INTRODUCTION

Acrylamide is a crystalline amide that polymerizes rapidly and is formed during the heating process of high-starch food products at high temperatures, above 120°C. The higher the temperature of the production process, the more acrylamide will be formed (Harimadi et al., 2018). The National Toxicology Program has classified acrylamide as a compound that causes cancer or is potentially carcinogenic in humans, in addition, acrylamide can damage nerves and cause fertility disorders and based on animal studies has been shown to cause damage to nerve cells (Rosita et al., 2021).

One of the cooking oils frequently used by the community is palm oil. Heated oil will produce acrylate aldehyde, also known as acrolein, which has a smell similar to burning fat and is characterized by white smoke. Acrolein is one of the acrylamide-forming compounds in addition to being formed from amino acids directly and from the dehydration or decarboxylation of some specific organic acids (Gunarti et al., 2016).

The Prevention of acrylamide formation can be done through the adsorption process. The material commonly used for adsorption is activated charcoal (Mulyati & Panjaitan, 2021; Priyanto et al., 2021; Tanasale et al., 2018). However, the use of activated charcoal as an adsorbent can increase acrylamide levels. The use of activated carbon as a cooking oil purifier has been proven to improve the quality of oil based on SNI 01-3741-2013 but on the other hand, increases acrylamide levels so it is not suitable for re-consumption because of its carcinogenic properties (Gunarti et al., 2016).

The adsorption process can be done by utilizing the remaining agricultural products as adsorbents, one of which is Water Hyacinth. Cellulose has been widely used as an adsorbent, cellulose that has been studied in addition to bagasse as an adsorbent in used frying oil, including research (Takarani et al., 2019) which uses corn husk cellulose as an adsorbent in entangling metal solutions, then research (Safrianti et al., 2012) which uses rice straw waste cellulose as lead adsorption.

Water Hyacinth cellulose which is quite high at 60% (Heriyanto et al., 2015) based on research (Moeksin et al., 2016) the cellulose content in water hyacinth is 64.51% where cellulose contained in this hyacinth has an OH group that can potentially bind acrylamide compounds in frying oil. However, the use of cellulose as an adsorbent for acrylamide has not yet been explored.

Abstract

Cooking oil frequently used by the community is palm oil. When heated repeatedly, this oil can produce acrolein, a compound that contributes to the formation of acrylamide. Water Hyacinth cellulose contains -OH groups capable of binding to acrylamide. The purpose of this study was to determine the effect of adding Water Hyacinth cellulose as an adsorbent and to evaluate the impact of soaking time and cellulose weight on acrylamide levels. The analysis was conducted using HPLC, with a mobile phase consisting of methanol and 0.1% phosphoric in a ratio of 5:95, at a flow rate of 1mL/min; the volume of the injected sample was 20 µL. Acrylamide in oil was identified at a retention time of 4.700 minutes. The regression equation obtained from the acrylamide calibration curve is y = 229.52 x + 10.472 with a correlation coefficient (R²) of 0.9988. This study concluded that soaking for 24 hours can reduce acrylamide levels by 69.13%, and using 6 grams of cellulose can reduce acrylamide levels by 75.15%.

Keywords: Water Hyacinth, acrylamide, cellulose, HPLC.
Therefore, this study aims to determine the effect of adding Water Hyacinth cellulose on acrylamide levels in frying oil.

**METHODOLOGY**

**Materials and Instrumentals**

**Materials**

Frying oil was obtained from fried chicken preparations. Water Hyacinth was collected from a lake in Tasikmalaya. The chemicals and reagents used included sodium hypochlorite NaOCl, sodium hydroxide NaOH p.a, 85% phosphoric acid, activated carbon from Sigma, Aqua pro injection, dichloromethane, and methanol for HPLC.

**Instrumentals**

The equipment used in this study included an HPLC (Agilent Technologies 1120 compact LC), UV-Vis spectrophotometer (Genesys 10s UV-Vis), injection tissue filter for HPLC bottles, micropipette, analytical scales, an ultrasonic measuring device, a hot magnetic plate stirrer, a sieve 40, 50 mL brown bottles, a Buchner funnel, a Buchner Erlenmeyer flask, a water bath shaker (18-one), a simplisa blender, and an FTIR (Agilent carry 630).

**Methods**

**Isolation of Cellulose**

The cellulose isolation method, as referenced from (Wulandari & Dewi, 2019a), includes several modifications. The dried Water Hyacinth is subsequently chopped into small pieces. The chopped hyacinths are ground into a powder. The Hyacinth powder is bleached with 250 mL of 0.735% (w/v) sodium hypochlorite for 6 hours with constant stirring at a temperature of 45°C. The residues are then washed with distilled water until a neutral pH is achieved. The neutralized residue is refluxed with 150 mL of 17.5% sodium hydroxide for 3 hours, with constant stirring at 45°C. Afterward, the residue is washed to reach a neutral pH and dried at room temperature for 2-3 days.

