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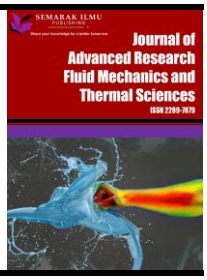

Hydrolysis Process of Red Sorghum Grains for Producing Bioethanol

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ABSTRACT

As part of the effort to continuously enhance the development and utilization of bioenergy/bioethanol and to ensure its sustainability, using sorghum as a raw material for bioethanol production requires a study to obtain efficient hydrolysis conditions and achieve optimal bioethanol conversion. This research method is divided into two main parts: samples with pretreatment and without pretreatment. The pretreatments used NaOH solution (0.05% and 0.1%); soaking temperatures at 35,40 and 45^{o}C; and soaking time at 30, 60, 90, and 120 minutes. The liquefaction and saccharification results with pretreatment showed a greater decrease in viscosity values after both liquefaction and saccharification, and the RS and DE results also demonstrated an increasing trend compared to samples without pretreatment. It is due to the presence of protein bodies in sorghum that inhibited hydrolysis without applying the pretreatment. The best result on the reduced sugar (RS) value achieved was 10.43%, and the DE value was 67.90%. Thus, the optimum operational condition was achieved with a sample with 0.05% NaOH soaking for 90 minutes at 45°C (A9). Subsequently, fermentation was carried out on sample A9 with pretreatment and sample without pretreatment (A0/B0). The alcohol obtained from fermentation with pretreatment was 9.5%, with a fermentation ratio (FR) of 86.97%. Meanwhile, the alcohol yield from fermentation for the sample without pretreatment was 8.9%, with an FR of 83.02%.

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1. Introduction

Sorghum (*Sorghum bicolor*) originates from Africa and belongs to the Poaceae family [1]. Sorghum is the world's fifth most important cereal crop after maize, wheat, rice, and barley [2]. This Sorghum plant can also withstand drought conditions better than other cereal crops, making it a viable alternative for food production and as an energy source [3,4,8,10].

Sorghum contains a high amount of carbohydrates (74%), protein (11.3%), and fat (3.3%). Sweet sorghum, in particular, has an even higher carbohydrate content [6]. The carbohydrate content in sorghum grains is not much different from wheat, and higher than rice and corn, making it a potential raw material for bioethanol production. A comparison of the nutritional composition of sorghum and other cereals is shown in Table 1 below.

Table 1
Comparison of nutritional content of cereals [6]

Nutrient Units	Sorghum	Wheat	Rice	Corn
Energy (Kkal)	339,0	342	360	365
Protein (gr)	11,3	11,3	6,61	9,42
Carbohydrate (gr)	74,6	75,90	74,26	72,56
Fat (gr)	3,3	1,71	0,58	4,74
Fiber (gr)	2,7	12,2	7,3	-

The sorghum grain consists of three different parts, namely the pericarp (outer layer), embryo (germ), and endosperm (storage tissue). The relative proportions of these parts vary based on hybrids and the environment (for example, the embryo may be larger than the endosperm if the grain develops under stress) [7]. The structure of sorghum grains is shown in Figure 1 in more detail. The chemical composition of each part of the sorghum grain can be seen in Table 2. Table 2 shows that the overall composition of sorghum grains contains 73.8% starch, 12.3% protein, and 3.6% fat.

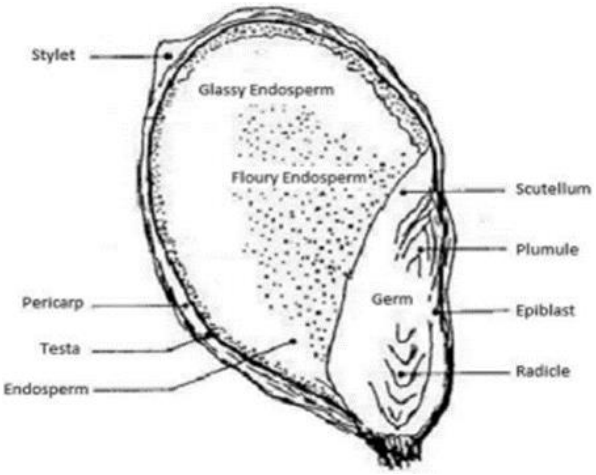

Fig. 1. Sorghum grain structure [5]

The overall chemical composition of sorghum grains is shown in Table 2.

Table 2
Chemical composition of whole sorghum [7]

Component	Whole Grain %
Sorghum grain	100
Protein	12.3
Protein Range	11.5 - 12.3
Total Protein	100
Fat	3.6
Fat Range	--
Total Fat	100
Starch	73.8
Starch Range	72.3 - 75.1
Total Starch	100
Ash	1.6
Ash Range	1.6 - 1.7
Total Ash	100

Among the factors influencing the visual color of grain sorghum are the genetics of pericarp color, pericarp thickness, the presence or absence of a testa, color, and thickness of the testa, and the endosperm color (Rooney and Miller, 1981). Pericarp thickness ranges from 8 to 160 um. Pericarp thickness influences grain color to range from shades of white to shades of pink, orange, red and even brown. In hybrids with very thin pericarp, the grain coat is almost transparent and the grain will have a translucent appearance.[7]. The explanation of the characteristics and color of sorghum grains can be seen in Table 3.

