EXTRACTING LIGNIN FROM CACAO POD HUSK

● TRAN THI TUONG AN - PHAM THI TUYET - VU LE VAN KHANH - LE THI THUY - TRAN THI HONG HANH

ABSTRACT:

Currently, the world faces the risk of energy exhaustion and environmental pollution due to agricultural and industrial wastes. Solving waste sources and reusing them as raw materials in production to minimize environmental pollution is interesting. Vietnam has a developed agricultural industry, and the country's annual production of food crops and industrial crops is increasing substantially. Especially, cacao production in Vietnam is gowing, and the amount of cacao shells released into the environment is increasing. This study examines the cacao pretreatment with the use of sodium hydroxide (NaOH) in combination with hydrogen peroxide (H_2O_2) to separate and collect lignin. The combination of NaOH with H_2O_2 has many advantages in the lignin separation process and it improves the performance of lignin recovery process. Cacao shell is pre-treated by using 1% H_2O_2 in 0.8% NaOH (w/v) with the solid/ liquid ratio of 1g/18mL for 3 hours at room temperature and the stirring speed of 150 rpm. The lignin content which is separated during the pretreatment process is 44.5%. The efficiency of lignin recovery process after the pretreatment process is 65.76%.

Keywords: cacao shell, hydrogen peroxide, lignin, separation, sodium hydroxide.

1. Introduction

Lignin is a complex polymer that is an abundant polyphenol biopolymeric material [1], [2]. This is a branched-chain, non-carbohydrate aromatic polymer with the monomers being insoluble phenylpropane units including p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) and challenging to degrade enzymatically [3] [4]. Lignin is one of three compounds found in plants and trees which form bonds with the hemicellulose to surround the cellulose. Lignin is often concentrated in wood tissues, acts as a cell adhesion agent, and increases the mechanical strength of cell walls [5]. The complex structure of lignin is made up of primary linkages: β-O-4, α-O-4, β-5, 5-5, 4-O-5, β-1, and β-β [6], [7].

Currently, extraction of lignin from plants can be done by physical and/or chemical and biochemical, and biological treatments [4]. The biological treatment has several advantages, such as being friendly with the environment and less harmful; however, the most significant limitation of this method is the low lignin separation efficiency. Meanwhile, chemical and physical treatment methods have been much attention due to their high ability to extract lignin and receive amorphous cellulose fibers [8]–[10]. Hence, this study focused on investigating the parameters that affect lignin removal efficiency from the cacao shells by the chemical treatment method. NaOH and H_2O_2 were used as analytical reagents due to their low cost and industrial scale.

Sodium hydroxide (NaOH) is known as a strong base that has been used in many research on lignin removals from plants. The advantage of NaOH pretreatment is that large amounts of lignocellulose glucan and xylan can be retained in the solid phase after pretreatment, which increases the yield of ethanol obtained due to both of them having the ability to ferment to obtain ethanol.

The combination of NaOH and H_2O_2 to pretreat plant cells can reach higher efficiency of lignin removal than pretreatment with each individual substance.

2. Materials and methodology

2.1. Materials

Cacao peels were harvested at confectionery processing factories in Ho Chi Minh City, Vietnam. The grounded peel was then dried in a dryer oven at 80°C for 2 days to reach the moisture content of 5 ± 0.5%. Subsequently, the dehydrated peels were grounded to powder by the food processor and packed polyethylene bags, sealed, and stored at 4°C until using.

2.2. Methodology

2.2.1. Chemical Analysis

Research some chemical composition of cacao peel which includes: moisture content; total soluble solids; pH; reducing sugar content; cellulose content; and lignin content.

2.2.2. Investigate the process of treating cacao shell

2.2.2.1. Effects of NaOH and H_2O_2 combination on lignin separation

NaOH at different concentrations of 0.2%, 0.4%, 0.6%, 1%, 2%, 3% was mixed with 1% H_2O_2 (w/v) which was investigated. About 2g of cacao shell was put in a 100 mL glass bottle and then added 40 mL of the active substance. The mixture was stirred at 150 rpm for 3 hours at room temperature. After the pretreatment stage, lignin content was analyzed by UV-vis.

2.2.2.2. Effects of liquid-solid ratio on lignin separation

About 2g cacao shell was put in a 100 mL glass bottle with was added the optimal concentration of NaOH and H_2O_2 mixture the different liquid-solid ratios of 10:1, 12:1, 14:1, 16:1, 18:1, 20:1 (mL/g). The mixture was stirred at 150 rpm, 3 hr. Lignin concentration was analyzed by UV-vis.

