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Hydrogen Cyanide (HCN) Detoxification of Crude Rubber Oil Seeds Using Ultrasonic Waves and Neutralization

To cite this article: Neswati and S D Ismanto 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **515** 012013

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Hydrogen Cyanide (HCN) Detoxification of Crude Rubber Oil Seeds Using Ultrasonic Waves and Neutralization

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Abstract. Rubber seeds contain high levels of Hydrogen Cyanide (HCN) and oils. Rubber seed oil contains a large number of free fatty acids. The uses of rubber seeds and oils could be more extensive if the HCN and free fatty acid content could be reduced. The HCN content can be reduced with ultrasonic waves with certain process conditions. while the high fatty acid content can be reduced by conducting several neutralization processes. The purpose of this study was to determine the optimal temperature and duration of these detoxification processes based on HCN content in distilled water. as well as the number of neutralization processes that must be carried out. This study consisted of 2 stages; 1) reducing the HCN levels of rubber seeds with ultrasonication varying temperature (30 ° C. 40 ° C. 50 ° C) and duration (20 minutes. 40 minutes. 60 minute); 2) reducing the levels of seed oil free fatty acids by neutralization processes conducted 1 or 2 times. The results showed that the best process conditions for detoxifying HCN rubber seeds was ultrasonication at 50 ° C for 60 minutes followed by 2 neutralization cycles.

Keywords: Rubber Seeds, Hydrogen Cyanide, Ultrasonic Waves, Neutralization

1. Introduction

Rubber (*Havea braziliensis*) is one of the leading plantation commodities in Indonesia. Rubber plantations are spread throughout almost every province in Indonesia. including West Sumatra. According to [1]. the area of rubber plantations in West Sumatra in 2016 was 130.000 hectares with total production of around 135.900 tons and which increased to 130.700 hectares with 159.700 tons by 2017. The main yield of rubber is latex. while the byproducts include wood or rubber tree trunks and rubber seeds. According to [2.3]. the most abundant byproduct is rubber seeds. Most rubber seeds are just thrown away [4] with a small portion is used for breeding purposes [5]. Rubber seeds contain about 35-40% oil [6.7.8.9.10]. This high oil content has attracted the attention of scientists as a potential raw ingredient in biodiesel and soap. In addition, rubber seed oil has potential to be processed into a food oil [11]. because the composition of omega 3 (linolenic acid). omega 6 (linoleic acid) and omega 9 (oleic acid) oils is very high [12]. However unprocessed rubber seed oil contains 111.19 ppm HCN in the form of linamarin [11] which is toxic to humans and animals. The amount of cyanide that enters the body must not exceed 1 mg per kilogram of body weight per day [13].

HCN is soluble in water and volatile at high temperatures so the HCN content in rubber seeds can be reduced by removing the seed coat. slicing. soaking with water and heating. The temperature and extraction time greatly affect the amount of HCN extracted and the quality of the rubber seed oil. However, heating of rubber seeds must be done carefully because of the high



content of unsaturated fatty acids which oxidize easily. One way to reduce the amount of heat necessary is sonication which uses ultrasonic waves (frequency 42 kHz) allowing the use of a lower temperature and a shorter heating time. Ultrasonic wave energy causes cavitation, the formation and collapse of small bubbles which help the diffusion of solvents into plant cell walls [14].

The quality of rubber seed oil is also affected by the refining process. The oil purification process includes purification, degumming, neutralization, decolorization and deodorization. The neutralization process is important in modifying rubber seed oil to meet the requirements for food oil by reducing the amount of free fatty acids. According to [15] free fatty acids make up about 17% crude rubber seed oil and acid number 34. According to [16] the acid number for cooking oil must be below 0.6 mg KOH / g equivalent and free fatty acids less than 0.3% [17]. High levels of free fatty acids from rubber seed oil can be reduced by a neutralization process using NaOH. High levels of free fatty acids can be reduced by neutralizing several times until the contents are in accordance with food oil standards.