Table 3
Grain characteristics of color sorghum [7]

Characteristics of Sorghum	The Color of Sorghum Grains
Thin Pericarp	White to pink, orange, red, brown
Thick Pericarp	Dull, white to brown, depending on the pericarp
Testa present	Depends on the thickness of the testa, may contain tannins
Testa absent	Usually tannin-free, depends on the pericarp
Transparent pericarp	Grain color results from the combination of pericarp and endosperm, e.g., bronze from red pericarp and yellow endosperm
Intensifier gene	Affects the intensity of pericarp color, hybrids with bright red or orange Grains
Spreader gene	Causes brown grains in hybrids with testa layers; in the absence of the spreader gene, high-tannin grains with thick white pericarp may appear white.

The composition and characteristics of sorghum grains will indeed influence the color of sorghum grains, and various combinations of these characteristics can result in different grain colors as shown in Table 4 [7].

Table 4
Grain characteristics and resulting color in sorghum [7]

Pericarp Color	Pericarp Thickness	Endosperm Color	Tannin Layer	Intensifier Gene	Spreader Gene	Grain Color
White	Thick	White	Absent	Absent	Absent	Opaque White
White	Thin	White	Absent	Absent	Absent	Pearly White
White	Thick	White	Absent	Absent	Absent	Chalky White
White	Thick	White	Absent	Present	Present	Brown
White	Thin	Yellow	Absent	Absent	Absent	Yellow
White	Thick	Yellow	Absent	Absent	Absent	White
Red	Thick	White	Absent	Absent	Absent	Light Red
Red	Thick	White	Present	Absent	Absent	Bright Red
Red	Thick	Yellow	Present	Absent	Absent	Bright Red
Red	Thin	Yellow	Absent	Absent	Absent	Bronze
Red	Thick	Yellow	Absent	Present	Present	Brown
Red	Thin	Yellow	Present	Absent	Absent	Bright Orange/Bronze
Red	Thick	White	Present	Present	Present	Intense Brown/Red
Yellow	Thin	White	Absent	Absent	Absent	Lemon Yellow
Yellow	Thick	Yellow	Absent	Absent	Absent	Lemon Yellow

With the different compositions and characteristics, the utilization of sorghum grains as animal feed and as an alternative energy source requires appropriate selection based on the intended use of the sorghum grains [3,4,10]. For example, in animal feed, the presence of high tannin levels in sorghum grains can be beneficial. However, as an alternative energy source, the presence of tannins can hinder the hydrolysis process in the pre-treatment of sorghum for bioethanol production.

Biomass that produces bioenergy holds great potential for the environment compared to fossil energy sources, making it an alternative energy source that needs to be developed. The use of bioenergy can help reduce pollution and greenhouse gas emissions. With its high carbohydrate content, resilience to dry conditions, and the possibility of up to three harvests per year, sorghum becomes a renewable energy alternative that should be further developed [8]. Sorghum is a multipurpose plant that has the potential to be food, feed, fodder, and biofuel [10,11].

1.1 Background

As part of the efforts to continuously enhance the development and utilization of bioenergy and its sustainability, the Indonesian Government encourages every investor to invest in the field of biofuel. The utilization of vegetable-based fuels (BBN) began in 2006 with the issuance of Presidential Instruction No. 1 in 2006 [12]. Starting in 2009, the Government implemented a mandatory policy for the utilization of BBN in the transportation, industrial, and power generation sectors through the Regulation of the Minister of Energy and Mineral Resources of the Republic of Indonesia No. 32 in 2008 concerning the Provision, Utilization, and Trade of Vegetable-Based Fuel (Biofuel) as an Alternative Fuel [13]. This regulation was subsequently updated through the Regulation of the Minister of Energy and Mineral Resources of the Republic of Indonesia No. 12 in 2015 [14]. Meanwhile, based on Presidential Regulation of the Republic of Indonesia No. 22 of 2017 [15] regarding the National General Energy Plan (RUEN), the Government set a target of achieving a 23% implementation of New and Renewable Energy (EBT) by 2025, with a target of 5% for biofuels. According to the 2017 Indonesian Energy Outlook, the achievement of EBT implementation in the energy mix reached 12%. Therefore, strategies in research, development, and policies are necessary to ensure that the established targets can be successfully met.

Biomass energy has emerged as a choice or priority among various renewable energy alternatives due to its environmental friendliness, assured availability, and sustainability. Biofuels, including biodiesel, bioethanol, and bio-oil, are examples of biomass energy sources that rely on cultivated crops as their raw materials [16]. The development of biofuels has the potential to reduce the need for petroleum imports while expanding employment opportunities. However, its success depends significantly on the availability of feedstock. The development of bioenergy technology still requires government support to compete with conventional energy technologies that have long been used by society, both in terms of reliability and economics. This is because there are few technology providers in the field of bioenergy, limiting investment choices for equipment [17].

The availability of feedstock is a primary requirement for investing in the bioenergy sector. However, sometimes feedstock derived from biological resources is not specifically designated for bioenergy or is a by-product of a business unit. Therefore, the source of feedstock determines the sustainability of bioenergy development projects [17]. Among the various biomass feedstocks, one with high potential as an alternative energy source is sorghum. Sorghum can produce 17.7 kiloliters per hectare per year. One of the advantages of sorghum compared to other biomass sources is its rapid growth. Unlike other biomass sources like sugarcane, which can only be harvested once a year, sorghum can be harvested up to three times a year [8]. Sorghum is a multipurpose plant that has the potential to be food, feed, fodder, and biofuel [3,10].