2.2.2.3. Effects of pretreatment time on lignin separation

In this experiment, the pretreatment time was investigated. The mixture of optimum NaOH concentration mixed with 1% H_2SO_4 at an optimum liquid-solid ratio at room temperature was used to set up this experiment. The lignin content was analyzed at survey time points, including 0, 1.5, 3, 4.5, and 6hr.

2.2.2.4. Effects of stirring the material on lignin separation

Evaluate the effect of stir speed on lignin removal, about 2g cacao shell was put in a 100mL glass bottle that was added an optimum volume at an optimum concentration of the reactant (NaOH mixed with 1% of H_2O_2). In the optimal stirring time, different stirring speeds including 0, 90, 120, 150, 180 rpm were investigated.

2.2.3. Investigate conditions for recovering lignin from pretreatment fluids

2.2.3.1. Effect of pH on lignin recovery

The effect of pH at different levels of 1, 2, 3, 4, 5 on lignin recovery was investigated. About 40 mL pretreatment solution was adjusted to survey points of pH. This solution was put into the 80°C thermostatic bath for 45 minutes. After that, the precipitate was collected, then washed with hot water and dried at 70°C, 24 hr.

2.2.3.2. Effect of temperature on lignin recovery

About 40 mL pretreatment solution that was adjusted at the optimum pH level was put in a thermostatic bath for 45 minutes for temperature investigation. The surveyed temperature points include 40 - 90°C.

2.2.3.3. Effect of time on lignin recovery

About 40 mL pretreatment solution was adjusted at the optimum pH level. This solution was put in the thermostatic bath that was set up at optimum temperature. The lignin recovery was collected at different incubation times of 10-60 minute.

3. Result and discussion

3.1. Composition of cacao shell

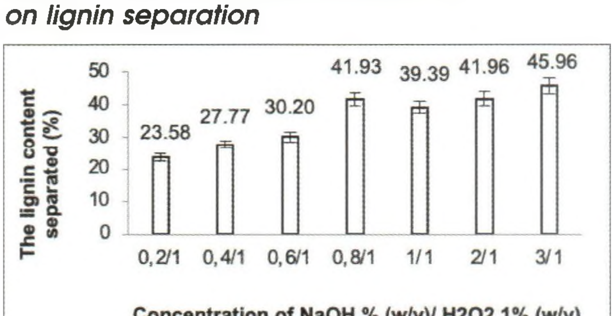
According to the analysis of the cacao shell, the highest cellulose content was 37.6% of the dry weight, and the lignin content was 32.4% of the dry weight. It was higher when compared with other lignocellulose materials, typically straw, banana peel [11]. The shell weight accounts for more than 50% of the total weight. The production and consumption of cacao in the world are pretty high, so the yield of cacao shells is large.

3.2. Investigate of the lignin separation conditions

3.2.1. NaOH and H_2O_2 combination on lignin separation

At optimum pH of 11.5-11.6, H_2O_2 has dissociated following the reaction $H_2O_2 \leftrightarrow HOO^- + H^+$, and quickly impact lignin and related phenolics to create a molecular low which these oxidation products are readily soluble in water [12]. Therefore, finding the suitable NaOH concentration in the environment H_2O_2 is important to promote this reaction. The oxidation capacity of lignin is highly dependent on the pH of the response.

Figure 1. Effect of sodium hydroxide on lignin separation



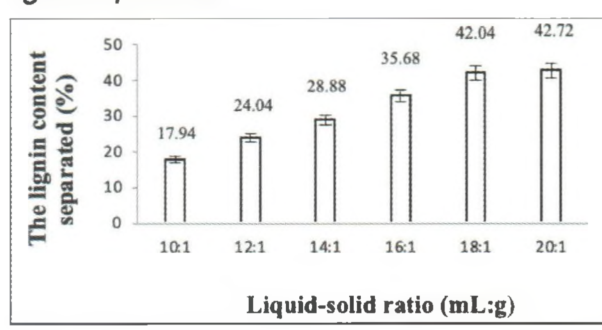
Source: Tran et al., 2022

According to ANOVA results, there was a difference in lignin content separated when changing NaOH concentration (P-value = 9.86 × 10-17 < α = 0.05). Survey results (Fig. 1) showed that NaOH concentration of 0.8% in H_2O_2 of 1% (w/ v) received the highest lignin content of 41.9%. When NaOH concentration decreased, lignin content separated significantly decreased, specifically 26.3%; 27.8%; 30.2% with NaOH concentrations of 0.2%; 0.4%; 0.6%, respectively. Conversely, when NaOH concentration increased to 1%, 2%, and 3%, the lignin content separated was negligible, in the range of 2% - 4.0%. Thus, 0.8% NaOH is the most suitable for the dissociation reaction of hydrogen peroxide (1%) ($H_2O_2 \leftrightarrow HOO^- + H^+$, pKa = 16).

