



Research Article

Effect of Alkaline Concentration and Temperature on Lignin Extraction from Sugarcane Bagasse for Potential Use as UV Absorber

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) primarily being extracted for its sugar and juice leaving the large quantities of bagasse as its fibrous waste. Since sugarcane bagasse consist high lignin content which have ultraviolet (UV) protection properties, it has led to further valorization of this biomass. The objective of this study is to optimize the alkaline pretreatment condition based on the concentration of sodium hydroxide and temperature to extract high yield of lignin for UV protection properties in cosmetic application using response surface methodology under Box-Behnken design and analysing the properties of lignin in sunscreen. The lignin composition in sugarcane bagasse was analyzed, giving 16.7 ± 4.8 wt% of sugarcane bagasse. Alkali extraction of lignin from sugarcane bagasse has been carried out two different parameters which are concentration of sodium hydroxide (NaOH) (2-10% w/v) and temperatures (60-140 °C) in water bath at a fixed time, 1 hour. The optimized pretreatment condition obtained is at 10% w/v NaOH concentration with temperature of 100 °C for 1 hour, as the highest yield of lignin can be extracted. Lignin yield achieved about 55.8 ± 1.92 wt%, with a purity of $28.1 \pm 10.75\%$. Ultraviolet protection properties of lignin had been analyzed by determining the sun protection factor (SPF). It was done by blending different concentration of lignin into a base cream. The SPF achieved for 5% lignin-blended cream was 4.76 and for 10% lignin-blended cream was 5.78 respectively.

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INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a major agricultural crop cultivated in tropical and subtropical countries in the world with estimated around 1.87 millions harvested in 2020 (Wan-Mohtar et al., 2023). Malaysia is one of the major producers of sugarcane in Southeast Asia, with an estimated production of over 25,032 Mt in 2022 (Department of Agriculture, 2022). According to Nazir et al. (2020), about 3000 tons of sugarcane were produced in Malaysia per year. Sugarcane mainly being consumed for its raw sugar and juice extracted from its stem. It also have been use for alcoholic beverages from the fermentation of the sugary juice of sugarcane (Muñoz et al., 2024).

From the processing of sugar, sugarcane bagasse is the primary waste generated with about 36 million tons annually (Ratanasumarn & Chitprasert, 2020). 1 tonnes of sugarcane would generate around 280 kg of bagasse (Aminudin et al., 2016). As the demand for sugarcane increases, the sugarcane bagasse (SB) waste may also escalates. This results in huge waste production which and causing environmental concerns in which SB waste are often being deposited in landfills or burned in sugarcane mills (Salatein et al., 2024). SB generated is largely burnt especially in some states of Malaysia that have large area of plantation of sugarcane, such as Perlis and Kedah (Nazir et al., 2020). The emissions from agricultural activities

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including sugarcane burning is one of the most abundant reasons that cause air pollution in Malaysia. These issues have led to the valorization of the SB waste and turn it into a value-added products. SB consists of lignocellulose in which nearly 50% cellulose, 25% hemicellulose, and 25% lignin (Su et al. 2015). These sugarcane bagasse wastes rich in lignin biomass and have the potential to be used in many value added product applications such as resin, cosmetic and food packaging film (Antunes et al., 2023; Chauhan et al., 2023; Kirar et al., 2024)

Lignin is a second most abundant phenolic, complex heterogeneous polymer found in most terrestrial plants, with among 15% to 40% dry weight found in plant cell wall. Lignin contains phenylpropane units made out of three monomers, including *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Kim & Um, 2020). The phenolic substructure derived from these monomers are phydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Kim & Um, 2020). It is also composed of several functional groups (phenolic, conjugated double bonds, ketone and other chromophore groups) which to be one of the factors contributing in antioxidants and UV properties of lignin (Lin et al., 2021). The mechanism of lignin as UV absorber is that the chromophore group on lignin structure contain π bond and unbonded electron which enhance the UV absorptivity (Lv et al., 2023). In addition, phenolic substructure on lignin able to scavenge free radicals and inhibit production free radical which also contributing to the stability of lignin as UV protector material (Lv et al., 2023). Those structure the main component in the lignin to absorb visible light, in order to block a broad spectrum of UV radiation, including UV A and UV B, making it highly promising for use in both day and night formulations.

