

# Thermal-Acid Hydrolysis of Sisal Boles Juice for Lactic Acid Production

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**Abstract** The effect of reaction temperature, time and pH on the maximum total sugar concentration during thermal-acid hydrolysis of sisal boles juice was investigated. The Minitab software (V. 17) was used to design a 2<sup>n</sup> full factorial design for sisal juice hydrolysis and analyse the effects of the variables on the response. Thermal-acid hydrolysis can be carried out at a moderate temperature of 60°C with a concentrated hydrochloric acid at pH of one within a period of 30 minutes to achieve a sugar yield of 39%. The breakdown of complex sugars to simple sugars of sisal bole juice is basically a function of acid concentration in sugar solution. Lowering pH of the juice contributed to increase in hydrolysis rate. The use of ANOVA analyses gave evidence that linear, 2- way interactions and 3-way interactions of all the variables: temperature (60-100°C), time (30-60 min) and pH (1-5) significantly affected the sugar yield. This amount of sugar yield from sisal boles juice is higher than the yield of 26% which has been reported. The hydrolysed juice was also subjected into fermentation for lactic acid and results showed favourable LA yield was obtained with the recommended thermal-acid hydrolysis in this study.

**Keywords** Thermal-acid hydrolysis, Sisal bole juice, Complex sugars breakdown, Lactic acid production

## 1. Introduction

Sisal is a semiarid and marginal land crop of the tropics whose leaves are used for extraction of fibres. In a typical sisal production process, a very small portion, about 2% of sisal plant (sisal fibres) is used to produce twines, packaging's, carpets, matts, threads, fine yarns, ropes and roofing tiles. The remaining bulk (98%) which include leaves decortication residue and sisal postharvest remaining (sisal poles, sisal boles with leave stubs) is discarded as waste [1-3]. Sisal fibres have also been used in the automotive sector and for specialist paper manufacturing [4]. Generally, production of one tonne of fibre generate waste amounting to 24 tonnes of leaf residues, 100 m<sup>3</sup> of wastewater and 4.7 tonnes of sisal boles [4]. On average a sisal bole weighs 40 kg and contains juice which consist of hydrolysable sugars (mostly fructose) that can be fermented to produce different bio-products such as citric acid, lactic acid and ethanol [1, 2, 5].

Extraction of juice from sisal boles is discussed in earlier study by Msuya *et al*, [5]. Before fermentation to other bio-products the extracted sisal bole juice need to be hydrolysed to break down the total sugars into simple monomer sugars that can be assimilated into microbial cells. Various researchers have worked on different methods for

breaking down the complex sugars in sisal boles into simple sugars [2, 6, 7]. Conversion process of polysaccharides (mainly fructans) to fermentable sugars from sisal juice can be done either by thermal, enzymatic or chemical hydrolysis. Enzymatic hydrolysis leads to high purity fructose yield but requires commercial hydrolytic enzymes such as *fructanase* which are expensive considering high volume production [2]. The enzymatic hydrolysis is also very slow [8]. In addition, some hydrolysis products such as glucose tend to inhibit the process and thus need to be removed, this increases complexity of the process [2].

Thermal hydrolysis involves heating the chopped boles to temperatures above 100°C by autoclaving or oven cooking. The boles are cooked or autoclaved to hydrolyze the fructans and release fermentable sugars, principally fructose [2, 9]. Here the hydrolysis is done before extraction of juice. Thermal hydrolysis has the advantage of ease of controlling, acceptable processing time and relatively low capital investments costs [2, 10]. The resulting fructose syrup has a relatively low purity and can form Maillard reactions due to high temperatures (above 100°C) used [1, 2]. A Maillard reaction is a non-enzymatic reaction between sugars and proteins that occurs upon heating and that produces browning colour into the product. These are fermentation inhibitors which reduces the microorganism activities hence low product yield [10-12].