1.2 Formulation of the Problem

There are several challenges faced and affecting the development of bioenergy, especially concerning investments in the field of bioenergy. These challenges are divided into four main groups: the availability of raw materials, technology, institutional management, and sources of funding development of bioethanol from sorghum lies in its hydrolysis process [20]. Further research is needed, particularly in the pre-treatment of sorghum grains, as the starch hydrolysis process is hindered by a matrix of protein or protein bodies that adhere to the sorghum starch granules. The obstruction of starch granule contacts with the α-amylase enzyme or the polymerization of proteins forming networks results in high viscosity in the sorghum flour suspension during liquefaction. To enhance the utilization of sorghum as a raw material for bioethanol production, it is necessary to conduct studies to obtain efficient hydrolysis process conditions and achieve optimum bioethanol conversion while also generating other valuable derivative products [8]. All parts of the sorghum plant, from the grains to the stalks (sorghum juice or "nira"), can be utilized as renewable sources of energy in the form of ethanol or as biomass in the form of bio-pellets [18].

2. Methodology

The pretreatment process of sorghum grains prepared for bioethanol fermentation involves various mechanical, physical, chemical, and biological treatments, each of which affects the efficiency of the bioprocess for converting the complex starch compounds into bioethanol. The flowchart of all these process stages is presented in Figure 2.

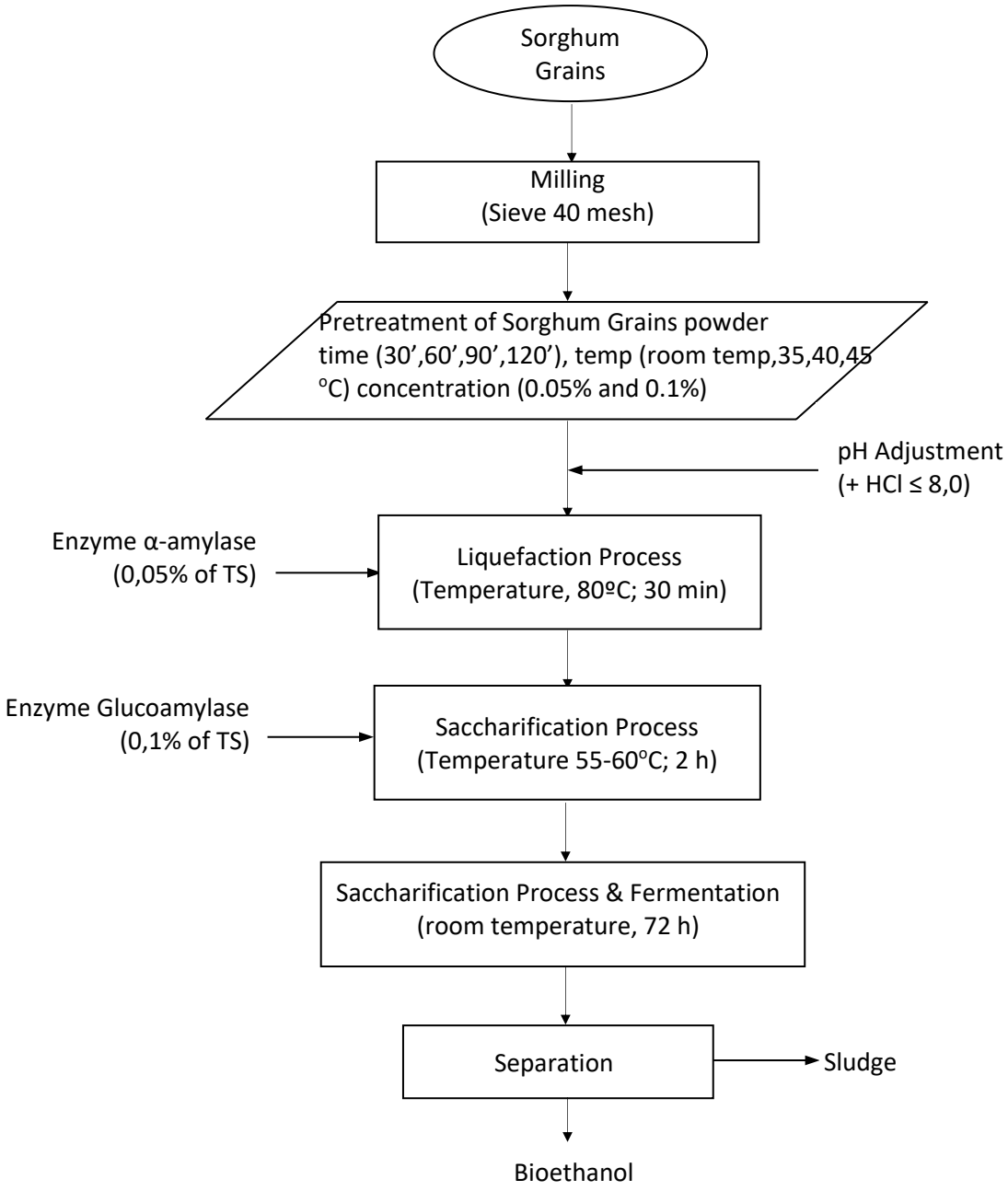

Fig. 2. Flowchart of the bioethanol production process from sorghum grains

The earliest stage of the process involves the mechanical treatment of crushing the grains with a disc mill to reduce their particle size. The reduction in particle size obtained will affect the disintegration of the grain coat tissue, which in turn influences the effectiveness of the starch compound hydrolysis process. To anticipate or minimize these effects, the distribution of particle sizes in the resulting flour will be determined. The size used in this study that passed through a mesh size of 40 from the sieve can be used in the hydrolysis stage. With this size, the surface area of the sorghum Grains is larger, which enhances the effectiveness of the enzymatic process.

