greater number of OH groups in the cellulose. In line with these structural modifications, Sul'man et al. [2010] reported that probe-type ultrasonication at 30 kHz applied to raw lignocellulosic biomass lowered the crystallinity index (CrI) and improves the recovery of cellulose more susceptible to enzymatic hydrolysis. However, Olughu et al. [2023] demonstrated that ultrasonication effect on enzymatic hydrolysis efficiency was relatively modest compared to fungal pretreatment.

Interpretation of chemical analysis

Table 3 showed that the treatments significantly affected cellulose, protein, and dietary fiber. Different superscript letters indicate significant differences ($p < 0.05$).

Protein contents of the fermentation treatment were significantly different from the cellulose as a control, and sonication alone. The approximately tenfold increase in protein after fermentation indicates the contribution of microbial biomass (single-cell protein) and extracellular enzyme production. Sonication alone does not significantly increase protein because it only causes physical changes. The protein value in *S. cerevisiae* is slightly higher than in *A. niger*, possibly because yeast biomass growth is more dominant even though its cellulose degradation activity is lower than that of mold. The cell wall of *S. cerevisiae* consists of mannoprotein (approximately 40% of the dry mass of the cell), b-glucan (approximately

Table 3. Chemical analysis

Treatments	Cellulose (%)	Reducing sugar (%)	Protein (%)	Dietary fiber (%)
Cellulose	33.92 ± 0.06	30.61 ± 0.1	0.82 ± 0.21	50.7
Cellulose+sonication+ fermentation *S.cerevisiae* (sample-1)	17.07 ± 0.00^{a}	35.28 ± 0.1^{a}	12.83 ± 0.54^{a}	38.54^{a}
Cellulose+sonication (sample-2)	47.64 ± 0.01^{b}	31.06 ± 0.2^{b}	1.28 ± 0.21^{b}	42.68^{b}
Cellulose+sonication+ fermentation *A.niger* (sample-3)	19.83 ± 0.02^{c}	41.43 ± 0.1^{c}	12.25 ± 0.35^{c}	36.57^{a}

Note: Data were mean values of four replicates ± standard deviation. Numbers followed by different superscript in the same column indicated non-significant difference ($p<0.05$).

60% of the dry mass of the cell wall), and N-acetylglucosamine polymers (approximately 2% of the dry mass of the cell wall), of which 40–60% of the polymers serve as a source of protein [Martin and Chan, 2024].

The decrease in dietary fiber indicates the conversion of complex polysaccharides into simpler and more soluble fractions. The combination of sonication + *A. niger* fermentation produced the lowest dietary fiber (36.57%), indicating more effective cellulase and hemicellulase activity. Although the protein in *S. cerevisiae* is higher, dietary fiber degradation is lower than in *A. niger*, because yeast does not produce significant amounts of cellulase.

Relationship between cellulose content, reducing sugar, CrI, and FTIR characteristics

Cellulose and reducing sugar contents varied significantly among treatments, reflecting different structural and enzymatic effects. Ultrasonication alone (Sample-2) produced the highest cellulose contents (47%) but moderate CrI (44.4%). Indicating mainly physical disruption of lignocellulose without substantial crystalline reorganization. FTIR supported this, showing crystallinity bands (1430–1445 and 1317 cm^{-1}) with moderate intensity and a pronounced amorphous band (~897 cm^{-1}), consistent with crystallinity indicators reported by Marchwicka et al. [2025].

Combined sonication and fermentation with *A. niger* (sample-3) resulted in lower cellulose (19%) but the highest CrI (55.5%) and the highest reducing sugar (41.43%). This indicates selective degradation of amorphous regions by cellulolytic enzymes, enriching crystalline domains. FTIR confirmed stronger crystallinity bands and a weaker amorphous signal. Similar finding was reported by Dixid and Sukha [2023].

In contrast, sonication followed by *S. cerevisiae* fermentation (Sample-1) showed the lowest CrI (39.4%), cellulose level, and high reducing sugar (35.28%), indicating extensive depolymerization. FTIR revealed a dominant amorphous band (~897 cm^{-1}), consistent with reduced crystallinity due to hydrogen bond disruption as described by Poletto et al. [2014].

Overall, sonication mainly enhanced cellulose release, while *A. niger* selectively removed amorphous fractions, increasing CrI, and *S. cerevisiae* promoted broader cellulose depolymerization, lowering CrI.

