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Influence Of Hydrochloric Acid Concentration On Reducing Sugar Yield And Fermentation Ph In Sweet Potato Peels Hydrolysis

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Abstract:
Introduction: *Acid hydrolysis remains one of the most effective methods for converting starchy and lignocellulosis feedstocks into fermentable sugars as compared to physical and thermal methods. This study determines the effect of HCl concentration on reducing sugars and pH after hydrolysis, after fermentation and the amount of reducing sugar consumed.*
Methodology: *Sweet potato peels (*Ipomoea batatas*) were collected, washed, dried for 10 days, and mechanically crushed to reduce particle size. Twenty grams of the sample was hydrolyzed using hydrochloric acid at concentrations of 0.2 M, 0.5 M, 1.0 M, 1.5 M, and 2.0 M, heated in a water bath at 98 °C for 2 hours, and autoclaved at 121 °C for 15 minutes. The hydrolysates were filtered and stored for analysis. pH was measured using a calibrated pH meter, while reducing sugars were quantified spectrophotometrically using the alkaline copper tartrate and arsenomolybdate method with standard glucose solutions for calibration.*
Results: *Hydrochloric acid concentration significantly influenced reducing sugar yield and pH (Kruskal–Wallis,* $p < 0.05$*). The highest reducing sugar after hydrolysis (28.77 g/L) and consumption during fermentation (21.83 g/L) occurred at 0.5 M HCl, while the lowest yield after hydrolysis was observed at 2.0 M (18.03 g/L). Post-hoc analysis revealed that most acid concentration pairs differed significantly, except for 0.2 M vs 2.0 M and 0.5 M vs 1.0 M in reducing sugar after fermentation. Higher acid concentrations (≥1.0 M) tended to maintain higher post-fermentation pH (3.70), whereas lower concentrations resulted in more acidic conditions (pH 3.20–3.30).*
Conclusion and Recommendations: *The findings indicate that hydrochloric acid concentration has a significant effect on the amount of reducing sugars released during hydrolysis, the quantity consumed during fermentation, and the resulting pH levels. It is therefore recommended that excessively high HCl concentrations be avoided during hydrolysis, as these can lower sugar availability and, in turn, reduce fermentation efficiency.*
Key Words: *acid hydrolysis, fermentation pH, reducing sugars, sweet potato peels*

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I. Introduction

Acid hydrolysis remains one of the most effective methods for converting starchy and lignocellulosis feedstocks into fermentable sugars as compared to physical and thermal methods. However, it requires treatment with temperatures that are high so as to degrade the feedstock and convert the polymeric sugars such as starch to monomeric sugars. Various factors are found to influence acid hydrolysis yield such as acid concentration, treatment duration and temperature[1]. Among these, acid concentration plays a critical role in determining the quantity of reducing sugars produced and the pH profile after hydrolysis and subsequent fermentation. Understanding this relationship is essential for optimizing bioethanol production processes, particularly when working with agricultural waste feedstocks such as sweet potato peels.

Based on previous studies, varying optimal acid concentrations have been reported. These depend on the feedstock and conditions of the process. Dilute sulphuric acid concentrations of 0–4% produced optimal monomeric sugars at lower temperatures (20-40 °C). On the other hand, higher temperatures (100–250 °C) were required when using weak acids. [3]found that 3.17% hydrochloric acid with an autoclavation time of 44.14 minutes yielded 29 g/L of reducing sugars from coconut leaflets. Similarly, [4]reported that 0.5 M HCl produced the highest fermentable sugar yield from sweet potato peels at 121^0C. Yields declined beyond this point due to excessive acid concentration breaking down starch inefficiently. Studies on other feedstocks, such as banana peels[5], have shown comparable trends, with higher reducing sugar yields achieved at lower acid concentrations.

Acid concentration also influences reducing sugar consumption and pH changes during fermentation. Excessive acid concentrations produce inhibitory by-products such as furfural, which impair microbial activity and reduce sugar utilization[6]. According to[7], reducing sugar yield increased up to 4.5–5% acid concentration

before declining. [5]found that high acid levels led to both lower reducing sugar content and reduced pH after fermentation. While some researchers argue that acid concentration primarily affects sugar release rather than consumption during fermentation, the consensus in literature indicates that optimal acid levels are necessary to balance sugar availability, microbial performance, and overall ethanol yield. This study builds on these findings by investigating the effect of hydrochloric acid concentration on reducing sugar yield and pH during hydrolysis and fermentation of sweet potato peels, aiming to identify conditions that maximize bioethanol production efficiency.

II. Materials And Methods

Reagents

The study utilized analytical-grade reagents as follows: Hydrochloric acid (HCl) at varying concentrations: 0.2 M, 0.5 M, 1.0 M, 1.5 M, and 2.0 M, standard glucose, sodium carbonate (Na_2CO_3), sodium bicarbonate ($NaHCO_3$), potassium sodium tartrate, anhydrous sodium sulfate (Na_2SO_4), ammonium molybdate, sulphuric acid (H_2SO_4), disodium hydrogen arsenate, and buffer tablets for pH meter calibration.