Acid hydrolysis (dilute or concentrated) involves use of large quantities of mineral acids, which has to be recovered,

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resulting in high cost of neutralization and gypsum disposal problem [13] if sulphuric acid is used. Unlike dilute acid hydrolysis, concentrated acids gives higher sugar yields with fewer degradation products which can favours microbial growth hence minimize inhibition during fermentation [11, 14, 15]. Combined acid and medium temperature hydrolysis (thermal-acid hydrolysis) can compromise use of larger quantities of mineral acid and the use of excessive heat applications [2, 16, 17] that can lead into production of fermentation inhibitors such as hydromethylfulfural. This work investigated the effect of reaction temperature, time and acid concentrations on the sugar yield during thermal-acid hydrolysis of sisal boles juice.

## 2. Material and Methods

### 2.1. Extraction of Juices from Sisal Boles

Samples of Agave H11648 boles 10-12 years old were randomly collected from one hectare of Katani Ltd sisal estate, Tanga, Tanzania. The boles were weighed before and after removing the leaf stubs. They were then cleaned using tap water. Juice extraction process involved chopping of sisal bole into small pieces (about 5-15 mm<sup>3</sup>) to increase the surface area during pressing. The juice was extracted from 1 kg sample of chopped boles using hydraulic pressing machine with a capacity of 16 tonnes. The extraction machine is made of stainless steel housing. Three alternatives were tried to maximize juice extraction. The first option involved direct pressing of chopped sisal boles without heating, the second involved heating the boles by autoclaving at 121°C for 5 minutes. The autoclaved chopped bole parts were then cooled to room temperature (30°C ± 3) before pressing.

The third option involved mixing the boles with 1L of boiled water (100°C) before pressing to increase solubility of soluble components in bole mass to bring about the easiness during extraction process. The biomass residue after the first juice collection in either option was tested for residual sugar concentrations. This was done by adding half a litre of hot water to enhance wet blending of the biomass residue and pressed to extract the juice to be tested. The process was repeated to ensure minimal sugar residual in the pulp by measuring sugar content of the wash liquor extract.

### 2.2. Hydrolysis of Sisal Boles Juice

Three options were tested for the suitable hydrolysis method on the sugar yield as per literature information. The first option involved autoclaving the juice at 121°C (thermal hydrolysis) for 15minutes without acid. The second option involved acid hydrolysis (pH 1-2) without heating and the third option combined both acid and temperatures (thermal-acid hydrolysis) for 15 minutes at 121°C. Total sugars concentrations were determined using UV Light Spectrophotometer at 540nm (SPECTRONIC 21D,

MILTON ROY) by DNS method [18]. The calibration standard curves were drawn using standard sugars from Sigma Aldrich. The sugar concentrations were calculated using equation (i).

$$\% \text{ Sugar} = \frac{\text{Absorbance}}{\text{Slope from calibration curve}} \times \text{Dilution factor} \times 100 \quad (1)$$

The thermal-acid hydrolysed juice was then subjected to Lactic acid fermentation to check the ferment ability of the sugars. The preparation of juice and inoculum for fermentation is reported in other study by Msuya *et al.*, [5]. The LA production was analysed at the end of the fermentation period using UV/IV digital spectrophotometer (Labtronics LT-31) as per method by Borshechskaya *et al.*, [19]. This method was selected because it is relatively cheap and has an error of less than 3% compared to HPLC method [19].

### 2.3. Design of Experiment for Thermal-Acid Hydrolysis

To maximize sugar extraction from the extracted juice through hydrolysis, the design of experiment was done on thermal-acid hydrolysis to identify the possible combination that could favour high sugar yields fermentable to lactic acid. The thermal-acid hydrolysis process involved transferring of about 50 ml of juice into 100 ml flat bottomed conical flasks followed by adjustment of the solution pH between the ranges of 1-5 using concentrated hydrochloric acid (36%). The low and high levels were adapted from reported information from literature [2, 17, 20] and are given in Table 1. The samples were placed in an oil bath at temperature range of 60 to 100°C and time range of 30 to 60 minutes.