2. Methods

2.1 Materials

Raw material: rubber seeds originating from the Nagari Sitanang area, Lareh Sago Halaban District, Lima Puluh Kota District. Chemicals: 0.1 N NaOH, alcohol, PP Indicator, chloroform, 15% KI, 0.1 N Na₂S₂O₃, starch indicator, 95% ethanol, KOH, 0.5 N HCl, distilled water, hexane solvent, anhydrous sodium sulfate.

2.2 Methodology

This study consisted of two stages: stage 1 reducing the levels of HCN rubber seeds with ultrasonic waves by using 2 treatment factors, namely: temperature (30 ° C, 40 ° C, 50 ° C) and duration (20 minutes, 40 minutes, 60 minutes); stage 2 reducing the levels of seed oil free fatty acids by the neutralization process which was conducted 1 time and then twice. Each treatment was carried out in triplicate. The data obtained was averaged and the standard deviation calculated.

2.3 Research Implementation

2.3.1 HCN rubber seed extraction using ultrasonic waves (modification [18])

10 grams of rubber seed meat that had been separated from the shell and broken up was added to 220 ml of distilled water (ratio of ingredients to solvent = 1 gr: 22 ml). Extraction of HCN with sonication then was conducted at the treatment temperature. HCN analysis of the result was carried out based on the official AOAC method 915.03 (part B, the alkaline titration method) calculated using the formula [19]:

$$X = C \times V \times 54 \times \frac{\text{dilution}}{\text{aliquot}} \times \frac{1000}{m} \quad 1$$

Where X is the content of cyanide acid (mg / kg); m represents the weight of the sample (g); C is the concentration of a standard solution of silver nitrate (mol / L); V is the volume of the standard silver nitrate solution.

2.3.2 Rubber Seed Oil Extraction (Modified [20])

Rubber seed meat with the lowest HCN content obtained from the Phase 1 study was then solar so that the oil came out when the seeds were pressed (moisture content 10%). This seed meat was placed in an aluminum foil container and then heated in an oven at 70 ° C for 30 minutes to facilitate the hydraulic pressing process. The temperature used is 70 ° C for 20 minutes. The containers with rubber seed meat were transferred to a felt tool to cool to room temperature. The meat was pressed for 20 minutes, at 250 kg / cm and the resulting oil placed in a 250 ml measuring

cup and filtered using filter paper. to remove impurities then stored in a tightly closed dark bottle to avoid oxidation.

2.3.3 Neutralization of Rubber Seed Oil (Modification of [21])

100 mL of oil was put into a 250 mL cup with 10 mL of 0.5 N NaOH and warmed up to 60 ° C while stirring. Warming was continued stirring slowly after the soap globules formed. When the soap particles settled. heating and stirring were stopped (at this time the soap was at 80-85 ° C). The mixture was left for some time so that the soap stock became separated on the surface. The clear oil was separation from the soap stock. For the second treatment this process was repeated twice. The neutralized oil free fatty acid levels were analyzed based on the AOCS Official Method Ca 5a – 40, free fatty acid % being calculated by the formula:

$$\% \text{ FFA as oleic acid} = \frac{\text{volume of alkaline (mL)} \times \text{normality of alkaline} \times 28.2}{\text{sample weight (g)}} \quad 2$$

3. Results and Discussion

3.1 Analysis of HCN dissolved in water and in Rubber seed meat. (Step 1)

Rubber seeds contain potentially levels of HCN at around 111.19 ppm [11]. To reduce this. day long heating and soaking are usually carried out. Ultrasonication speeded up the process. The resulting levels of HCN are shown in Table 1 and 2.

Table 1. HCN content dissolved in aquades (ppm)

Treatment	B1=20 minutes	B2=40 minutes	B3=60 minutes
A1=30°C	165.00 ± 0.11	223.67 ± 0.06	289.67 ± 0.05
A2=40°C	194.33 ± 0.06	231.00 ± 0.01	282.33 ± 0.03
A3=50°C	227.3 ± 0.06	271.33 ± 0.03	326.33 ± 0.06

Table 1 shows HCN concentrations between 165.00 ± 0.11 ppm and 326.33 ± 0.06 ppm. The lowest value was from 20 minutes at 30°C and the highest after 60 minutes at 50°C.