3.2.2. Effect of liquid-solid ratio on lignin separation

One of the essential factors that affect lignin separation is the liquid-solid ratio. It is necessary to identify the optimal liquid-solid ratio so that the efficiency of lignin separation reaches the highest.

Figure 2. Effect of Liquid - solid ratio on lignin separation



Source: Tran et al., 2022

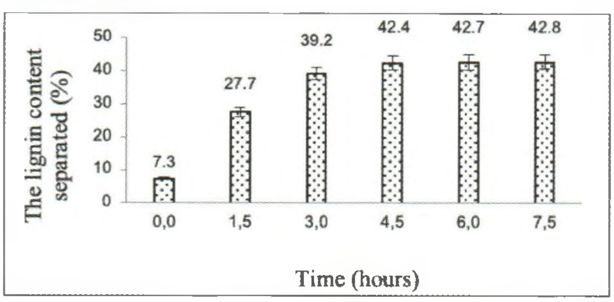
ANOVA results and Fig.2 showed a significant difference when changing the ratio of added substances leads to a change in the lignin content separated (P-value = 2.39 × 10-8). When the liquid-solid ratio varies from 10:1 to 18:1, the lignin separation efficiency increases significantly from 17.94% to 42.04%. Cacao shell has quite a low moisture content of 13.68%, so if the amount of substance added a little gave, the volume that compensates for the material's moisture content would be lost. The remaining liquid was not enough for the hydrolysis process entirely, so suspension was formed, making filtration very difficult.

On the other hand, when the ratio of raw materials from 18:1 to 20:1, the lignin separation increased from 42.04% to 42.72%, but according to ANOVA results, there was no difference between the two ratios (P-value = 0.727723159> α = 0.05). This result is appropriate with the theory because the more material, the more reactant, consequence the lignin content separated increased. However, when the solution reaches the saturation value, the lignin content that separates will change insignificantly if a continued increase in the amount of substance added [13]. Therefore, the optimum liquid-solid ratio was 18:1 (mL/g).

3.2.3. Effect of time on lignin separation

Hydrogen peroxide (H_2O_2) is a readily soluble substance in alkaline media. Therefore, investigating the hydrolysis time for the complete lignin oxidation by hydroxyl (-OH) and superoxide (O_2-) radicals that are generated from H_2O_2 is necessary. ANOVA results showed the difference significant in lignin content (%) at different pretreatment times (P-value = 2.16 × 10-11).

Figure 3. Effect of time on lignin separation

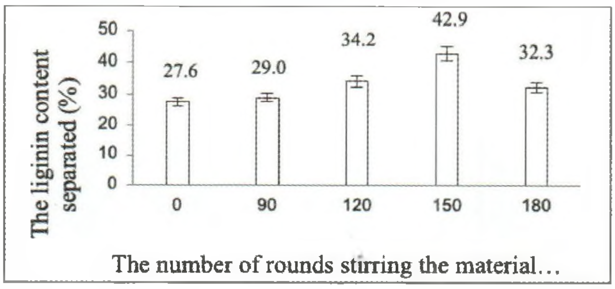


Source: Tran et al., 2022

The survey results (Fig.3) shown that increasing the pretreatment time of the sample, the lignin content separated was increased, specifically 7,3%; 27,7%; 39,2%; 42,4%; 42,7%; 42,8%, which corresponding to the hydrolysis time of 0.5 hours; 1.5 hours; 3 hours; 4.5 hours; 6 hours; 7.5 hours. The lignin content increased when increasing hydrolysis time from 0 hr to 3 hrs, and then almost unchanged with further increased hydrolysis time from 4.5 - 7.4 hrs. It can be explained that H_2O_2 is less stable in alkaline media; hydroxyl (OH-) radicals and superoxide (O_2-) were generated from H_2O_2 decrease as time increases, resulting in a reduction in oxidation rate. After 3 hours, the lignin content separation was still increased but not significantly because the solution has an alkaline residue that can hydrolyze a small portion of lignin in the material slowly [14]. Thus, 3hr of hydrolysis time is the optimum condition.