UVB rays (280-320 nm) primarily target the outermost epidermal cell layer, leading to sunburn and skin cancer (Wu et al., 2023). UVA rays (320-400 nm) accounts for over 95% of UV light and penetrates the skin through the upper epidermal layers at a rate 7-9 times higher than UVB radiation (Wu et al., 2023). According to the World Health Organization (2017), there are 132,000 cases of melanoma and 2 to 3 million cases of non-melanoma skin cancer are reported annually worldwide. Therefore, sunscreen is important to be utilized by people daily to avoid skin damage. Nowadays, most cosmetic products in the market containing chemical UV filters in their composition. However, the safety of those chemical UV filters are kept in question due to their harmful side effects such that it could penetrate into skin that it has been reported that there are detection of UV filters in blood (Frederiksen et al., 2020) and breast milk (Molins-Delgado et al., 2018). Thus, the demand for sunscreens derived from natural sources having the characteristic to absorb UV protection increases. Most of the cosmetic industry is also studying and developing breakthrough formulations of UV protection products, especially by utilizing plant antioxidants as the raw materials. Since lignin has low cytotoxicity and antimicrobial agent, it is safe to be used to produce eco-friendly lignin-based products which is sunscreen (Ariyanta et al., 2023).

Additionally, it is preferable if the lignin are produced by an eco-friendly and sustainable method for the preservation of nature. This alternative not only assists customers looking for eco-friendly products, but it also advances a healthier beauty industry while preserving our planet's natural resources. Alkaline pretreatment is regarded an effective method to extract lignin from agricultural residues due to its

low cost, ease of preparation and achieving high yield of lignin compared to the other methods. Sodium hydroxide (NaOH), one of the most common alkaline reagents (Moubarik et al., 2013). Alkaline pretreatment using NaOH has been proven by several literature to have high yield in lignin (70 - 82%) with severe reaction condition of NaOH concentration (7-11% w/v), temperature (100-135 °C) and time (40-75 mins) (Muñoz et al., 2024; Ratanasumarn & Chitprasert, 2020). The mechanism of alkaline pretreatment is the saponification of intermolecular ester bonds crosslinking lignin and hemicellulose, the lignin to be depolymerised. The linkage between lignin and carbohydrates will be disrupted in lignocellulosic biomass (Morales et al., 2018). The crystallinity of cellulose can be reduced, and the degree of carbohydrate polymerization is decreased (Xu et al., 2010).

Even though there are few studies regarding the optimization of sugarcane bagasse, however, each of the study differ due to the complexity of the biomass structure and lignin composition. Factors such as growing conditions, including types of soil used for the plantation of sugarcane, climates conditions, crop varieties and genetics of the plant will affect the lignin composition and also the suitable conditions of yield of lignin extraction (Barciela et al., 2023). Therefore, in this study, able to identify optimization parameters with diverse range of concentration and temperature for the respective sugarcane bagasse from the local vendor to obtained high lignin extraction. The aim of the study was to provide insight on determining optimize condition of alkaline pretreatment (concentration of sodium hydroxide and temperature) to extract high yield of lignin as well as with high purity. Furthermore, to study the incorporation of lignin extract from SB as an active ingredient in skincare formulation with UV-protection properties and proven to be stable under specific conditions. This also helps in reduce SB waste and decreases the air pollution caused by the burning of sugarcane bagasse by adding value on the waste, at the same time contribute to the research of lignin in cosmetic industry.