Higher temperatures and longer extraction times lead to more of the HCN being dissolved out into the water. This is because the effect of the heat on the cell wall makes it easier for the cell to release the HCN. [18].

Longer extraction times lead to more HCN dissolving out as the longer the particles have in contact with the water the greater the number of cell walls that break down due to cavitation allowing the HCN to pass into solution [18].

Table 2. HCN content of Rubber Seed Meat (ppm)

Treatment	B1=20 minutes	B2=40 minutes	B3=60 minutes
A1=30°C	72.67 ± 0.11	55.00 ± 0.03	50.67 ± 0.01
A2=40°C	7.00 ± 0.17	55.33 ± 0.06	48.00 ± 0.03
A3=50°C	58.00 ± 0.05	49.67 ± 0.06	46.00 ± 0.02

Table 2 shows that 60 minutes treatment at 50°C reduced HCN content by 88% to 46.00 ± 0.02ppm. This value is lower than the results of [23] who obtained 1500 ppm by using maceration and an hour of processing at 100°C in hexane [23]. A series of extraction and clarification and neutralization processes is expected to further reduce HCN levels. According to [24] after crude rubber seed oil was purified with 3% bentonite there was a 30% decrease in HCN content.

3.2 Neutralization (Step 2)

The best HCN detoxification process was at 50 ° C for 60 minutes. The neutralization process purifies the oil by using alkali to remove free fatty acids which can cause lipid oxidation by forming soap [25]. This neutralization process can help remove dyes and impurities like sap [26].

With the right concentration of KOH all of this alkaline and free fatty acids can be removed in the form of soap.

Table 3. Effect of Number of Neutralization Processes on Water Content and Volatile Compounds, Free Fatty Acid Levels, Acid Numbers, Peroxide Numbers and HCN Levels in ultrasonicated detoxified Rubber Seed Oil

Treatment	Water content and volatile compounds (%)	Free fatty acids (%)	Acid number (mg KOH / g)	Peroxide Number (meq O ₂ / kg)	HCN (content (ppm))
One step neutralization	0.49±0.02	19.77± 0.05	28 ± 0.01	1.69 ± 0.25	5.1 ± 0.02
Two step neutralization	0.48±0.01	15.25 ± 0.07	21.6 ± 0.01	0.00	4.9 ± 0.01

3.2.1 Water content and volatile compounds

Water content and evaporated material are calculated based on the weight lost during heating in the oven at a temperature (130 ± 1) ° C [16]. Table 3 shows that the more extensive the neutralization process, the lower the water content and volatile compound content of the rubber seed oil. This is because the neutralization process can reduce the content of volatile compounds in oil. [25] states that purification with alkali can reduce the content of volatile compounds in oil, free fatty acids, pigments (such as carotene and chlorophyll), metals, oxidation products, phospholipids and soap.

Water and volatile compound content obtained from the study ranged from 0.48 ± 0.01 to 0.49 ± 0.02%. [27] found the water content of rubber seed oil compressed at 70 ° C was 0.2%. According to the SNI 3741: 2013 standard the water and volatile compound content of cooking oil should be less than 0.15% [16]. The high value in this study was due to the fact that some of the aquades used to extract HCN bound to the fibers in the rubber seeds. When the rubber seeds are extracted by a hot press method, water that is bound to the rubber seed fiber comes out with the oil as the 70 ° C pressing temperature is not enough to evaporate this water. 70 ° C was chosen to protect the unsaturated fatty acid rich oil. Furthermore, HCN being also volatile at high temperatures can also increase this water and volatile compound content value. Vegetable oil consists of a complex mixture of glycerides (mainly triacylglycerols with some diacylglycerol and monoacylglycerol), hydrocarbons, alcohol, phospholipids, free fatty acids, pigment, sterol, tocopherol, and volatile compounds [25]. Some volatile compounds contained in vegetable oils are in the form of polycyclic aromatic hydrocarbons.

3.2.2 Free fatty acid content

Free fatty acids are fatty acids that are not bound to triglycerides and are hydrolysis products from oils and fats [28]. The presence of water in oil and the heating process will encourage a hydrolysis reaction that involves breaking of the triglyceride bonds producing glycerol, di- and mono-acylglycerol and free fatty acids [29]. The free fatty acid content is an indication of damage to fat or oil and can be reduced by the process of neutralization which can be carried out several times depending on the content of free fatty acids.