A suspension of sorghum flour with a total sugar content of approximately 15% w/v was placed in a 2 liters fermenter, The addition of NaOH was varied to obtain a specific concentration (0.05 and 0.10 %), for a specific duration (30 min, 60 min, 90 min, and 120 min), and at a specific temperature (room temperature, 35, 40, and 45°C). After adjusting the pH of the suspension to ≤8, the liquefaction process was initiated. α-amylase enzyme was added at a dose of 0.1% of the Total Sugar (TS) content.

The mixture was heated to reach the gelatinization temperature (80-90°C) and maintained for 30 minutes. Sampling was performed at the end of the liquefaction process to measure viscosity and the dextrose equivalent (DE) value before lowering the temperature to 55 - 60°C.

Once the temperature reaches 55-60°C, the saccharification process begins by adding glucoamylase enzyme at a dose of 0.2% of the TS content. The temperature was maintained for 120 minutes, and sampling was done to determine the achieved DE value. The saccharification process continues after cooling to room temperature or 30-33°C, which occurs simultaneously with the fermentation process. After incubating for 72 hours, the fermentation processes were stopped. To determine the efficiency of the starch bio-conversion process into bioethanol (Sugar Consumption Ratio and Fermentation Ratio), the alcohol content (gravimetric method), total sugar content, and reducing sugar content (Modified Somogy's method).

The hindrance in the liquefaction process can be overcome through enzymatic or chemical treatments [19]. To ensure that the additional cost of the alkali hydrolysis treatment remains economically viable, the alkali concentration and the minimum time required for the hydrolysis of the protein body must be determined. The adequacy conditions for the success of the alkali hydrolysis process are determined by viscosity and DE (Dextrose Equivalent) values measured at the end of the first-stage hydrolysis process (liquefaction). It should also be considered that in industrial-scale implementation, each stage of hydrolysis and fermentation is conducted in different reactor tanks. The transfer of fluids from one reactor to another is done by using pumps. The smoothness of fluid transfer is influenced by the viscosity of the fluid.

As the saccharification process approaches, the temperature of the medium is lowered to 60°C, and glucoamylase enzyme is added [20]. The temperature was typically held at 60°C for 1-2 hours. After this, the starch slurry was cooled to 30°C, and yeast culture was added for the fermentation process. The adequacy of the saccharification process is determined by the parameter of a DE (Dextrose Equivalent) value ≥ 40% to ensure the yeast's nutritional needs for growth and efficient fermentation [20]. The achievement of the DE value within the two-hour timeframe depends on the enzyme dosage. The subsequent activity of the glucoamylase enzyme, which occurs simultaneously with the fermentation process at 30°C, may be lower, but the rate of hydrolysis was expected to be sufficient to maintain the supply of glucose, which was converted by the Saccharomyces cerevisiae yeast into bioethanol [20]. The efficiency of the simultaneous hydrolysis and the fermentation process will be determined by calculating the Sugar Consumption Ratio and Fermentation Ratio [20].

2.1 Process Optimization of Starch Hydrolysis

The optimized conditions for the alkaline hydrolysis process (protein matrix hydrolysis) were kept constant. The optimization of the degradation process of starch compounds into bioethanol was based on the Sugar Consumption Ratio (SCR) and Fermentation Ratio (FR), with variations in the dosage of α-amylase and glucoamylase enzymes. The selected dosage for α-amylase is 0.1% of the TS content, while the dosage for glucoamylase enzyme is 0.2% of the TS content.

The pH, temperature, and incubation time for each stage of the hydrolysis processes were aimed to be the same as the testing conditions for the protein matrix hydrolysis on a flask scale. Sampling was done to determine the achieved DE value at each stage of the hydrolysis process by measuring the formation of reducing sugars. The fermentation rate, SCR, and FR are determined at the end of the 72-hour incubation. Sampling was done every 12 hours to measure the remaining TS content (modified Somogy's method), the amount of bioethanol formed (alcohol meter), pH, and acidity level.

Dextrose Equivalent (DE) is an indicator of the level of starch degradation into simple sugars, calculated as the percentage of reducing sugar content to the total sugar content. The Sugar

Consumption Ratio is calculated based on the fact that yeast cannot consume starch before it is hydrolysed into simple sugars. The percentage of glucose utilized by yeast in the media is calculated using the following formula (modified Somogy's method):

$$\text{SCR} = \frac{Tsi - TSf}{TSi}\ x\ 100\ \% \quad (1)$$

SCR: Sugar Consumption Ratio
TSi: Initial Total Sugar content in the media
TSf: Final Total Sugar content in the media

The efficiency of alcohol formation by yeast is expressed as the Fermentation Ratio and calculated using the following formula:

$$FR = \frac{Vi.Ceth}{Vf.TSi\ x\ (0.6439)}\ 100\% \quad (2)$$

FR: Fermentation Ratio
Vi: Initial media volume
Vf: Final media volume
Ceth: Alcohol concentration (% vv)
TSi: Initial Total Sugar value (% wv)

3. Results and Discussion

3.1 Hydrolysis Experimental of Sorghum Grain Flour

In Table 5 below, it can be seen that after the liquefaction process using α-amylase at 80°C and the addition of 0.05% NaOH and 0.1% NaOH concentration based on total sugar for 30 minutes, sample A1 showed a viscosity reduction of 5% (from 100 cP to 95 cP). Samples A2 and A3 showed a viscosity reduction of 10%, sample B1 showed 15%, sample B2 showed 23%, and sample B3 showed a 40% reduction. However, no viscosity reduction occurred in the A0/B0 (blank) sample. This indicates that in the liquefaction process, samples A2 and A3 experienced minor cleavage of amylopectin chains from branched chains to linear chains (amylose) compared to samples B1, B2, and B3. The best liquefaction process conditions were achieved in sample B3, meaning a greater cleavage of amylopectin chains into amylose [22].