The Minitab software (V. 17) was used to design a 2<sup>n</sup> factorial for sisal juice hydrolysis and analyse the effects of the variables on the responses. Three factors: pH, Temperature and Time were analysed for their main and interaction effects on response (sugar yield). The experiments were performed in random order using one replicate to estimate the pure error. Equation (ii) was used to model the relationship between factors and response where  $X_1$ ,  $X_2$  and  $X_3$  are independent variables: Temperature, Time and pH respectively;  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$  are linear coefficients;  $\beta_{12}$ ,  $\beta_{13}$ ,  $\beta_{23}$  are two way interaction coefficients,  $\beta_{123}$  three way interaction regression coefficients and  $Y$  is the response function (% Sugar).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{123} X_1 X_2 X_3 \quad (2)$$

**Table 1.** Matrix of Experiments 2<sup>3</sup> Factorial Design for Thermal-Acid Hydrolysis

Variables	Coded levels		
	Low -1	Middle 0	High 1
Temperature, (°C)	60	80	100
Hydrolysis time, (min)	30	45	60
pH	1	3	5

### 3. Results and Discussion

#### 3.1. Results of Juice Extraction and Sugar Yield on the Tested Hydrolysis

The calibration curve for the standard sugars gave a linear relationship with  $R^2$  of 0.9971. Juice extraction results were  $567 \pm 2.9$ ,  $791 \pm 0.6$  and  $653 \pm 2.9$  volumes (ml) of juice per kg of sisal bole for chopped and pressed, autoclaved, and mixed with boiling water, respectively. Thus, the autoclaved sisal boles gave the highest volume of juice compared to the other methods. This can be attributed to higher temperature ( $121^\circ\text{C}$ ) used under autoclaving at pressure of 1.5 bar, which led to rupture of sisal boles microfibril structure providing more surface area for juice extraction. The sugar yield results from the tested hydrolysis options are presented in Table 2.

**Table 2.** Sugar yield (% w/v) results for the tested hydrolysis options (Mean $\pm$ SDV)

Juice sources	Without hydrolysis	Thermal hydrolysis	Acid Hydrolysis	Thermal-acid hydrolysis
Bole 1	$0.03 \pm 0.01$	$0.17 \pm 0.02$	$34.00 \pm 3.61$	$37.00 \pm 4.58$
Bole 2	$0.03 \pm 0.01$	$0.12 \pm 0.02$	$29.79 \pm 0.50$	$36.86 \pm 4.73$