MATERIALS AND METHOD

Materials

Sugarcane bagasse (*Saccharum officinarum* L.) obtained from Taman Universiti, Skudai, Johor, Malaysia, concentrated 72% H₂SO₄, concentrated 96% H₂SO₄, distilled water, NaOH (2%, 4%, 6%, 8%, 10%), and base cream. Materials used included oven, beaker, grinder, electronic balance, thermostat, vortex, Buchner funnel, filter paper, UV-visible spectrophotometer, quartz cuvette, magnetic stirrer, centrifuge, and 3M Transpore Tape.

Preparation of Sugarcane Bagasse

Sugarcane bagasse cut into smaller pieces. Then, it was dried in oven at 50 °C for 24 hours until it is fully dried. Then, the dried- SB was milled to a particle size smaller than 0.4 mm by grinder, and was stored at room temperature in a dark and dry place for further use (Morales et al., 2018).

Lignin Composition Analysis of Sugarcane Bagasse

Klason lignin can be defined as the insoluble residue after the ash has been removed from the plant tissues by concentrated acid hydrolysis, which is also a method to determine the lignin composition present in biomass. Following the extensive research done to determine the

lignin composition, Klason lignin is the most direct and reliable method for quantitative analysis (Chen, 2015). It was determined by two-stage acid hydrolysis of the SB samples. A 200 mg of SB was added into 3 mL of 72% H₂SO₄ slowly and incubated in a water bath at 30 °C for 1 hour. Then, distilled water was added to dilute the mixture to 3%. After being diluted, the mixture was heated under reflux until the boiling temperature, it is left to let the insoluble residue to be sedimented. Filtration was carried out at a weighted sintered crucible, and washed with 500 mL distilled hot water. After washing, it was dried in an oven at 105 °C for 12 hours. The residue was Klason lignin and is then weighed (Jöul et al., 2022). The lignin composition was determined and calculated by using the formula below.

$$\text{Lignin Composition (\%)} = \frac{\text{Weight of Lignin}}{\text{Weight of sugarcane bagasse}} \times 100\%$$

Extraction of Lignin from Sugarcane Bagasse

Extraction of lignin was carried out in NaOH solution. The liquid to solid ratio (LSR) was fixed in 1:10, for different concentration of NaOH and SB is utilized. Alkaline extraction was carried out in water bath by varying the NaOH concentration (2%, 4%, 6%, 8%, and 10%) while the temperature and time was fixed at 100 °C and 1 hour, respectively. The product was then filtered by using vacuum filtration. The liquid phase from the experiment was acidified with 96% H₂SO₄, until pH 2 for lignin precipitation. It was then centrifuged at 4200 rpm, and the resultant pellet consist of the precipitated lignin. Following the filtration, the extracted lignin was washed with distilled water, air-dried at 45 °C for 24 hours, and stored for characterization analysis. The final product was weighted. Next, the project was proceeded by varying the temperatures, at a fixed NaOH concentration which gave the highest yield of lignin at a fixed time, 1hour. The temperature range was at (60 °C, 80 °C, 100 °C, 120 °C, 140 °C). The same steps above were repeated for alkaline extraction of lignin. Then, the percentage of delignification can be determined (Morales et al., 2018).

Purity Test of Lignin Extracted from Sugarcane Bagasse

The purity test of lignin is conducted using the lignin composition analysis (two-stage acid hydrolysis). The sample use is lignin sample that is extracted from the previous alkaline pretreatment. Two purity tests were carried out for the lignin product extracted, one was from the experiment studied on the effect on NaOH concentration, and another was experiment of the effect on extraction temperature.

The purity of lignin was determined by two-stage acid hydrolysis. A 200 mg of lignin sample was added into 3 mL of 72% H₂SO₄ slowly and incubated in a water bath at 30 °C for 1 hour. Then, distilled water was added to dilute the mixture to 3%. After being diluted, the mixture was heated under reflux until the boiling temperature, it is left to let the insoluble residue to be sedimented. Filtration was carried out at a weighted sintered crucible, and washed with 500 mL distilled hot water. After washing, it was dried in an oven at 105 °C for 12 hours. The residue is Klason lignin and was then weighed (Jöul et al., 2022).