In Table 3 it can be seen that the free fatty acid content of rubber seed oil that had been neutralized in a 2 step process was 15.25 ± 0.07%. Free fatty acid levels in rubber seed oil are around 0.84 - 42.412% [30], 28.47% [31]. The more extensive the neutralization process, the lower the free fatty acid content because the neutralization process uses alkali which reacts with free fatty acids to form soap. This soap is then separated from the oil using a centrifuge [25].

Rubber seed oil has a high free fatty acid content and the 2 stage neutralization process was not sufficient to significantly reduce this. According to [32] the free fatty acid content of fresh rubber seed oil is 17%. The free fatty acid content obtained from this study is slightly lower than the 17%

found by [15]. This is due to the high water content of rubber seeds due to preliminary treatment to reduce HCN levels by using distilled water. The fibers in the rubber seeds bound to aquades and the air drying process is not able to evaporate this water optimally. Water in the rubber seeds and heat during compression resulted in hydrolysis producing free fatty acids.

This 15.25% free fatty acid content means that this treatment does not result in rubber seed oil that meets the requirements for cooking oil (a maximum of 0.3%) [17] or for biodiesel raw materials (around 2%) [15]. Further neutralization would be necessary to reach these levels but with each neutralization stage the oil yield reduces.

3.2.3 Acid numbers

Acid numbers indicate the amount of total acids found in oil, both free fatty acids and fatty acids that are bound to triglycerides. In Table 3 it can be seen that the rubber seed oil acid number ranges from 28 ± 0.01 mg KOH / g to 21.6 ± 0.01 mg KOH / g. According to [30] the acid number of rubber seed oil ranges from 1.68 to 42.41 mg KOH / g. With more neutralization the acid number will reduce as more fatty acids are neutralized by KOH and removed as soap and neutralization can reduce the acid number in rubber seed oil by almost 50%. According to [33] the rubber seed oil acid number is 82 mg KOH / g. Acid numbers could be reduced further by carrying out the neutralization process more than 2 times and carrying out other refining processes such as degumming, decolorization, and deodorization to bring the acid numbers down to the standard for cooking oil (a maximum of 0.6 mg KOH / g).

3.2.4 Peroxide Number

Oils that contain lots of unsaturated fatty acids are very easily undergo auto-oxidation [34]. According to [35], the higher the unsaturation of fatty acids, the easier an oil is oxidized. For example, linoleic acid (18: 2) is more easily oxidized than oleic acid (18: 1). [36] states that rubber seed oil is high in unsaturated fatty acids, especially oleic acid and linolenic acid. In this experiment a lower peroxide number was obtained because the preliminary treatment process (to reduce the HCN content at 50°C) and the pressing process (60°C) took place at low temperatures.

In Table 3 it can be seen that the peroxide number obtained is 0.00 meq O_2 / kg - 1.69 ± 0.25 meq O_2 / kg. The second neutralization process eliminated the peroxide content in rubber seed oil by reducing or even eliminating the peroxide content. According to [25] the neutralization process can also reduce the oxidation products contained in oil.

Cooking oil must have a peroxide number lower than 10 meq O_2 / kg based on SNI 3741: 2013 and the two stage neutralization process resulted in rubber seed oil met that requirement.

3.2.5 HCN Content

[11] states that raw rubber seeds have HCN levels of 111.19 ppm. Preliminary treatment using ultrasonic and neutralization significantly reduced HCN content in rubber seed oil to 5.1 ± 0.02 ppm - 4.9 ± 0.01 ppm.

In Table 3 it can be seen that the two stage neutralization process lowered the levels of HCN the most because it not only neutralizes free fatty acids but also HCN. The reaction of HCN with a base will produce solid cyanide which is then easily separated out.