The saccharification data below show a reduction in viscosity values across all sample types. However, samples with pretreatment experienced a more significant viscosity reduction than those without pretreatment. The viscosity reduction for the A0/B0 sample (without pretreatment) was 60%. Meanwhile, the viscosity reductions for samples with pretreatment were as follows: sample A1 by 70% cP, A2 and A3 by 75%, B1 by 90%, and B2 and B3 by 95%.

Table 5
The analysis for the hydrolysis experiment of sorghum flour pretreatment in different concentrations of NaOH solution for 30 minutes

Parameters	Blank	Concentration of NaOH 0.05 %			Concentration of NaOH 0.1 %		
	Room Temp	35°C	40°C	45°C	35°C	40°C	45°C
	A0/B0	A1	A2	A3	B1	B2	B3
Initial Total Sugar	15.36%	15.36 %	15.36%	15.36%	15.36 %	15.36%	15.36%
Liquefaction pH	6.50	6.50	6.50	6.50	6.50	6.50	6.51
Viscosity after liquefaction (cP)	100 cP	95 cP	90 cP	90 cP	85 cP	75 cP	60 cP
RS after liquefaction	1.16%	1.35%	1.45%	1.88%	1.50%	1.69%	1.88%
Saccharification pH	6.32	6.48	6.48	6.47	6.48	6.50	6.32
Viscosity after saccharification (cP)	40 cP	30 cP	25 cP	25 cP	10 cP	5 cP	5 cP
RS after saccharification	8.11%	9.70%	9.70%	9.85%	9.70%	9.85%	9.85%
DE (Dextrose Equivalent)	52.80%	63.15%	63.15%	64.13%	63.15%	64.13%	64.13%

The RS value in the sample without pretreatment (blank) A0/B0 was 8.11%, while samples A1, A2, and B1 showed values of 9.7%, and samples A3, B2, and B3 showed values of 9.85%. The DE values in the sample without pretreatment (blank) A0/B0 was 52.80%, in samples A1, A2, and B1 was 63.15%, and in samples A3, B2, and B3 was 64.13%. The DE value is calculated by dividing the reducing sugar content (RS after saccharification) by the total sugar content (TS) and multiplying by 100%. The DE value indicates the degree of hydrolysis of starch or complex carbohydrates into simpler sugars, like glucose. The DE scale ranges from 0 to 100, where: DE= 0 means the carbohydrate remains in complex starch form (unhydrolyzed), DE =100 means the carbohydrate is fully hydrolyzed into pure glucose.

Higher DE values correspond to increased sweetness, solubility, and reduced reactivity of the carbohydrate. Based on Table 5, with a pretreatment time of 30 minutes, the highest RS and DE values were found in samples A3 and B2, with RS values of 9.85% and DE values of 64.13%. Sample B2 is considered the best sample, as it has a lower post-saccharification viscosity of 5 cP, compared to sample A3's viscosity of 25 cP. A lower viscosity sample is easier to handle in solution transfer systems for subsequent processes.

According to Table 6, after the liquefaction process using α-amylase at 80°C and the addition of 0.05% NaOH and 0.1% NaOH concentration based on total sugar for 60 minutes, sample A4 showed a viscosity reduction of 40% (from 100 cP to 60 cP). Samples A5 and B4 showed a 50% reduction, samples A6 and B6 showed an 85% reduction, and sample B5 showed a 70% reduction. No viscosity reduction occurred in the A0/B0 (blank) sample. This indicates that, in the liquefaction process, sample A4 experienced minor cleavage of amylopectin chains from branched chains to linear chains (amylose) compared to samples A5, B4, A6, B5, and B6. The best liquefaction process conditions were achieved in samples A6 and B6, indicating a greater cleavage of amylopectin chains into amylose.

Next, the saccharification process showed a reduction in viscosity values across all sample types. However, samples with pretreatment had a more significant reduction in viscosity compared to those without pretreatment. The viscosity reduction for the A0/B0 sample (without pretreatment) was

60%. Meanwhile, the viscosity reductions for samples with pretreatment were as follows: samples A4 and B4 by 85%, samples A5 and B5 by 90%, and samples A6 and B6 by 95%.

For RS values, the sample without pretreatment (blank) A0/B0 showed a value of 8.11%, while samples A4, A5, A6, and B4 showed values of 9.85%. Samples B5 and B6 showed RS values of 10.14%. For DE values, the sample without pretreatment (blank) A0/B0 had a value of 52.80%, while samples A4, A5, A6, and B4 showed values of 63.15%, and samples B5 and B6 showed values of 66.02%.