Ultraviolet-Protection Properties of Lignin

The extracted lignin is blended with the base cream using a magnetic stirrer at speed in a dark room of 600 rpm for 24 hours. The lignin-blended cream with 5 wt% of lignin undergoes centrifugation at 10,000 rpm for 1 hour, to make sure the lignin was dissolved and suspended in the cream. Then, it was applied at 2 $\frac{\text{mg}}{\text{cm}^2}$ to the 3M Transpore Tape which is attached to the surface of a clean quartz plate. The lignin-blended cream was spread over the entire surface by slowly rubbing the slide surface with a thimble-coated finger. After that, it was dried in a dark room for 20 min. UV transmittance was measured by a spectrophotometer. The lignin-blended cream was scanned at four spots in the range from UVB (290–320 nm) to UVA (320–400 nm) (Lee et al., 2019). After determining the UV transmittance, sun protection factor (SPF) was calculated using the equation below (Sadeghifar et al., 2020).

$$SPF = \sum_{290}^{400} \frac{E_{\lambda} S_{\lambda}}{E_{\lambda} S_{\lambda} T_{\lambda}}$$

Where

E_{λ} = CIE erythral spectral effectiveness,

S_{λ} = Solar spectral irradiance

T_{λ} = Spectral transmittance of the sample

RESULTS AND DISCUSSION

Lignin Composition

The total lignin composition in the sugarcane bagasse is determined using Klason lignin method (acid hydrolysis). This was also being used as a reference for the yield of lignin extracted. Before carrying out the pretreatment of sugarcane bagasse, the lignin composition of the SB was determined. According to **Table 1**, the lignin composition of SB was analysed. For scientific experiment, the replicate should be done 3 times to make sure the result is reproducible and low error bar. The average of lignin composition is between 16.7% in 1 gram of SB. Based on the studies conducted and reviewed in literatures, the range of lignin in SB was between 19% to 26%, and the average value was nearly 23% (Carvalho et al., 2021; Ji et al., 2021).

The lignin composition of the result obtained from this experiment was still within the expected range. However, it was differed from the expected average value which is 23% from previous studies (Ratanasumarn & Chitprasert, 2020). This was because the lignin composition of SB was affected by several factors, including the growing conditions, fertilization method, soil management and chemical composition of soil (Mokhena et al., 2018). Another than that, the climate conditions such as the humidity, climate seasons and the method used to test the lignin compositions are also one of the factors affecting the composition of lignin (Barciela et al., 2023).

Table 1 Lignin Composition of Sugarcane Bagasse

Sample	Lignin Composition in Dry Basis (%)
Sample 1	20.1
Sample 2	13.3
Average	16.7 ± 4.8

Yield of Lignin Extracted at Different Alkaline Concentration and Temperature

Yield of lignin extracted from SB by various concentration of sodium hydroxide and temperatures were shown in **Table 2** and **Table 3**. There were two sets of experiments were done, which are vary on NaOH concentration and temperature range. From the result of both sets of experiment, it can be concluded that the highest lignin yield was achieved at the condition of 10% NaOH, at the temperature of 100 °C within 1 hour.

The range of lignin yield obtained were varied from 55.79% to 203.39% for the concentration of NaOH of (2%, 4%, 6%, 8% and 10%) at fixed temperature (100 °C) and time (1 hour). As the NaOH concentration increased, the yield of lignin extracted also increased. This is in accordance to the study from [Ratanasumarn et al., \(2020\)](#) where they stated that lignin yield of sugarcane bagasse increased as the concentration of the NaOH solution increases. In this work, the highest yield of lignin extracted was achieved by utilizing 10% of NaOH.

On the other hand, when studying the temperature parameter, as the temperature was set at 100 °C, the highest yield of lignin had been achieved, which is 198.58%. However, the yield of lignin obtained was already over the limit and higher than 100%, this may due to the exist of impurities. In fact, in the extraction of lignin, there was some components which were not being separated from the lignin in SB. For instance, polysaccharides with the C=O broadening of acetyl and uronic ester group, hemicellulose and cellulose ([Asem et al., 2023](#)).