3.2.6 Fatty acid profile

Based on Table 3 it can be seen that rubber seed oil which has been neutralized in a two-step process produced the rubber seed oil with the better chemical properties. This oil was then analyzed to determine the type and content of each fatty acid. A GCMS chromatogram of rubber seed oil can be seen in Figure 1. The profile and levels of fatty acids in rubber seed oil can be seen in Table 4.

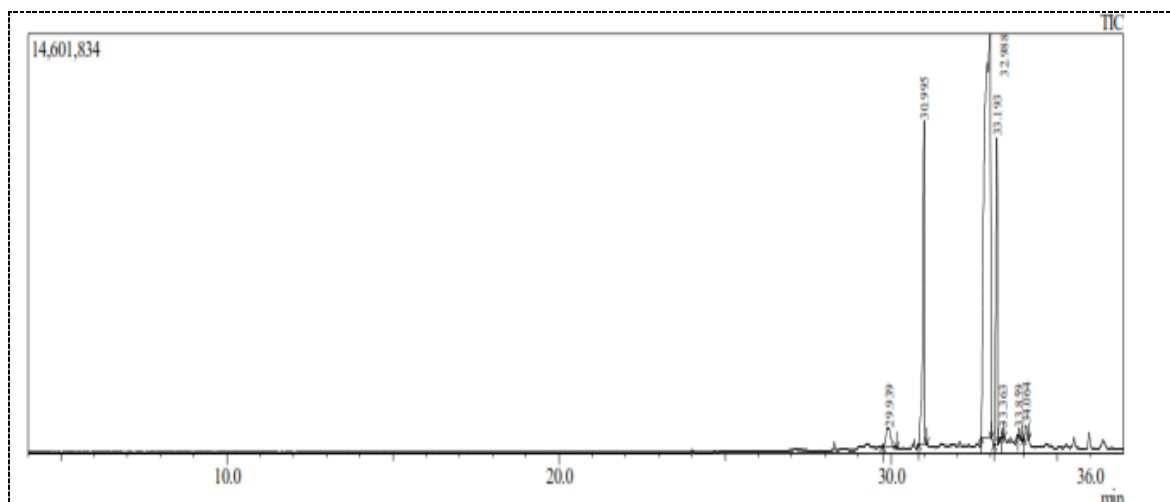


Figure 1. GCMS chromatography of rubber seed oil

Table 4. Rubber seed oil Fatty Acid content and Profile

Fatty Acid	Content (%)
Polyunsaturated fatty acids (omega 3 fatty acids)	
Linolenic Acid (C18: 3)	0.29
Polyunsaturated fatty acids (omega 6 fatty acids)	
Adrenic Acid (22: 4)	0.92
Monounsaturated fatty acids (omega 9 fatty acids)	
Eicosenoic Acid11 (C20:1)	2.88
Oleic Acid (C18: 1)	67.32
Saturated fatty acidsPalmitic acid (C16: 0)	
Palmitic acid (C16: 0)	14.95
Heptadecanoic Acid (17: 0)	0.14
Stearic acid (C18: 0)	12.42
Myristic acid (14: 0)	0.25
Arachidic acid (20: 0)	0.83
Total fatty acids	100

In Table 4 above it can be seen that the main types of fatty acids that make up rubber seed oil are unsaturated fatty acids (71.41%) including oleic acid (67.32%), eicosenoic acid 11 (2.88%), adrenic acid (0.92%) and linolenic acid (0.29%). The high unsaturated fatty acid content means rubber seed oil is easily oxidised so processing and storage need to take account of this. The high Polyunsaturated Fatty Acid (PUFA) content in rubber seed oil can be added to egg yolks to reduce cholesterol content in egg yolks [37].

4. Conclusion

The best HCN detoxification process of rubber seeds used ultrasonic waves at 50 ° C for 60 minutes resulting in a HCN content in aquades of 326.33 ppm solution and 46 ppm in the pulp. The two step neutralization was the best treatment with regard to moisture and volatile substances content of 0.48%, free fatty acid levels of 15.25%, Acid Number 21.6 mg KOH / g, Peroxide number 0 meq O₂ / kg and 4.9 ppm.

Acknowledgements

Gratitude is expressed to the Manager of the DIPA research program Faculty of Agricultural Technology Andalas University who helped by funding this research.

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