3.2.4. Effect of stirring the material on lignin separation

Figure 4. Effect of stirring material on lignin



Source: Tran et al., 2022

The survey results are shown in Fig. 4, and ANOVA results show that the number of material stirring cycles significantly affects the lignin content separation (P-value = 0.000437 < α = 0.05). When the stirring speed increased from 0 rpm to 150 rpm, the lignin content increased from 27.6% to 42.9%. However, when the stirring rate was 180 rpm, the lignin content was reduced to 32.3%. This may be because solvent evaporation is promoted under high, stirring speed and high-temperature conditions.

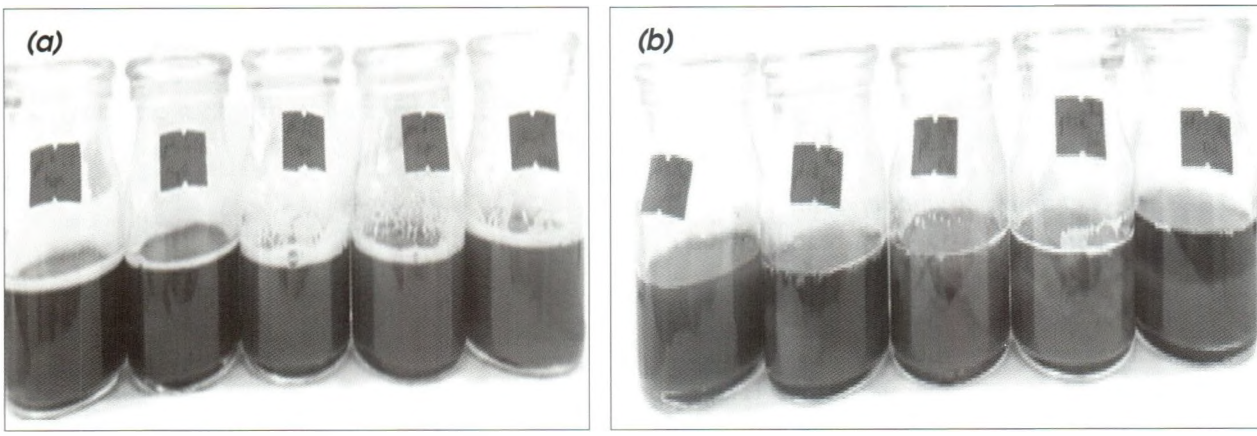
3.3. Investigate conditions for recovering lignin from pretreatment fluids

At the optimal treatment conditions (0,8% NaOH (w/v) in 1% H_2O_2 (w/v), the liquid-solid ratio of 18: 1, time of 3 hours, material stirring speed of 150 rpm), the lignin content separation was 44.5%. According to the calculation results, the lignin content in the liquid was 7mg/mL.

Experimental results showed that the color of the solution changed from dark brown to light brown when the pH was reduced (Fig. 5a). The dark brown color of the solution was from chromophoric groups in lignin structure such as quinone, carbonyl, carboxylic acid, hydroperoxy-based, and phenolic hydroxyl groups. Moreover, when the pH is reduced, the lignin solubility decreases, so the color of the solution will be brighter.

The change in pH affects lignin recovery

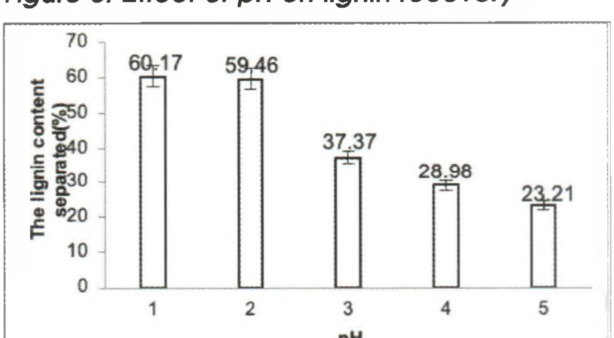
Figure 5. a, The color of the solution fades as the pH value decreases from 5 to 1; b, The color of the solution from pH of 5 to pH of 1 after treating



Source: Tran et al., 2022

efficiency (Fig. 5b). The lower the pH, the higher the lignin recovery efficiency. The pH decreased from 5, 4, 3, 2, 1, the efficiency increases to 23.21%, 28.98%, 37.37%, 59.46%, 60.17%, respectively. The lignin dissolves well in an alkaline environment because it contains acid functional groups (phenolic, carboxylic acid). However, the solubility will decrease as the pH of the medium decreases.