**Infrared spectroscopy (IR) analysis**

The water hyacinth and ultraviolet FTIR spectrometers were recorded on the Agilent carry 630 Infrared Spectrometer. The sample preparation for cellulose is to place a portion of hyacinth and water hyacinth cellulose on the sample compartment. Wavenumber from 4000 cm⁻¹ to 600 cm⁻¹.

**Preparation of The Mobile Phase**

The mobile phase consists of a mixture of methanol and 0.1% phosphoric acid in 5:95. The mixture is homogenized and filtered using an eluent filter. The air that is in the mobile phase is removed using an ultrasonic stirrer.

**Calibration Curve Creation**

A standard solution of acrylamide is injected in the amount of 20 µL into the column. The concentration range of the standard acrylamide solution used is 1, 2, 3, 4, 5, and 6 ppm.

**Impact of Soaking Duration and Water Hyacinth Cellulose Adsorbent Weight on Acrylamide Reduction Levels**

To assess the impact of adding Water Hyacinth cellulose on acrylamide levels, experiments were conducted by incorporating 2, 4, and 6 grams of water hyacinth cellulose into used frying oil that had been spiked with 50 ppm acrylamide and allowing it to soak for 24 hours. Immersion of used frying oil using 2 grams of Water Hyacinth cellulose was carried out for 24, 48, and 72 hours.

**Determination of Acrylamide Content in Used Frying Oil Samples**

The sample was spiked with an acrylamide comparator then added 2 grams of Water Hyacinth cellulose adsorbent and stirred with a magnetic stirrer for 10 minutes and then left to stand for 24 hours. After that, the extraction process was carried out. The samples were dissolved in 60 mL of dichloromethane and 3 mL of ethanol was added. It was shaken on an orbital shaker at 120 rpm for 10 minutes. The solution was subsequently filtered, and 25 mL of the mobile phase was added to the filtrate. The dichloromethane and ethanol were evaporated by heating in a water bath at 80°C for 5 hours. Afterward, the mixture was centrifuged for 15 minutes, and the bottom layer was collected and tested using HPLC.

**Data Analysis**

To evaluate the reduction in acrylamide levels in used frying oil, the linear regression equation of the calibration curve. Then to determine the percent decrease in acrylamide levels, the formula is used:

\[
\frac{\text{Initial level} - \text{Final level}}{\text{Initial level}} \times 100\% \quad \text{........................................(1)}
\]
RESULTS AND DISCUSSION

Isolation of Cellulose

Cellulose isolation is divided into two stages, during the lignin removal process a solution of sodium hypochlorite (NaOCl) is used which is a strong oxidizer so that it can break the ether bonds in the lignin structure (Rachmawaty et al., 2013). The process of removing hemicellulose involves using a sodium hydroxide (NaOH) solution, a strong base capable of severing the hemicellulose chain from the main cellulose chain. This isolation process causes a change in the physical form of Water Hyacinth powder, which is initially green in color to a grayish white color.

![Figure 1. Cellulose of Water Hyacinth](image1)

The yield produced from this cellulose isolation process averaged 32.57%, the percent yield was greater when compared to the Water Hyacinth yield in the study (Pratama et al., 2019) which only produced a yield of 6-20%.

Infrared spectroscopy (IR) analysis

The loss of lignin and hemicellulose was determined using FTIR characterization. Based on Figure 2

![Figure 2. FTIR Spectrum of Water Hyacinth and Its Cellulose](image2)

In the IR spectrum results, two peaks are present in water hyacinth but absent in cellulose. The peak at wave number 1732.5 cm\(^{-1}\) indicates the presence of acetyl and ester groups in the carboxyl group chain, signifying the presence of lignin and hemicellulose. The peak at wave number 1244.37 cm\(^{-1}\) indicates C-O vibrations for aryl groups in lignin. This aligns with research conducted by Rachmawaty et al., (2013), which shows that the wave number 1734.5 cm\(^{-1}\) indicates the presence of lignin and hemicellulose. In the research by (Wulandari & Dewi, 2019b), it is mentioned that wave number 1730.57 cm\(^{-1}\) indicates the presence of carboxylic groups in hemicellulose and lignin, while wave number 1249.80 cm\(^{-1}\) indicates aryl groups in lignin. From this, it can be concluded that the resulting cellulose is free of lignin and hemicellulose. Additionally, the IR spectrum results for cellulose show peaks at wave numbers 3278.72 cm\(^{-1}\) and 2920.79 cm\(^{-1}\), indicating the presence of -OH and C-H groups, which are the primary functional groups of cellulose.

