EVALUATION OF CHEMO-MECHANICAL METHODS FOR EXTRACTING CELLULOSE FROM WASTE CABBAGE (*BRASSICA OLERACEA* VAR. *CAPITATA* L.) TRIMMINGS

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This study investigates the cellulose yield and purity of waste cabbage (*Brassica oleracea* var. *capitata* L.) trimmings obtained through three extraction methods: (a) alkali hydrolysis and bleaching treatment with high shear homogenization (AHBH), (b) alkali hydrolysis with ultrasonication (AHU), and (c) chemical extraction with mechanical assistance (CEMA). The cellulose yield varied significantly among the methods, with AHBH yielding the highest at 7.11%, followed by CEMA at 6.56%, and AHU at the lowest at 5.08%. Analysis of α-cellulose content revealed AHU's purity at 63%, attributed to acid hydrolysis and sonication facilitating higher α-cellulose purity. In contrast, AHBH and CEMA exhibited lower purity at 58% and 55%, respectively. Morphological analysis indicated distinct characteristics for each method: alkaline treatment in AHBH caused disintegration of cellulose fibers, AHU's ultrasonication led to fibrillation, and CEMA displayed densely packed irregular fibers, suggesting potential residual hemicelluloses and lignin. These findings highlight the significant impact of extraction techniques on cellulose yield, purity, and structure.

Keywords: cellulose extraction, cellulose yield, cellulose purity, α-cellulose, hemicellulose, lignin

INTRODUCTION

A pressing environmental concern worldwide is posed by agricultural waste. According to the Food and Agriculture Organization of the United Nations, a staggering 30% of the global food production intended for human consumption is wasted annually, with a total of 1.3 billion tons. These losses signify a significant waste of resources.[1]

Residues generated from agro-industrial processes, such as agricultural fibers, present a promising alternative raw material for various industrial applications due to their significant natural fiber content.[2] As a result, the utilization of agricultural waste for the development of value-added products can lessen its burden on the environment. For example, some fibers from agricultural waste can be added to reinforced polymer composites for commercial use, such as coir, banana, and sisal fibers.[3] Additionally, rice straw and bagasse fibers have been utilized in the production of writing and printing papers.[4] Given their inherent properties, these fibers can be processed to enhance environmental friendliness and cost-effectiveness of products by substituting synthetic fibers.

Research exploring the potential of environmentally friendly and sustainable products, including fibers from agricultural waste, has garnered significant interest within the scientific community. In a study conducted by A. Singh *et al*.,[5] the potential of vegetable waste was investigated for bioenergy generation. Through fermentation, vegetable waste can be harnessed for biofuel production under controlled conditions. This biomass, abundant in carbohydrates, holds promise as a source of renewable energy. Despite the abundance of natural fibers from agricultural waste, only a small fraction, approximately 10%, is currently utilized as alternative raw material across various industries, including biocomposites, building components, automotive parts, biomedical applications, and packaging.[5]

In the Philippines, particularly in the Cordillera Administrative Region, cabbage trimmings constitute a significant portion of the agricultural waste produced. According to research on the vegetable industry in the Philippines by S. Concepcion *et al*.,[6] the Cordillera Administrative Region accounts for 73% of cabbage production in the country. In 2002, a total of 66,875 tonnes of cabbage was harvested. Before the cabbages reach the market, most outer leaves often sustain damage due to factors such as faulty delivery methods and insect damage, necessitating their removal. Data from the trading post in La Trinidad indicates that up to 17 tons of vegetable waste are collected daily.

To harness this resource, this present work aims to investigate potential uses for waste cabbage trimmings. Cabbage, like other vegetables, contains lignocellulosic content. According to the study by B. Rani and K. Kawatra,[7] the composition of dietary fiber in cabbage (per 100 g dry weight) includes 63.02% cellulose, 15.20% hemicelluloses, 14.88% lignin, and 6.79% pectin. Furthermore, the possibility of using cabbage's outer leaves as a feedstock for the production of nano-fibrillated cellulose was examined by Khutkutapan *et al.*[8] The study yielded 36.5% cellulose on a dry basis, with the extracted sample composition consisting of 67.4% cellulose, 13.6% hemicelluloses, and 6.7% lignin.[7]

This present work aims to determine the most effective chemo-mechanical method in extracting cellulose from waste cabbage (*Brassica oleracea* var. *capitata* L.), trimmings using different chemo-mechanical methods: (a) alkali hydrolysis and bleaching treatment with high shear homogenization (b) acid hydrolysis with ultrasonication, and (c) chemical extraction using formic acid and hydrogen peroxide with mechanical assistance.