Figure 6. Effect of pH on lignin recovery



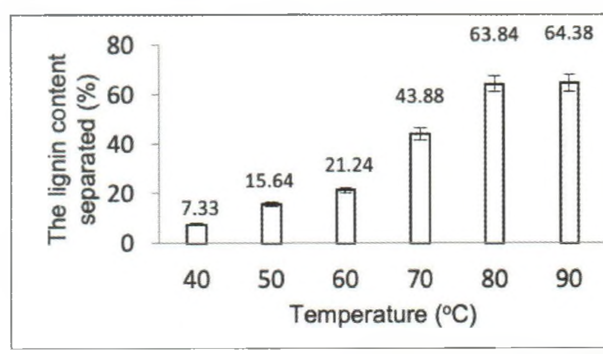
Source: Tran et al., 2022

Fig. 6 showed that at pH of 1, the lignin yield was highest; however; there is no significant difference in lignin content between pH of 1 and pH of 2 (P-value = 0.82527077> α = 0.05). Hence, a pH of 2 was optimal.

3.3.1. Effect of temperature on lignin recovery

As shown in Fig.7 the higher the temperature, the higher the lignin recovery efficiency;

Figure 7. Effect of temperature on lignin recovery



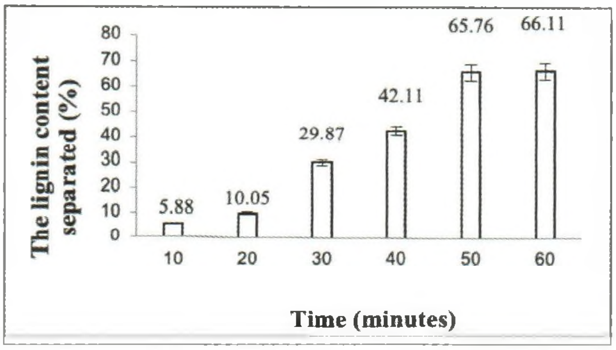
Source: Tran et al., 2022

specifically, the temperature increased from 40°C to 90°C, the lignin recovery efficiency was increased from 7.33% to 64.38%, respectively. At 90°C, the lignin recovery efficiency was the highest (64.38%). But there was not significantly different from the efficiency at a temperature of 80°C (63.84%) (P-value = 0.753751 > α = 0.05); Therefore, 80°C was the optimal temperature.

3.3.2. Effect of time on lignin recovery efficiency

The time increased from 10 minutes to 60 minutes, the lignin content recovered from 5.88% to 66.11% of the amount of lignin in the solution (Fig. 8). Besides, after 50 minutes, the lignin content recovered only increases by about 0.5%. This result also showed the limits of lignin content recovered in the pretreatment fluid. At pH of 2 and

Figure 8. Effect of time on lignin recovery



Source: Tran et al., 2022

80°C, the lignin was cut off and affected by a thermal. So 50 minutes was the most effective time.

4. Conclusion

The research has found the optimal conditions for pretreatment of cacao shells with sodium hydroxide (NaOH) combined with hydrogen peroxide (H_2O_2) to lignin separation and recovery. Cacao shell is pretreated with 0.8% NaOH combine with 1% H_2O_2, liquid-solid ratio of 18:1 (mL/g), time of 3 hours, the number of rounds stirring of 150 rpm would be separated 44.5% lignin in raw materials. After cacao shell pretreatment, the solution was further processed at pH of 2, at 80°C, for 50 minutes. The result was 65.76% lignin recovered. The lignin after recovery will apply as an additive in paper production ■

Acknowledgment:

We acknowledge the support of time and facilities from Ho Chi Minh University of Technology (HCMUT), VNU-HCMUT and fund of Murata Science Foundation.

REFERENCES:

1. H. Ludmila, J. Michal, Š. Andrea, and H. Alš. (2015). Lignin, potential products and their market value. *Wood Research,* 60(6), 973-986.

2. S. G. Wettstein, D. Martin Alonso, E. I. Gürbüz, and J. A. Dumesic. (2012). A roadmap for conversion of lignocellulosic biomass to chemicals and fuels. *Current Opinion in Chemical Engineering,* 1(3), 218-224.

3. G. F. Calvo-flores and J. A. Dobado. (2010). Lignin as Renewable Raw Material. *ChemSusChem,* 3, 1227-1235.

4. S. Laurichesse and L. Avérous. (2013). Chemical modification of lignins: Towards biobased polymers. *Progress in Polymer Science,* 849, 3-25.