Table 6
The analysis for the hydrolysis experiment of sorghum flour pretreatment in different concentrations of NaOH solution for 60 minutes

Parameters	Blank	Concentration of NaOH 0.05%			Concentration of NaOH 0.1 %		
	Room Temp	35°C	40°C	45°C	35°C	40°C	45°C
	A0/B0	A4	A5	A6	B4	B5	B6
Initial Total Sugar	15.36%	15.36 %	15.36%	15.36%	15.36 %	15.36%	15.36%
Liquefaction pH	6.50	6.47	6.51	6.52	6.53	6.49	6.52
Viscosity after liquefaction (cP)	100 cP	60 cP	50 cP	15 cP	50 cP	30 cP	15 cP
RS after liquefaction	1.16%	2.46%	2.60%	3.33%	2.46%	2.60%	3.33%
Saccharification pH	6.32	6.07	6.20	6.22	6.04	6.08	6.22
Viscosity after saccharification (cP)	40 cP	15 cP	10 cP	5 cP	15 cP	10 cP	5 cP
RS after saccharification	8.11%	9.85%	9.85%	9.85%	9.85%	10.14%	10.14%
DE (Dextrose Equivalent)	52.80%	63.15%	63.15%	63.15%	63.15%	66.02%	66.02%

With a 60-minute pretreatment time, the best results were obtained for sample B5, with an RS value of 10.14% and a DE value of 66.02%. Sample B5 is considered the best because it achieved maximum hydrolysis results with a pretreatment temperature of just 40°C, indicating effective hydrolysis without requiring the highest temperature.

According to Table 7, data from the liquefaction process using α-amylase at 80°C, with the addition of 0.05% NaOH and a NaOH concentration of 0.1% relative to total sugar for 90 minutes, shows that sample A7 experienced a 70% viscosity reduction (from 100 cP to 30 cP). Samples A8 and A9 had an 80% reduction, sample B7 had an 85% reduction, and samples B8 and B9 had a 90% reduction. However, no viscosity reduction occurred in the A0/B0 (blank) sample without pretreatment. This indicates that in the liquefaction process, all samples with pretreatment experienced cleavage of amylopectin chains from branched chains to linear chains (amylose). The best liquefaction process conditions were achieved in samples B8 and B9, indicating more extensive cleavage of amylopectin into amylose.

Next, in the saccharification process, all types of samples showed a reduction in viscosity values. However, samples with pretreatment experienced a more significant viscosity reduction than those without pretreatment. The viscosity reduction for the A0/B0 sample (without pretreatment) was 60%. Meanwhile, the viscosity reductions for samples with pretreatment were as follows: sample A7 by 85%, samples A8, A9, and B7 by 90%, and samples B8 and B9 by 95%.

Table 7
The analysis for the hydrolysis experiment of sorghum flour pretreatment in different concentrations of NaOH solution for 90 minutes

Parameters	Blank	Concentration of NaOH 0.05%			Concentration of NaOH 0.1 %		
	Room Temp	35°C	40°C	45°C	35°C	40°C	45°C
	A0/B0	A7	A8	A9	B7	B8	B9
Initial Total Sugar	15.36%	15.36 %	15.36%	15.36%	15.36 %	15.36%	15.36%
Liquefaction pH	6.50	6.48	6.48	6.47	6.47	6.48	6.48
Viscosity after liquefaction (cP)	100 cP	30 cP	20 cP	20 cP	15 cP	10 cP	10 cP
RS after liquefaction	1.16%	2.31%	2.31%	2.60%	3.04%	3.33%	3.47%
Saccharification pH	6.32	6.44	6.44	6.35	6.73	6.60	6.54
Viscosity after saccharification (cP)	40 cP	15 cP	10 cP	10 cP	10 cP	5 cP	5 cP
RS after saccharification	8.11%	9.85%	10.14%	10.43%	9.85%	10.14%	10.43%
DE (Dextrose Equivalent)	52.80%	63.15%	66.02%	67.90%	63.15%	66.02%	67.90

The RS value for the sample without pretreatment (blank) A0/B0 was 8.11%, samples A7 and B7 showed values of 9.85%, samples A8 and B8 showed values of 10.14%, and samples A9 and B9 showed values of 10.43%. For DE values, the sample without pretreatment (blank) A0/B0 was 52.80%, samples A7 and B7 were 63.15%, samples A8 and B8 were 66.02%, and samples A9 and B9 were 67.90%.

From the data above, sample A9 is considered the best for the hydrolysis process as it achieved the highest RS and DE values with a lower NaOH concentration (0.05%). Sample A9 demonstrated optimal liquefaction and saccharification hydrolysis data and will therefore be used in further fermentation processes to produce ethanol (bioethanol).

In Table 8, the longest soaking time (pretreatment) of 120 minutes was used. The liquefaction process was conducted with α-amylase at 80°C, with 0.05% NaOH and 0.1% NaOH concentration relative to total sugar for 120 minutes. Samples A10, A11, and A12 showed an 85% viscosity reduction (from 100 cP to 15 cP), sample B10 showed a 90% reduction, and samples B11 and B12 showed a 95% reduction. No viscosity reduction occurred in the A0/B0 (blank) sample. This result suggests that in the liquefaction process, all pretreatment samples underwent cleavage of amylopectin chains from branched chains to linear chains (amylose). The best liquefaction process conditions were achieved in samples B11 and B12, indicating more extensive cleavage of amylopectin into amylose.