Utilizing different methods, these extraction processes have been conducted, each offering distinct advantages and disadvantages in terms of the quantitative and qualitative properties of the produced cellulose. Consequently, the primary objectives of this present work are to extract cellulose from waste cabbage (*Brassica oleracea* var. *capitata*) utilizing three different methods, to analyze the characteristics of the resulting cellulose fibers, and to compare the efficacy of the extraction methods.

EXPERIMENTAL

Collection of cabbage trimmings

The raw material utilized in this study consisted in the outer leaves of cabbage collected from the La Trinidad trading post located at Kilometer 5, Pico Road, Barangay Pico, La Trinidad, Benguet.

Preparation of raw material

The study was conducted at the Saint Louis University, Chemical Engineering Laboratory and Natural Sciences Research Laboratory.

Three different extraction methods were studied: AHBH - alkali hydrolysis and bleaching treatment with high shear homogenization, AHU - acid hydrolysis with ultrasonication, CEMA - chemical extraction with mechanical assistance.

The collected cabbage trimmings were washed under running tap water. For methods AHBH and CEMA, the leaves were blanched for 10 minutes using boiling water to soften its tissues. The leaves were subsequently diced into smaller pieces, each less than half an inch in size. These chopped cabbage leaves were then laid out in a single layer on a tray and left to dry under the sun until they reached below 10% moisture.

Extraction method 1: Alkali hydrolysis and bleaching treatment with high shear homogenization (AHBH)

This method was adapted from the study of Khukutapan *et al*.,[8] on characterization of nano fibrillated cellulose from cabbage outer leaves. Ten grams of prepared waste cabbage leaves were placed in 500 mL of deionized water and heated in an autoclave for 2 hours at a temperature of 130 °C. The autoclaved sample was then allowed to cool down at room temperature, then proceeded through filtration using a PTFE membrane filter. The filtered sample was then added to 500 mL of 5% w/v KOH and stirred for 8 hours. Upon another filtration, the sample was washed until it was neutralized to a pH of 7. Using 50%v/v sodium chlorite solution, the insoluble residue was further delignified for one hour. The mixture was then placed on a hot plate heated at 70 °C with constant stirring for one hour. The sample was then filtered and washed with deionized water until it was neutralized to pH 7. After washing, it was dispersed in water for 5 minutes to form a homogenous fiber suspension. It was then defibrillated using high-shear (HS) homogenization for 15 min. The final extracted sample was dried and tested.

Extraction method 2: Acid hydrolysis with ultrasonication (AHU)

Method AHU was adopted and modified from the extraction of rice straw cellulose nanofibers by Nasri *et al.*[9] Washed vegetable trimmings were soaked for 2 hours in a 17.5% w/w sodium hydroxide solution. The sample were then washed with distilled water and subjected to high shear homogenization for 30 min. Using a diluted hydrochloric acid solution with an acid to sample ratio of 25 mL/g, the obtained material underwent hydrolysis to eliminate other extractive materials. This hydrolysis treatment was performed using a 2M hydrochloric acid solution at 80 °C with constant stirring for 2 hours. After which, the pulp was neutralized to pH 7 using distilled

water. The processed pulp underwent treatment with 2% w/w sodium hydroxide for a duration of 2 hours at 80 °C, while being continuously stirred, followed by thorough washing. This step aimed to eliminate soluble lignin, residual hemicelluloses, and pectin. Subsequently, the fibers were subjected to sodium chlorite at 50 °C for 1 hour to finalize the bleaching procedure. The quantity of sodium chlorite applied was determined according to the kappa number of the fibers.

The bleached fibers were rinsed with distilled water then left to air dry. The cellulose fibers were soaked in 125 mL distilled water, then subjected to sonication at 400 W for 30 minutes within a water bath maintained at a temperature of 25 °C. The resulting sample was filtered, then air-dried in preparation for testing.

Extraction method 3: Chemical extraction using formic acid and hydrogen peroxide with mechanical assistance (CMA)

Compared to previous methods, this process gives a more environmentally friendly method of cellulose extraction using fewer toxic reagents. This is based on the study by Nazir *et al.*[10] on eco-friendly extraction of cellulose from oil palm empty fruit bunches. The previously prepared sample was milled to reduce its size. Then, 10 grams of the fibers were immersed in 100 mL of 10% w/w NaOH solution and 100 mL of 10%v/v H_2O_2, covered with aluminum foil. The set-up was placed in a water bath, maintained at boiling point temperature for one hour. Afterward, the supernatant liquid was separated from the fibers, which were then washed until clear. Subsequently, the fibers were soaked in a solution consisting of 20%v/v formic acid and 10%v/vH_2O_2 at a 1:1 ratio (v/v). This mixture was heated in a water bath at 85 °C for 2 hours. Then, the mixture underwent filtration and was subsequently rinsed with 10% formic acid and distilled water. The extracted cellulose fibers were re-suspended in 10% hydrogen peroxide for 90 minutes at 60 °C. The pH was adjusted to pH 11 with 10% w/w NaOH, then filtered and washed again. Finally, the filtered sample was air-dried.