5. Q. Liu, L. Luo, and L. Zheng. (2018). Lignins : Biosynthesis and Biological Functions in Plants. *International Journal of Molecular Sciences,* 19(335), 1-16.

6. H. D. S. Abreu, A. M. Do Nascimento, and M. A. Maria. (1999). Lignin structure and wood properties. *Wood and Fiber Science,* 31(4), 426-433.

7. G. W. Huber, S. Iborra, and A. Corma. (2006). Synthesis of Transportation Fuels from Biomass: Chemistry, Catalysts, and Engineering. *Chemical Reviews,* 106(9), 4044-4098.

8. G. Brodeur, E. Yau, K. Badal, J. Collier, K. B. Ramachandran, and S. Ramakrishnan. (2011). Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review. *Enzyme Research,* 2011, 1-17.

9. R. Sun, J. M. Lawther, and W. B. Banks. (1995). Influence of alkaline pre-treatments on the cell wall components of wheat straw. *Industrial Crops and Products,* 4, 127-145.

10. Y. Sun and J. Cheng. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A review q. *Bioresource Technology,* 83, 1-11.

11. A. K. Kumar and S. Sharma. (2017). Recent updates on different methods of pretreatment of lignocellulosic feedstocks: a review. *Bioresources and Bioprocessing,* 4(7), 1-19.

12. J. M. Gould. (1984). Studies on the Mechanism of Al ka I i ne Peroxide Del ig n if icat ion of Agricultural Residues. *Biotechnology and Bioengineering,* 27, 225-231.

13. H. R. Ten and P. J. M. Teunissen. (2001). Oxidative mechanisms involved in lignin degradation by white-rot fungi. *Chemical Reviews,* 101(11), 3397-3413.

14. Y. Yamashita, M. Shono, C. Sasaki, and Y. Nakamura (2010). Alkaline peroxide pretreatment for efficient enzymatic saccharification of bamboo. *Carbohydrate Polymers,* 79(4), 914-920.

Received date: September 2, 2022
Reviewed date: September 17, 2022
Accepted date: October 10, 2022

Author's information:

1. TRAN THI TUONG AN[1]

2. PHAM THI TUYET[1]

3. VU LE VAN KHANH[1]

4. LE THI THUY[1]

5. TRAN THI HONG HANH[1]

[1] Faculty of Chemical Engineering at Ho Chi Minh City University of Technology

CHIẾT SUẤT LIGNIN TỪ VỎ CACAO

● **TRẦN THỊ TƯỞNG AN[1]**
● **PHẠM THỊ TUYẾT[1]**
● **VŨ LÊ VÂN KHÁNH[1]**
● **LÊ THỊ THÚY[1]**
● **TRẦN THỊ HỒNG HẠNH[1]**
[1]Khoa Kỹ thuật Hóa học, Trường Đại học Bách khoa
Đại học Quốc gia Thành phố Hồ Chí Minh

TÓM TẮT:

Hiện nay, thế giới đang phải đối mặt với nguy cơ cạn kiệt năng lượng và ô nhiễm môi trường do chất thải nông nghiệp và công nghiệp. Việc giải quyết nguồn thải và tái sử dụng làm nguyên liệu trong sản xuất nhằm giảm thiểu ô nhiễm môi trường là điều đáng quan tâm. Việt Nam là nước có nền nông nghiệp phát triển, sản lượng cây lương thực và cây công nghiệp hàng năm rất đáng kể. Trong số đó, sản lượng cacao ở Việt Nam đã tăng lên hàng năm. Lượng vỏ cacao thải ra môi trường ngày càng nhiều. Nghiên cứu này được thực hiện để kiểm tra việc xử lý sơ bộ cacao bằng natri hydroxit (NaOH) kết hợp với hydro peroxit (H_2O_2) để tách và thu hồi lignin. Kết quả khảo sát cho thấy sự kết hợp NaOH với H_2O_2 có nhiều ưu điểm trong việc tách lignin và tăng hiệu suất thu hồi lignin. Vỏ cacao được xử lý sơ bộ với H_2O_2 1% trong NaOH 0,8% (w/v), tỷ lệ rắn/lỏng 1g/18mL trong 3 giờ ở nhiệt độ phòng, tốc độ khuấy 150 vòng/phút. Hàm lượng lignin tách ra trong quá trình tiền xử lý là 44,5%. Lignin thu hồi từ quá trình tiền xử lý đạt được hiệu suất 65,76%.

Từ khóa: vỏ cacao, nước oxy già, lignin, phân tách, natri hydroxit.