Table 8
The analysis for the hydrolysis experiment of sorghum flour pretreatment in different concentrations of NaOH solution for 120 minutes

Parameters	Blank	Concentration of NaOH 0.05%			Concentration of NaOH 0.1 %		
	Room Temp	35°C	40°C	45°C	35°C	40°C	45°C
	A0/B0	A10	A11	A12	B10	B11	B12
Initial Total Sugar	15.36%	15.36 %	15.36%	15.36%	15.36 %	15.36%	15.36%
Liquefaction pH	6.50	6.48	6.47	6,48	6.49	6.48	6.48
Viscosity after liquefaction (cP)	100 cP	15 cP	15 cP	15 cP	10 cP	5 cP	5 cP
RS after liquefaction	1.16%	2.31%	2.31%	2.40%	3.04%	3.33%	2.17%
Saccharification pH	6.32	6.43	6.45	6.40	6,59	6.50	6.51
Viscosity after saccharification (cP)	40 cP	10 cP	10 cP	10 cP	10 cP	5 cP	5 cP
RS after saccharification	8.11%	10.14%	10.43%	10.43%	10.14%	10.43%	10.43%
DE (Dextrose Equivalent)	52.80%	66.02%	67.90%	67.90%	66.02%	67.90%	67.90%

For saccharification, the table shows a viscosity reduction across all sample types. However, samples with pretreatment showed a more significant reduction than those without pretreatment. The viscosity reduction for the A0/B0 sample (without pretreatment) was 60%, while the viscosity reductions for samples with pretreatment were as follows: samples A10, A11, A12, and B10 had reductions of 90%, while B11 and B12 had the highest reduction at 95%.

For RS values, the sample without pretreatment (blank) A0/B0 showed an RS value of 8.11%, samples A10 and B10 had a value of 10.14%, and samples A11, A12, B11, and B12 all had RS values of 10.43%. For DE values, the sample without pretreatment (blank) A0/B0 was 52.80%, samples A10 and B10 were 66.02%, and samples A11, A12, B11, and B12 were 67.90%.

With a 120-minute pretreatment time, sample A11 achieved the best results for hydrolysis, as it yielded the highest glucose conversion with a relatively lower pretreatment temperature (40°C) and lower NaOH concentration (0.05%). This sample demonstrated maximum carbohydrate hydrolysis efficiency, converting complex carbohydrates to glucose effectively.

3.2 Fermentation Experimental of Bioethanol from Hydrolyzed Sorghum Grain Flour at 2-Liter Scale

3.2.1 Experiment of bioethanol fermentation from red sorghum variety grain flour hydrolysis result with 0.05% NaOH pretreatment treatment for 1.5 hours at a pretreatment temperature of 45°C, scale 2 liter

The sample used in the fermentation process is sample A9, which underwent pretreatment with 0.05% NaOH and a pretreatment temperature of 45°C. This sample was carried through the liquefaction and saccharification hydrolysis processes and then used in the fermentation stage. The Fermentation Ratio (FR) was calculated from the fermentation results, yielding an FR value of 86.97%. The Fermentation Ratio indicates the efficiency of alcohol formation by the yeast. This FR was obtained using the calculation formula (2).

Referring to Table 9 and Figure 3, alcohol production was observed after 24 hours of fermentation, reaching 5.7%, and by the end of the fermentation period (72 hours), the alcohol

concentration reached 9.3%. At this point, yeast cell count had decreased, and residual reducing sugars (RS) remaining in the solution measured 0.48%.

Table 9
Bioethanol fermentation from hydrolysed red sorghum Grain flour with 0.05% NaOH soaking treatment for 90 minutes at a soaking temperature of 45°C on a 2 liters scale

Time (Hours)	Temperature (°C)	pH	RS (%)	Number of Cells (x 10^7)	Acidity (ml NaOH 0.1N)	Total Sugar (%)	Alcohol (%)
0	31.2	6.06	10.14	0.81	1.1	16.80	
12	28.0	5.27	8.11	8.75	2.5	13.33	
24	31.6	4.88	4.05	11.81	8.5	7.97	5.7
36	26.2	4.39	0.87	10.75	7.4	2.61	8.5
48	29.3	4.75	0.72	15.58	7.0	2.46	8.6
60	27.2	4.51	0.57	15.63	6.9	2.31	8.9
72	29.0	4.90	0.48	14.38	4.9	2.02	9.3

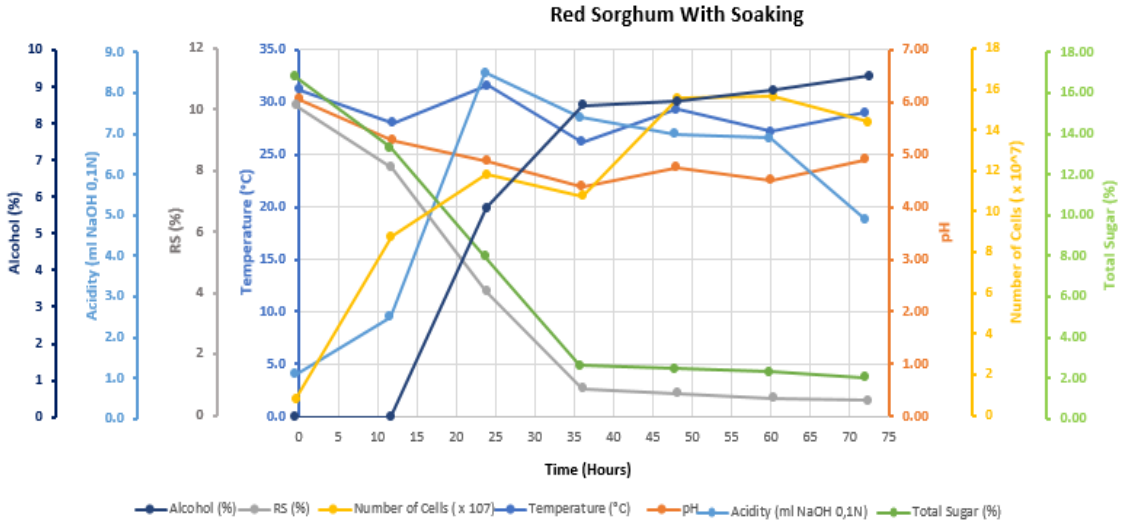

Fig. 3. Bioethanol fermentation graph from the hydrolysis of red sorghum flour with NaOH 0.05% immersion treatment for 1.5 hours at a pretreatment temperature of 45°C, scale 2 liters