Cellulose characterization

The full analysis of chemical composition of the samples was determined using the modified NREL method developed by Designer Energy Ltd. (DE).[11] Acid-insoluble lignin (AIL), cellulose content, and hemicelluloses were determined utilizing the proposed DE methods. Initially, 0.3 grams of the extracted sample underwent pre-hydrolysis in an Erlenmeyer flask with 5 mL of sulfuric acid (72 wt%) for two hours at 25 °C. Afterward, 45 mL of distilled water was added to the solution, which was then heated for an additional 2 hours in a reflux condenser. The mixture was subsequently cooled for 30 minutes at room temperature, poured into a polypropylene (PP) tube, and centrifuged for 15 minutes. Following the settling of lignin at the tube bottom, it was washed successively with hot water, 5% w/w sodium bicarbonate, and finally with distilled water. The liquid phase was separated by centrifugation before drying to a constant weight, and the amount of acid-insoluble lignin present in the cellulose was recorded.

For cellulose and hemicellulose content determination, a sample of 0.5 grams was mixed with 40 mL distilled water, 0.5 grams of sodium chlorite and 1 mL of glacial acetic acid in a flask. The mixture was subjected to water bath with constant stirring at 90 °C for 45 minutes. Subsequently, an additional 0.5 grams of sodium chlorite and 1 mL of acetate buffer were added, followed by stirring for an additional 45 minutes and cooling for 30 minutes. The resulting mixture was poured into a 50 mL tube and centrifuged for 10 minutes. The solid particles were washed with hot water before undergoing another stage of centrifugation and then dried at 105 °C to a constant weight, representing the amount of holocellulose in the sample.

To determine the amount of cellulose and hemicelluloses, the dried sample was mixed with 45 mL of 2 wt% hydrochloric acid. The mixture underwent hydrolysis at boiling temperature in a reflux condenser for two hours to remove the hemicellulose content of the sample. After cooling at room temperature for 30 minutes, the mixture was poured into a 50 mL tube and centrifuged for 10 minutes prior to washing. Then, the treated sample was washed with hot water, 1%w/w sodium bicarbonate, and distilled water for final washing to achieve a pH of 7. The washed cellulose was centrifuged and then dried at 105 °C to a constant weight. The weight difference between the empty tube and the tube with dried α-cellulose represented the weight of the α-cellulose, which was subtracted from the previously measured weight of holocellulose to obtain the weight of hemicelluloses.

For morphology, dried samples were brought to the University of Santo Tomas – Analytical Laboratory Services for testing. A Hitachu TM3000 Scanning Electron Microscope was used to examine the surface morphology of the samples.

Treatment of data

The data was gathered and recorded to account for cellulose that was extracted from the cabbage trimmings. The data was tabulated and compared for the determination of what method gave the highest yield of cellulose. The data from this study was analyzed using the Statistical Tool for Agricultural Research (STAR) software, which includes one-way analysis of variance (ANOVA). Significance was determined in all data analyses using P-values of 0.05 or below. The Tukey test was also used to evaluate whether there was a significant difference between each approach.

RESULTS AND DISCUSSION

Influence of the extraction methods used on cellulose yield and purity

By dividing the weight of the extracted sample by the original weight of the sample on a dry basis,

the percentage of cellulose yield was determined. The average percent yield of cellulose extraction using the three distinct methods is shown in Figure 1. The extraction method AHBH demonstrated the highest average percent yield, achieving 7.11%, followed by CEMA with an average percent yield of 6.56%. Conversely, AHU yields the lowest percentage at 5.08%.

As indicated by Garcia-Garcia *et al*.,[12] the study suggests that higher yields are obtained with longer hydrolysis times. In this study, the first method underwent hydrolysis for eight hours, while the remaining two methods were hydrolyzed for only two hours each.

According to Table 1, AHU extracted cellulose exhibits the highest α-cellulose content, averaging at 63%, indicating its superior purity among the methods employed. The use of acid hydrolysis in combination with sonication likely contributes to this method's elevated yield. Acid hydrolysis effectively disrupted hydrogen bonds within the sample, facilitating the separation of desired α-cellulose from other lignocellulosic components. Sonication assists in aggregating alpha-cellulose within the slurry solution by employing sound waves, thereby enhancing the purity of the collected sample.[13] The method AHBH follows closely with 58% alpha-cellulose, while CEMA extracted cellulose exhibits the lowest cellulose purity at 55%.