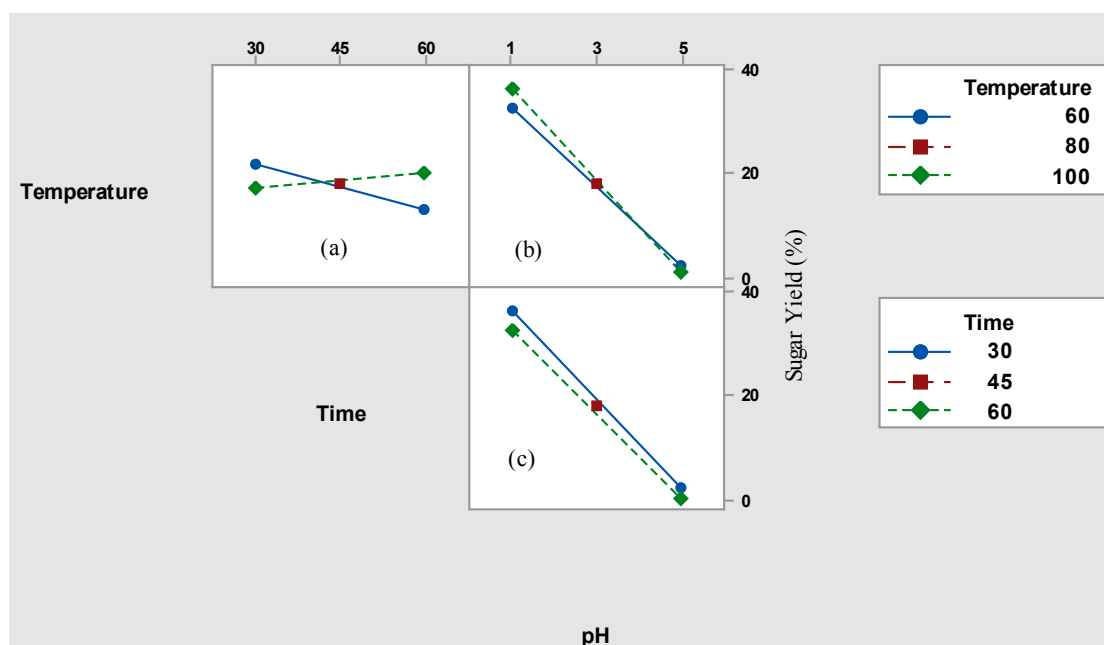
From Table 2, it is clearly seen that the combination of heat and acid (thermal-acid hydrolysis) has impact on the sugar yield. In both juice sources (boles), the total sugar yield was highest~37% (w/v). This is almost 42% higher than what has been reported by Ngonyani, [2]. Thermal-acid hydrolysis gave about 15% higher sugars than acid hydrolysis alone, although almost no significant sugar yield was obtained with thermal hydrolysis alone. Combining the two gave the best sugar yield due to increased mobility (enhanced mass transfer) of the hydrolysed sugars.

#### 3.2. Effect of Temperature, Time and pH on Sugar Concentration for the Designed Hydrolysis

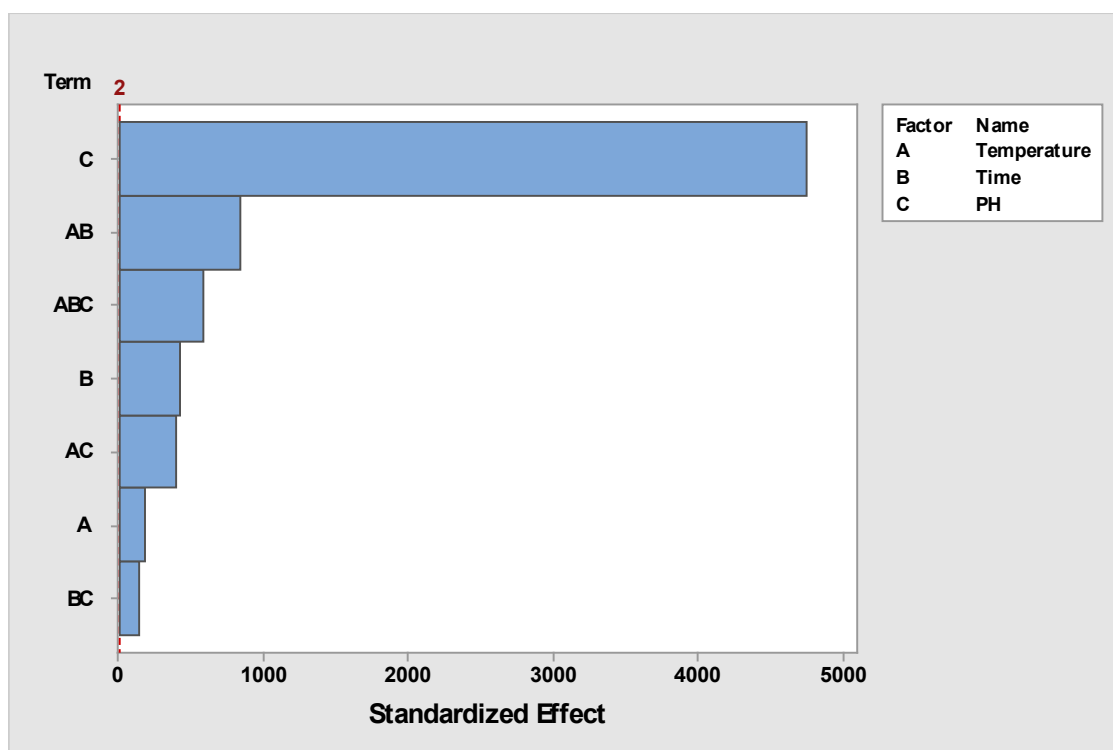
The effect of temperature, time and pH on acid hydrolysis of sisal bole juice was investigated. Variation of the percentage increase in sugar concentration with hydrolysis at different pH, heating time and temperatures is shown in Table 3.