CONCLUSIONS

This study demonstrated that combination of ultrasonication and microbial fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae* effectively modified the crystallinity and chemical composition of cellulose derived from palm fruit fiber. Ultrasonication disrupted the lignocellulosic structure, while fermentation further enhanced biochemical transformation, resulting in changes in cellulose content, reducing sugar, and other chemical components. The findings in the study fills an important gap in the valorisation of palm oil biomass by linking structural modification with compositional changes. Moreover, these findings are also highlighting the potential of palm fruit fiber as a sustainable source of value-added cellulose, and provide a promising strategy for the efficient utilization of agro-industrial residues in food, feed, and biotechnological applications.

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REFERENCES

1. Al-Rajabi M.M., and Haan T.Y., (2022). Green extraction method of cellulose fibers from oil palm empty fruit bunches. *Jurnal Kejuruteraan, 34*(5), 851–860. https://doi.org/10.17576/jkukm-2022-34(5)-12.
2. Anggoro D.D., Prasetyaningrum A., Udaibah W., Nabilah F., LeMonde B.U., (2024). Effect of ultrasound-advanced oxidation processes for pretreatment of oil palm mesocarp fiber for cellulose extraction. *International Journal Renewable Energy Development, 13*(3). https://doi.org/10.61435/ijred.2024.60027
3. Azmi H., Octaviani N.F., Musa'adah., (2026). Inovasi produksi bioetanol berbasis lignoselulosa limbah kulit kopi (*Coffea* sp.) dengan konsep biorefinery menggunakan saccha romyces cerevisiae. *Current Biochemistry, 12*(1). https://doi.org/10.29244/cb.12.1.3
4. Baksi S., Saha D., Sarkar U., Basu D., Kuniyal J.C., (2023). Pre-treatment of lignocellulosic biomass: review of various physico-chemical and biological methods influencing the extent of biomass depolymerization. *International Journal of Environmental Science and Technology*, https://doi.org/10.1007/s13762-023-04838-4
5. Chatterjee S., Sharma S., Prasat R.K., et al., (2015). Cellulase enzyme based biodegradation of cellulosic materials: an overview. *Sout Asian Journal, 5*(6), 271–282. http://doi.org/10.38150/sajeb.5(6).p271-282
6. Chen W. L., Liang J. B., Jahromi M. F., Abdullah N., Ho Y. W., Tufarelli V., (2024). Enzyme treatment enhances release of prebiotic oligosaccharides from palm kernel expeller. *BioResources, 10*(1). https://doi.org/10.15376/biores.10.1.196-209
7. Data, Rahul., (2024). Enzymatic degradation of cellulose in soil: A review. *Heliyon, 10*(2024), e24022. https://doi.org/10.1016/j.heliyon.2024.e24022
8. Dixit M., Shuka P., (2023). Multi-efficient endoglucanase from Aspergillus niger MPS25 and its potential applications in saccharification of wheat straw and waste paper deinking. *Chemoshere, 313*(2023), 137298. https://doi.org/10.1016/j.chemosphere.2022.137298
9. Dungani R., Owolabi A.F., Saurabh C., Khalil H.P.S.A., et al., (2016). Preparation and fundamental characterization of cellulose nanocrystal from oil palm fronds biomass. *J Polym Environ.* https://doi.org/10.1007/s10924-016-0854-8
10. Fang L., Catchmark J.M., (2014). Structure characterization of native celluloseduring dehydration and rehydration. *Cellulose, 21*, 3951–3963. http://doi.org/10.1007/s10570-014-0435-8
11. Ferreira K C., Bento J.A.C., deJesus L. S., Bassinello Z., (2018). Dietary fibers: analysis methods. *Multidisciplinary Journal, 5*(3), 174–179. https://doi.org/10.29247/2358-260X.2018v5i3.p174-179
12. Gavrila A.I., Vartolomei A., Calinescu I., Vinatoru M., et al., (2024). Ultrasound Assisted Alkaline Pretreatment of Biomass to Enhance the Extraction Yield of Valuable Chemicals. *Agronomy.* http://doi.org/10.20944/preprints202404.0583.v1
13. Ilyas R.A., Sapuan S.M., Ishak M.R., Zainudin E.S., (2017). Effect of delignification on the physical, thermal, chemical, and structural properties of sugar palm fibre. *BioResources, 12*(4), 8734–8754.
14. Junior D.B.S., Kelbert M., deAraujo P.H.H., deAndrade C.J., et al., (2025). Application of physical pretreatment of lignocellulosic biomass to produce fermentable sugars. *Sustain. Chem. 6*(2), 13. https://doi.org//10.20944/preprints202501.0170.v1
15. Kustyawati M. E., Fadhallah E. G., Hidayati S., Pramesti A., Hidayat L., (2024). The potential of oil palm mesocarp fiber waste as a prebiotic material – chemical and microbial evaluation using *Probiotic Saccharomyces cerevisiae, Lactobacillus casei* and *Escherichia coli*. *Ecological Engineering & Environmental Technology, 25*(10), 339–346. https://doi.org/10.12912/27197050/191799
16. Kustyawati M.E., (2018). *Saccharomyces cerevisiae: Metabolit dan agensia modifikasi Pangan.* Graha Ilmu Publishing.
17. Marchwicka M., Vybohova E., and Radomski A., (2025). Ultrasound-induced structural modification of cellulose in poplar wood: effects on crystallinity and enzymatic hydrolysis efficiency. *Materials, 18*(22), 5156. https://doi.org/10.3390/ma18225156
18. Martin G.J.O., and Chan S., (2024). Future production of yeast biomass for sustainable proteins: a critical review. *Sustainable Food Technology, 2*(6), 1585–1772. https://doi.org/10.1039/d4fb00164h
19. Nguyen T.Y., Cai C.M., Kumar R., and Wyman C.E., (2017). Overcoming factors limiting high-solids fermentation of lignocellulosic biomass to ethanol. *PNAS, 114*(44), 11673–11678. https://pnas.org/cgi/doi/10.1073/pnas.1704652114
20. Ofongo R.T.S., Ohimain E.I., and Iyayi E.A., (2019). Cellulase and hemicellulase activity under submerged fermentation of rice mill feed by fungi. *International Journal of Environment, Agriculture and Biotechnology (IJEAB), 4*(1), 233–238. http://dx.doi.org/10.22161/ijeab/4.1.35
21. Oh S. Y., Yoo D. I., Shin Y., Seo G., (2005). FTIR analysis of cellulose treated with sodium hydroxide and carbon dioxide. *Carbohydrate Research, 340*, 2376–2391.
22. Olughu O.O., Tabil L.G., Dumonceaux T., Mupondwa E., Cree D., (2023). Synergistic effect of ultrasonic and fungal pretreatment on enzymatic sacchari cation of switchgrass. *Research Square*. https://orcid.org/0000-0002-8435-2366