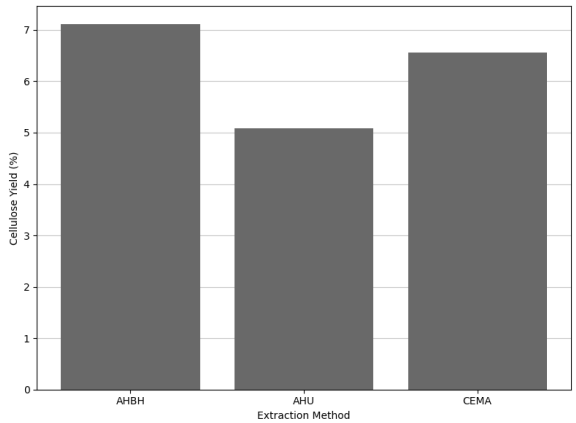

Figure 1: Yields of cellulose obtained by the three methods

Table 1
Chemical composition of cellulose extracted from cabbage leaves

Treatment	% Lignin	% Hemicelluloses	% α-cellulose
AHBH	10.34^{b}	33^{ns}	58^{b}
AHU	9.84^{b}	29^{ns}	63^{a}
CEMA	15.50^{a}	33^{ns}	55^{b}
CV, %	4.29%	4.47%	1.97%
Level of significance	**	ns	*

Means with the same letter are not significantly different ($p \leq 0.05$); ns – not significant

Morphological properties of the extracted cellulose through SEM analysis

Prolonged alkali treatment and high-temperature treatment of the fiber in the first method have led to the disintegration of cellulose fibers, resulting in low cellulose content and reduced productivity.[14] For AHBH, the substantial change brought about by alkali treatment leads to the breaking of OH bonds in the fiber network structure, which separates the cellulose fibers from the interfibrillar areas, as seen in Figure 2 (A and B).[15] Meanwhile, the AHU method employs ultrasonication, which appears to disintegrate the cellulose into nanofibrils, as evidenced by the visible structure in Figure 2 (C) and 2 (D), indicating fibrillation. It is apparent that the ultrasonic treatment effectively fibrillates cellulose microfibrils into nanofibrils, irrespective of ultrasonication power or duration.[16] The cellulose extracted using the method CEMA and shown in Figure 2 (E) and 2 (F) displays an irregular arrangement of intertwined fibers that are densely packed. The dense composition observed implies the potential presence of residual hemicelluloses and lignin compared to the cellulose obtained by the method AHU, where their removal appears more thorough.

The morphology of each sample was greatly affected by how the extraction was performed due to the different extraction methods employed.

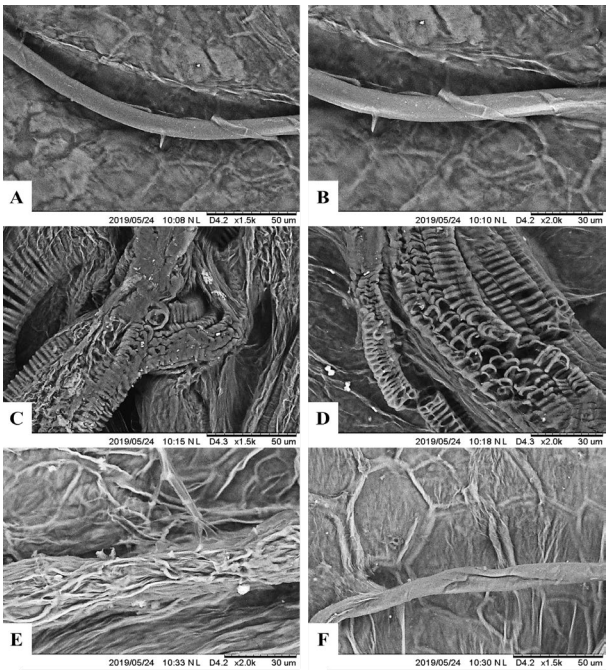

Figure 2: SEM imaging of cellulose extracted using: (A) AHBH x1500 magnification, (B) AHBH x2000 magnification, (C) AHU x1500 magnification, (D) AHU x2000 magnification, (E) CEMA x1500 magnification, (F) CEMA x2000 magnification

CONCLUSION

This study successfully extracted cellulose from waste cabbage trimmings using three different methods: alkali hydrolysis and bleaching treatment with high shear homogenization (AHBH), acid hydrolysis with ultrasonication (AHU), and chemical extraction with mechanical assistance (CEMA). The data obtained in this study indicates that AHBH yielded the highest cellulose among the three methods, averaging 7.11% extracted from whole cabbage trimmings. This method is suitable for producing cellulose derivatives, such as cellulose ethers and cellulose esters, for pharmaceutical applications.[17] As regards the purity of cellulose, AHU extracted cellulose has the highest α-cellulose purity averaging 63%. This sample is suitable for manufacturing dietary fibers or paper,[18] which requires cellulose with high purity. This study suggests further investigation of the crystallinity index of the extracted cellulose, which influences the physical, mechanical, and chemical properties of cellulosic materials, as well as their processing and other applications.

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