**Table 3.** Matrix of Experimental  $2^3$  factorial design for thermal-acid hydrolysis

Runs	Temperature, $^\circ\text{C}$	Time (min)	pH	%Sugar
1	60	60	5	0.290
2	100	60	5	0.590
3	80	45	3	18.000
4	100	30	1	33.300
5	60	60	1	25.560
6	100	30	5	0.750
7	100	60	1	39.240
8	100	30	5	0.750
9	60	60	5	0.285
10	60	30	1	39.100
11	100	30	1	33.300
12	100	60	1	39.240
13	100	60	5	0.585
14	60	30	5	4.050
15	60	30	1	39.150
16	80	45	3	18.000
17	60	30	5	4.020
18	60	60	1	25.560



**Figure 1.** Interaction Plots for Thermal-acid hydrolysis of sugars from sisal boles juice



**Figure 2.** Pareto chart for standardized effects (response is % Sugar, Alpha = 0.05)

**Table 4.** Analysis of Variance (ANOVA) for Sugar Yield with 95% confidence interval

Source of Variation	DF	Adj SS	Adj MS	F-Value	P-Value
Model	8	4596.04	574.51	2997417.69	
Linear	3	4366.53	1455.51	7593966.24	
Temp.	1	5.93	5.93	30935.09	0.000
Time	1	33.26	33.26	357.88	0.000
pH	1	4327.34	4327.34	22577412.03	0.000
2-Way Interactions	3	166.11	55.37	288893.45	
Temp.*Time	1	133.29	133.29	695410.57	0.000
Temp.*pH	1	29.38	29.38	153268.17	0.000
Time*pH	1	3.45	3.45	18001.60	0.000
3-Way Interactions	1	63.36	63.36	330582.26	
Temperature*Time*PH	1	63.36	63.36	330582.26	0.000
Curvature	1	0.03	0.03	180.18	
Pure error	9	0.00	0.00	3.01	
Total	17	4596.04			

DF= Degree of Freedom, Adj SS = adjacent sum of squares, Adj MS =Adjacent Mean squares.

**Table 5.** Lactic acid production V/s hydrolysis condition of the sisal boles juice

Sample No.	Hydrolysis Temp (°C)	Hydrolysis Time (min)	Hydrolysis pH	Sugar Yield (% g/100ml)	LA Conc. (g/L)	LA Yield (g/g)
1	100	60	1	39.24	24.2	0.93
2	60	30	1	39.15	24.3	0.93
3	121	120	1	28.20	6.0	0.43
4	121	15	1	37.00	13	0.54
5	121	120	3	25.90	2.0	0.25
6	121	15	3	29.80	3.7	0.31

Thermal-acid hydrolysis at 100°C for 60 minutes and at 60°C for 30 minutes resulted at highest percentage of total concentration of 39%. This shows the breakdown of complex sugars to simple sugars can be achieved at 60°C at a low pH of 1 within 30 minutes. Increasing pH from 1 to 5 at any temperature showed a decreasing % sugar yield, that is, it contributed to decrease in hydrolysis rate. This is also elaborated by the interaction plots in Figure 1 that indicate a degree of interaction of the three factors: Temperature, time and pH on the sugar yield. The degree of interaction is justified by interaction lines that departed from being parallel. Big departure from parallel occurred when combining temperature and time (Figure 1a).