Table 2 Yield of Lignin Extracted from SB by Different NaOH Concentration (2%, 4%, 6%, 8%, 10%)

NaOH Concentration (% w/v)	Yield of Lignin Extracted (%)
2	55.79 ± 0.64
4	90.35 ± 1.10
6	130.08 ± 0.93
8	150.39 ± 10.20
10	203.39 ± 8.32

Table 3 Yield of Lignin Extracted from SB at Different Temperature (60 °C, 80 °C, 100 °C, 120 °C, 140 °C)

Temperature (°C)	Yield of Lignin Extracted (%)
60	98.98 ± 0.40
80	149.68 ± 5.10
100	198.58 ± 1.92
120	124.52 ± 3.00
140	109.96 ± 5.68

Purity of Lignin Extracted

In order to determine the pure lignin existed in the product, the lignin products were subjected to purity test. In both sets of experiments, the purity test had been conducted on the best result of the parameters that gave the highest yield of lignin. **Table 4** shows the purity of the lignin extracted from 10% NaOH, the average purity was 26.53 ± 2.02%. In addition, the purity of lignin extracted at temperature of 100 °C, the average purity was 28.10 ± 10.75% (**Table 5**).

Comparing to the purity of lignin extracted by using the similar alkaline extraction method, it composed of 63.5% of lignin ([Morales et al., 2018](#)). It was clearly shown that the delignification in this research resulted in lower purity. This

may due to some factors including human error and difference in equipment. The equipment used for lignin extraction is autoclave, which can conduct the extraction in a high-pressure condition, supported by saturated steam, in a more sterilized condition, and thus reduce the impurities in the lignin sample ([Yaakob & Roslan, 2021](#)). Other than that, the low purity may due to the complexity of lignin and hemicellulose segmentation. The lignin was covalently bond to cellulose and hemicellulose. In fact, carbohydrates were covalently anchored by lignin in plant cell walls, reducing the area of cellulose accessible for enzymatic attacks for cleavage ([Tarasov et al., 2018](#)). Therefore, there are multiple complex bonds and interactions between the components and makes the extraction of pure lignin difficult.

Table 4 Purity of Lignin Extracted from SB at 10% NaOH

Sample	Purity of Lignin Extracted (%)
Sample 1	25.10
Sample 2	27.95
Average	26.53 ± 2.02

Table 5 Purity of Lignin Extracted from SB at 100 °C

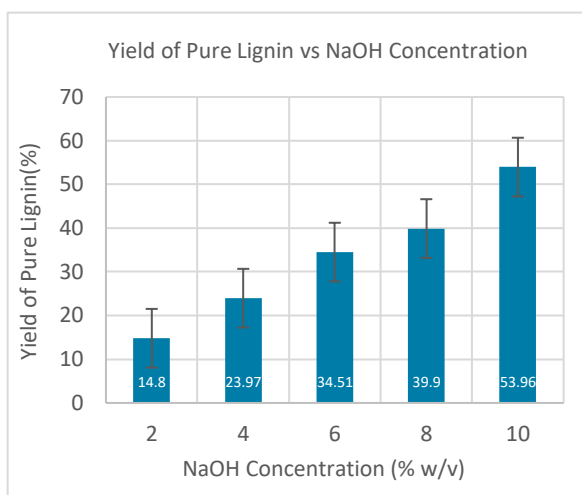
Sample	Purity of Lignin Extracted (%)
Sample 1	35.70
Sample 2	20.50
Average	28.10 ± 10.75

Yield of Pure Lignin Extraction from Sugarcane Bagasse at Different NaOH Concentration

Referring to **Figure 1**, it shows the yield of pure lignin extracted from SB. It was observed that the graph showed an increasing trend as the NaOH increased. The higher the concentration of NaOH used for extraction, the higher yield of pure lignin were able to be extracted. This indicated that NaOH concentration had a significant impact on the delignification percentage. The highest yield, 53.96% can be obtained when using 10% w/v of NaOH concentration.. On the other hand, 6% of NaOH concentration gave only 14.8% of lignin purity.