3.2.2 Experiment of bioethanol fermentation from red sorghum variety grain flour hydrolysis result without treatment, 2 liter scale

The sample used in this fermentation process was one without pretreatment, proceeding directly from the liquefaction and saccharification hydrolysis stages to fermentation. The Fermentation Ratio (FR) was calculated from the fermentation results, yielding an FR of 83.02%, which reflects the efficiency of alcohol formation by the yeast. This FR value was obtained using the calculation formula (2).

Referring to Table 10 and Figure 4, the final alcohol produced was 8.9%. Alcohol production was first observed after 24 hours of fermentation at a concentration of 5.6%, and by the end of the 72-hour fermentation period, the alcohol concentration reached 8.9%. Residual reducing sugars (RS) was measured at 0.29%, with total sugars at 2.31%. Additionally, the yeast cell count had decreased, indicating cell lysis and the cessation of bioethanol production.

Table 10
Bioethanol fermentation from hydrolyzed red sorghum grain flour without treatment on a 2 liter scale

Time (Hours)	Temperature (°C)	pH	RS (%)	Number of Cells (x 10^7)	Acidity (ml NaOH 0.1N)	Total Sugar (%)	Alcohol (%)
0	33.3	5.98	9.27	0.8	1.5	16.65	
12	28.2	4.93	7.24	9.3	4.6	13.04	
24	31.7	4.73	3.18	11.6	11.2	7.24	5.6
36	27.0	4.38	0.72	10.0	7.4	3.33	8.2
48	29.2	4.59	0.58	11.9	7.3	3.04	8.4
60	27.3	4.38	0.29	14.8	7.1	2.46	8.85
72	28.5	4.84	0.29	14.6	5.3	2.31	8.9

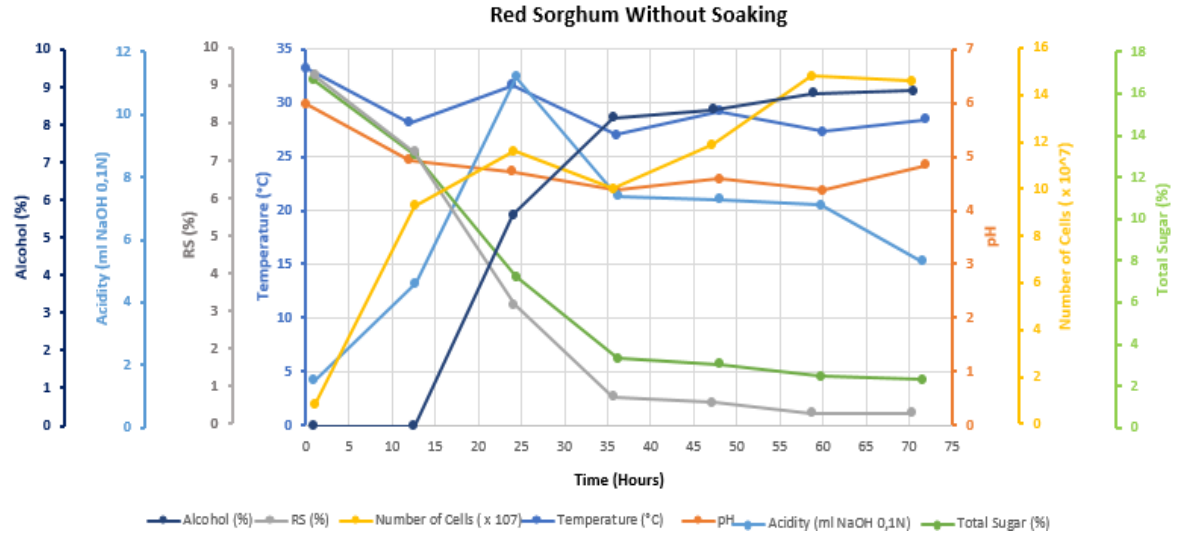

Fig. 4. Bioethanol fermentation graph from the hydrolysis of red sorghum flour without treatment, 2 liter scale

4. Conclusions

The effect of pretreatment in the liquefaction and saccharification hydrolysis process generally had a significant impact on samples with pretreatment compared to those without pretreatment. This is evident from the greater reductions in viscosity and increases in RS (Reduced Sugar) and DE (Dextrose Equivalent) values in pretreatment samples. The study on pretreatment of red sorghum for bioethanol production by using 0.05% NaOH solution for 90 minutes and a pretreatment temperature of 45°C demonstrated that the best results during the liquefaction and saccharification process with the RS value of 10.43% and a DE value of 67.90%. Furthermore, the fermentation with pretreatment produced an alcohol concentration of 9.3% with a Fermentation Ratio (FR) of 86.97%. Comparing those, the fermentation without treatment resulted in an alcohol concentration of 8.9% with an FR of 83.02%. The enzymatic reactions in each sample reflected different enzyme performances, showing variations in effectiveness depending on pretreatment conditions.

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