Extraction of Acrylamide in Used Frying Oil

In the extraction process using dichloromethane solution because acrylamide is polar so that it can bind acrylamide and to increase the solubility of acrylamide in dichloromethane, ethanol is added (Gunarti et al., 2016). While the addition of 0.1% phosphoric acid and methanol aims to bind acrylamide stored in dichloromethane when the heating process takes place to evaporate dichloromethanes, the results of acrylamide extracts are clear solutions and a slight smell of oil. Then after that, the analysis of the determination of acrylamide levels in used frying oil was carried out. The results of acrylamide extraction from used cooking oil are shown in Figure 3.

![Figure 3. Acrylamide extracted](image3)

Maximum Wavelength of Acrylamide

The reference acrylamide was dissolved in a mobile phase composed of methanol and 0.1% phosphoric acid in a 5:95 ratio. The measurements
using UV-Vis Spectrophotometry showed maximum absorption at a wavelength of 198 nm, which is consistent with the research conducted by (Wardani et al., 2022) where the maximum absorption of acrylamide is at a wavelength of 198 nm. Acrylamide can be analyzed using UV-Vis spectrometry due to the presence of chromophore groups (which have conjugated double bonds) and auxochrome groups (which possess free electron pairs). The chromophore group in acrylamide is represented by the C = C-C = O (dienone) group, while the auxochrome group consists of NH₂ (amine). The maximum absorption spectrum of acrylamide is shown in Figure 4.

**Figure 4. Absorption spectrum of acrylamide**

**Calibration Curve**

The acrylamide calibration curve is made to determine the relationship between the level of the sample solution and the instrument response expressed as a straight line (linear). The linearity used in this test starts from concentrations of 1, 2, 3, 4, 5, and 6 ppm. Data from the calibration curve determination yielded a regression equation of $y = 229.52x + 10.472$ with a correlation coefficient ($R^2$) of 0.9988. This linearity test aims to prove that the analyte can give a response that is directly proportional to the concentration. The calibration curve graph is shown in the figure 5.

**Figure 5. Calibration curve of Acrylamide**

**Effect of Soaking Time and Adsorbent Weight on the Reduction of Acrylamide Levels**

Soaking time of Water Hyacinth cellulose as adsorbent has a great effect on the amount of acrylamide adsorbed. The longer the soaking time, the lower the acrylamide content absorbed. This is illustrated in Figure 6, which shows the effect of time on the percentage decrease (%) in acrylamide content.

**Figure 6. Impact of soaking Duration of Water Hyacinth cellulose on adsorbed acrylamide**

Figure 6 shows that the maximum amount of acrylamide that can be absorbed by Water Hyacinth cellulose is at a soaking time of 24 hours with a percent reduction of 69.31%. This is because Water Hyacinth cellulose contains -OH groups that can absorb NH₂ groups on acrylamide. This is following research conducted by (Wardani et al., 2022) where the -OH group can bind to acrylamide compounds, and the high polarity of acrylamide causes it to be bound to polar groups such as the -OH group.

**Figure 7. Interaction between Acrylamide and Cellulose**
Figure 6 shows that the percentage of acrylamide adsorbed decreases with increasing soaking times, suggesting that the optimal adsorption time is 24 hours. After 24 hours, an equilibrium condition is reached, leading to the release (desorption) of acrylamide and a subsequent reduction in the adsorption percentage (Hasmalina Nasution, 2016; Sera et al., 2019).

Regarding the variation in the weight of Water Hyacinth cellulose adsorbent, increasing the adsorbent weight significantly reduces acrylamide levels in used frying oil. The graph showing the relationship between the addition of adsorbent weight and the reduction in acrylamide levels can be seen in Figure 8.

![Graph](image)

Figure 8. Impact of increasing Water Hyacinth cellulose weight on acrylamide adsorption

The figure above indicates that adding Water Hyacinth cellulose results in a significant decrease. A high enough decrease occurred in the weight variation of 6 grams with a percent decrease in content of 75.15%. This occurs because, as the mass of the adsorbent increases, its capacity to absorb acrylamide also increases. This is consistent with research conducted by (Hossain et al., 2012) and (Alfiany & Bahri, 2013) which found that as the mass of the adsorbent increases, the amount of compound absorbed by the adsorbent also increases. This is proportional to the increase in the number of particles and the surface area of the adsorbent, leading to higher adsorption efficiency. According to (Anjani and Koestiari, 2014), the quantity of adsorbent plays a crucial role because it tends to enhance the adsorption capacity of the adsorbate. Based on FDA (2004), it is stated that the maximum tolerance limit of acrylamide in the body is 2 ppm so the adsorption process using adsorbents needs to be done to reduce the acrylamide levels contained in used frying oil.

**CONCLUSION**

Water Hyacinth cellulose can reduce acrylamide levels in used frying oil. Weight addition of water hyacinth cellulose and soaking time can reduce acrylamide levels in used frying oil. The weight variation of 6 grams can reduce acrylamide levels by 75.15% while in the variation of soaking time, the 24-hour variation can reduce acrylamide levels by 69.31%.

**REFERENCES**


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