Less effect was seen when combining pH and time (Figure 1c). Figure 1(b) shows that an increasing in pH from 1-5 reduced the yield drastically regardless of the temperature used. Figure 1(c) shows that, regardless of time used sugar hydrolysis yield were drastically reduced by increasing pH from 1 to 5. In Figure 1(a), increasing time at different temperatures had interactive effect in that, at shorter time of 30 minutes at a temperature of 60°C a higher yield was obtained than that at 100°C while at longer time of 60 minutes a higher yield was obtained for the higher temperature of 100°C than at 60°C. The high interaction of time and temperature is also depicted by the Pareto chart for standardized effects in Figure 2.

It is clearly seen in Figure 2 that; all variables extend past the reference line at significance ( $\alpha = 0.05$ ). Results show that the largest effect is coming from pH. Less effect is shown when Time and pH (BC) are interacted and on temperature (A) alone. For economical reason therefore, it is logical to consider the low temperature and short time since the yield at 30 minutes and pH of 1 are almost the same at temperature of 60°C and 100°C. The maximum sugar obtained of 39% is higher than 26% that obtained by Ngonyani, [2] who did thermal-acid hydrolysis at higher temperature of 121°C for 2 hrs.

Percentage sugar yield model is represented by Equation (iii). In order to evaluate if the models are statistically significant with confidence level of 95% one criterion is to perform an F-Test. The ANOVA results in Table 4 show that, all interactions had significant effect on the sugar yield. It is evident that linear, 2- way interactions and 3-way interactions of all the variables (temperature, time and pH) significantly affected the sugar yield at 95% confidence level with p-value =0.00. Sugar yield presented a correlation coefficient of 0.999, therefore the linear model correlated well with the experimental data.

$$\% \text{ Sugar} = 108.83 - 0.75 \text{ Temp.} - 1.71 \text{ Time} - 18.15 \text{ pH} + 0.02 \text{ Temp.} * \text{Time} + 0.12 \text{ Temp.} * \text{pH} + 0.28 \text{ Time} * \text{pH} - 0.003 \text{ Temp.} * \text{Time} * \text{pH} + 0.14 \text{ CtPt} \quad (3)$$

Table 5; clearly show that, sample no. 5 has not only the lowest lactic acid yield but also lowest sugar yield compared to other samples. Generally, prolonged heat treatment at temperatures above 100°C does not only break down complex carbohydrate (polysaccharides) molecules, but also

enhance hydrolysis with maximum occurrence of Maillard reactions and caramelisation of the reducing sugar [1, 7, 21]. Longer time at high temperature may also lead into formation of hydromethylfulfural (HMF). Not only that but also cooking at high temperatures above 100°C for hours means higher use of energy hence not economical. Low lactic acid yield can therefore be attributed to presence of inhibitors like hydromethylfulfural as a result of longer heating time at higher temperature.

## 4. Conclusions

Thermal-acid hydrolysis can be carried out at moderate temperature of 60°C with concentrated hydrochloric acid at pH of 1 within a period of 30 minutes to achieve a sugar yield of 39%. A combination of strong acid and increased temperature above 60°C appeared to have improved mass transfer effecting the hydrolysis followed by increased mobility of the hydrolysed sugars to the solution. Thus, the breakdown of complex sugars to simple sugars is basically a function of acid concentration (hydrogen ion concentration) and its distribution in solution. At lower juice pH therefore increase in hydrolysis was achieved as elaborated and analysed by the interaction plots. ANOVA analyses gave an evidence that linear, 2- way interactions and 3-way interactions of all the variables (temperature, time and pH) significantly affected the sugar yield at 95% confidence level with p-value =0.00. The hydrolysed juice was also subjected into fermentation for lactic acid and results showed favourable LA yield was obtained with the recommended thermal-acid hydrolysis in this study.

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