Alkaline treatment of the biomass able to break the lignin framework and disrupt the linkages between lignin and carbohydrate which able to facilitate carbohydrate separation ([Dhara et al., 2023](#)). NaOH can separate lignin from cellulose and form bonds within the lignin itself. Alkaline hydrolysis occurs between the hydrogen bonds that link lignin to cellulose. As the concentration of NaOH used was directly correlated to the amount of lignin extracted, so at identical conditions, increasing the concentration of base should lead to an increased recovery of lignin from the cellulose fibers. Therefore, when the NaOH concentration was high, the availability of hydroxyl ions also high, more intramolecular ester-linkages in biomass could be broken by increasing alkali concentration by saponification, resulting in the extraction of lignin ([Jung et al., 2020](#)).

However, further increase in NaOH concentration may not have a distinct effect on lignin extraction. This was because the structure of the lignin would be destroyed by NaOH. In fact, not only the C-O bond between lignin and carbohydrates would be hydrolysed, hydroxyl ions are also believed to disrupt the alpha and beta-aryl ether bonds and carbon-carbon bonds in lignin resulting in depolymerization of lignin ([Jung et al., 2020](#)). Thus, the lignin complex may re-aggregate to form undissolved state ([Yang et al., 2018](#)).

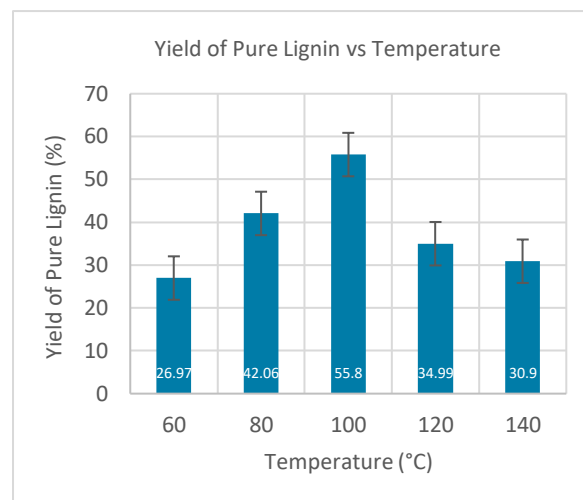
Figure 1 Yield of Pure Lignin Extracted vs NaOH Concentration

Yield of Pure Lignin Extraction from Sugarcane Bagasse at Different Temperature

Figure 2 illustrates the graph of yield of pure lignin extracted from SB according to different temperature. It was observed that the lignin yield shows an increasing trend from 60 °C to 100 °C, and decreases from 120 °C to 140 °C. The highest pure lignin yield was achieved at 100 °C which is 55.8% compared to other temperature. Meanwhile, the lowest yield of pure lignin is 26.97% at 60 °C.

It was observed that when the temperature reaching 100 °C, higher lignin yield obtained. This was because high temperature has a positive impact on the lignin yield at certain range (Morales et al., 2018). Extraction temperature was believed to be more effective in carbohydrate removal, promoting the weakening of carbohydrate-lignin bonds in lignin-carbohydrate complex (LCC). The recovery yield and the stability of lignin during isolation treatment can improve the delignification process, at a high temperature condition in which a study from Ratanasumarn et al., (2020) stated that at high temperature able to have a positive reaction rate but prolong exposure may decrease the lignin content.

However, if the extraction was carried out at temperature that is too high, it will bring an adverse effect on the lignin yield. The quality of the final structure of lignin will be affected, breaking the ether linkages and consequently, resulting in the formation of other undesired compounds (Lobato-Peralta et al., 2021). To be clear, prolong exposure to high temperatures of the SB decreases the total phenolic content in lignin product. This might cause undesired effect on final lignin structure (Ratanasumarn et al., 2020). At a harsh condition such as 120 °C and 140 °C, lignin was degraded into low molecular weight fragments as previously reported by Ugartondo et al., (2008). Therefore, the lignin yield decreases.