23. Poletto M., Zattera A.J., Santana R.M.C., (2014). Structural differences between wood species by FTIR. *Materials Research*, *17*, 1065–1072.
24. Rizal N.F.A.A., Ibrahim M.F., Zakaria M.F., et al., (2018). Pre-treatment of oil palm biomass for fermentable sugars production. *Molecules, 23*(6), 1381. https://doi.org/10.3390/molecules23061381
25. Stanciu M-C., Tanasa F., and Teaca C-A., (2024). Crystallinity changes in modified cellulose substrates evidenced by spectral and x-ray diffraction data. *Polysaccharides, 2025,* 6, 30. https://doi.org/10.3390/polysaccharides6020030
26. Suchova K., Feher C., Ravn J. L., Bedo S., Biely P., Geijer C. (2022). Cellulose- and xylan-degrading yeasts: Enzymes, applications and biotechnological potential. *Biotechnol. Adv. 59*, 107981. https://doi.org/10.1016/j.biotechadv.2022.107981
27. Sun L., Lu X., Xue Y., Wang X., Yu Z., Du X., Zhang R., Xiong J., (2021). Production of fermentable sugars by hydrolysis of cellulose using biomass-derived and recyclable 2,5-furandicarboxylic acid in green solvent system. *SSRN Electronic Journal.* https://doi.org/10.2139/ssrn.3951010
28. Sul'man E.M., Sul'man M.G., and Prutenskaya E.A., (2011). Effect of ultrasonic pretreatment on the composition of lignocellulosic material in biotechnological processes. *Biocatalysis, 3*(1), 28–33. https://doi.org/10.1134/S2070050411010120
29. Thapa S., Mishra J., Arora N., Mishra P., Li H., et al., (2020). Microbial cellulolytic enzymes: diversity and biotechnology with reference to lignocellulosic biomass degradation. *Rev Environ Sci Biotechnol*. https://doi.org/10.1007/s11157-020-09536-y
30. Van Soest P.J. (1963). Use of detergents in the analysis of fibrous feeds, II. A rapid method for the determination of fiber and lignin. *Journal of the Association of Official Agricultural Chemist, 46*(5), 829–835.
31. Yuniar R.A., Purwati E., Samudera B.B., Hidayat J.P., (2025). Efektivitas delignifikasi limbah mesokarp kelapa sawit untuk meningkatkan potensi selulosa. *Jurnal Serambi Engineering, x*(1), 11602–11608.