Figure 2 Yield of Pure Lignin Extracted vs Temperature

Sun Protection Factor (SPF) of Extracted Lignin

Sun Protection Factor (SPF) value had been determined to obtain the UV-Protection properties of the lignin. The sample used was the lignin obtained at the condition of 6% w/v of NaOH concentration, 100 °C and 1 hour of extraction time. **Table 6** shows the SPF achieved by different concentration of lignin-blended cream. Firstly, the base cream without lignin blended in gave SPF of 0.78. The SPF of 5 wt% lignin-blended cream was 4.76. When 10 wt% of lignin was blended into the base cream, the SPF increases up to 5.24. At higher wt% of lignin, blended into the cream, the colour of the cream became darker. The dark color of lignin is due to the structural changes causes by extraction and separation process (Sadeghifar & Ragauskas, 2020). This is due to during the process, a variety of chromophore group introduced into lignin causes the color becomes dark (Lee et al., 2020). In addition, lignin structure which is unsaturated functional groups that absorb visible light makes the lignin colour turns darker (Sadeghifar & Ragauskas, 2020). This is undesirable to be incorporate into sunscreen due to sunscreen in the market have a white pale colour. **Figure 3a** and **Figure 3b** shows the base cream blended by 5 wt% and 10 wt% of lignin respectively.

When varying the lignin concentration, 10 wt% of lignin in cream resulted in the highest SPF, which indicates the best UV-protection properties towards UVA and from the wavelength of 290 nm to 490 nm. Lignin can be used as a bioingredient in sunscreen as it contained unsaturated functional groups including carbon-carbon double bonds, aromatic rings, phenolic compounds and other chromophores which were UV-protective, anti-aging, and for skin-whitening capabilities (Widsten 2020). Therefore, when there is more lignin in the cream, the more unsaturated functional group like aromatic rings presents to adsorb the UV light, resulting in a higher UV-absorbing ability, and the SPF increases (Paulsson and Parkäs 2012). However, the SPF was low due to the low purity of the lignin sample.

Table 6 Sun Protection Factor (SPF) of Extracted Lignin from Sugarcane Bagasse

Sample	SPF
Based Cream	0.78
5wt % Lignin-blended cream	4.76
10wt % Lignin-blended cream	5.24

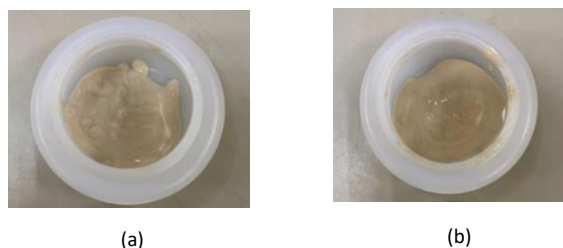


Figure 3 Appearance of: (a) 5 wt% of Lignin-Blended Cream, (b) 10 wt% of Lignin-Blended Cream

CONCLUSION

The research demonstrates the extraction of lignin from SB (*Saccharum officinarum* L.) could be done by alkaline hydrolysis method by using NaOH. The objective of the project was achieved in which optimization of the lignin extraction based on NaOH concentration and temperature was determined and moderate sun protection factor (SPF) obtained. After conducting the study on effect of NaOH concentration and temperature on yield of lignin extracted, it can conclude that the best condition selected for extraction of lignin is 10% NaOH solution, 100 °C, 1 hour. The extraction of lignin from sugarcane bagasse can add value to the waste, able to reduce air pollution problem that caused by burning it. It can also be used in the cosmetic industry for the natural UV-protection product. The SPF of 5.24 can be achieved by 10 wt% lignin-blended cream. However, the dark colour of lignin still remain a challenge to be commercial in the market. There are several studies that could be develop in future to lighten the lignin colour which is by bleaching or decreases the lignin size into nanoparticles.

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