Apparatus/Equipment

500 ml Erlenmeyer conical flasks (labeled A–E), Analytical balance (for weighing samples and reagents), Water bath (capable of maintaining 98 °C), Autoclave (set at 121 °C), Refrigerator (for sample storage), Whatman filter paper No. 1, pH meter (with calibration buffers), Test tubes and racks, Beakers (various sizes), Mechanical crusher or grinder (for particle size reduction), Spectrophotometer (for absorbance measurement), Boiling apparatus (e.g., hot plate or heating mantle), Thermometer (if separate from water bath), Measuring cylinders and pipettes (for accurate liquid measurements)

Sample Collection

The species of the sweet potatoes were identified by a botanist using the leaves in the farm before harvesting the tubers for fermentation. The sweet potatoes were then transported to Murang'a University of Science and Technology Laboratory for hydrolysis preparation.

Sample Preparation

The sample of sweet potatoes was washed to remove the soil and other dirt from farm. The sweet potatoes were then peeled off. The peels were dried for about 10 days. The quantity of the sweet potato peels was about 5 Kilograms. The sweet potato peels underwent physical pretreatment. This was achieved through mechanical crushing of the dried peels so as to reduce them into small particle sizes.

Hydrolysis of sample

Hydrochloric acid hydrolysis was adopted for the study. 20g of samples was weighed into five different conical flasks (500ml Erlenmeyer) and labeled A, B, C, D and E[8]. 120ml of 0.2M, 0.5M, 1.0M, 1.5M and 2.0M of HCl was added to flasks respectively. The mixture in the flasks was heated in water bath for 2hours at 98^0C, sterilized in the autoclave at 121^0C for 15mins and the flask allowed to cool. The samples were filtered through Whatman filter paper No.1 and stored in refrigerator to be used later.

Determination of PH

The sample's pH was determined by use of well calibrated pH meter. The calibration of pH meter was done using a specific buffer tablet.

Determination of Reducing Sugar

Reducing sugar in sweet potatoes peels, hydroryzed peels, cellulose degraded sweet potatoes peels and fermented peels was determined. 25 grams anhydrous Na_2CO_3, 2 g $NaHCO_3$, 2.5 g potassium sodium tartrate and 20 g anhydrous sodium was weighed into 80 ml and made up to 100 ml. Arsenomolybdate reagent was prepared by dissolving 2.5 g ammonium molybdate in 45 ml water and 2.5 ml sulphuric acid added, then mixed with 0.3 g disodium hydrogen arsenate dissolved in water. The solution was incubated at 37^0C for 24 hours.

Standard glucose solution was made by dissolving 1g of glucose in 100ml distilled water. Working standard of 100 µg/ml was prepared by adding 1ml of glucose solution to 100ml of water. 0.2 ml, 0.4 ml, 0.6 ml, 0.6 ml and 1.0 ml of working standard was measured into five well-labeled test tubes. 0.1g of each test sample was measured into three beakers then 100ml of water added to each beaker. 1 ml of test samples was measured into four test tubes. Distilled water was then added followed by 1ml of alkaline copper tartrate reagent and shaken for proper mixing. Test tubes were then boiled for 10 minutes then cooled in water followed by addition of arsenomolybdate reagent and shaken well for proper mixing. 7 ml of distilled water was added to each test tube to make up to 10 ml. The absorbance of the blue color in each sample was determined by

spectrometer, and then graph of absorbance against concentration plotted to obtain the amount of reducing sugar. The following quantities were determined:

% reducing sugar = Concentration of sugar in test tube

Concentration of sugar = $\frac{Absorbance\ of\ test\ sample}{Absorbance\ of\ standard\ sample}$

III. Results

At 0.2 M, the average reducing sugar after hydrolysis was 24.17 g/L, the average reducing sugar after fermentation was 6.50 g/L while the average reducing sugar consumed was 17.67 g/L; average pH after fermentation was 3.20. At 0.5 M, the average reducing sugar after hydrolysis was 28.77 g/L, the average reducing sugar after fermentation was 6.93 g/L while the average reducing sugar consumed was 21.83 g/L; average pH after fermentation was 3.30. At 1.0 M, the average reducing sugar after hydrolysis was 25.70 g/L, the average reducing sugar after fermentation was 6.95 g/L while the average reducing sugar consumed was 18.75 g/L; average pH after fermentation was 3.70. At 2.0 M, the average reducing sugar after hydrolysis was 18.03 g/L, the average reducing sugar after fermentation was 6.50 g/L while the average reducing sugar consumed was 11.53 g/L; average pH after fermentation was 3.70 (see Table 1).

Table 1: Average Reducing Sugar after Hydrolysis, after Fermentation, pH after fermentation and Reducing Consumed

Variable	Acid concentration (M)			
	0.2	0.5	1.0	2.0
Reducing Sugar after hydrolysis (g/L)	24.17	28.77	25.70	18.03
Reducing sugar after fermentation (g/L)	6.50	6.93	6.95	6.50
pH after fermentation	3.20	3.30	3.70	3.70
Reducing Sugar Consumed (g/L)	17.67	21.83	18.75	11.53

An examination of the data in Table 1 reveals that 0.5 M HCl has the highest reducing sugar after hydrolysis and the highest reducing sugar consumed. The data also reveals that 1.0 M and 2.0 M maintained a higher pH (less acidic). The data further reveals that 0.2 M and 2.0 M HCl concentrations have the lowest reducing sugar after fermentation. This is also shown in Figure 1.

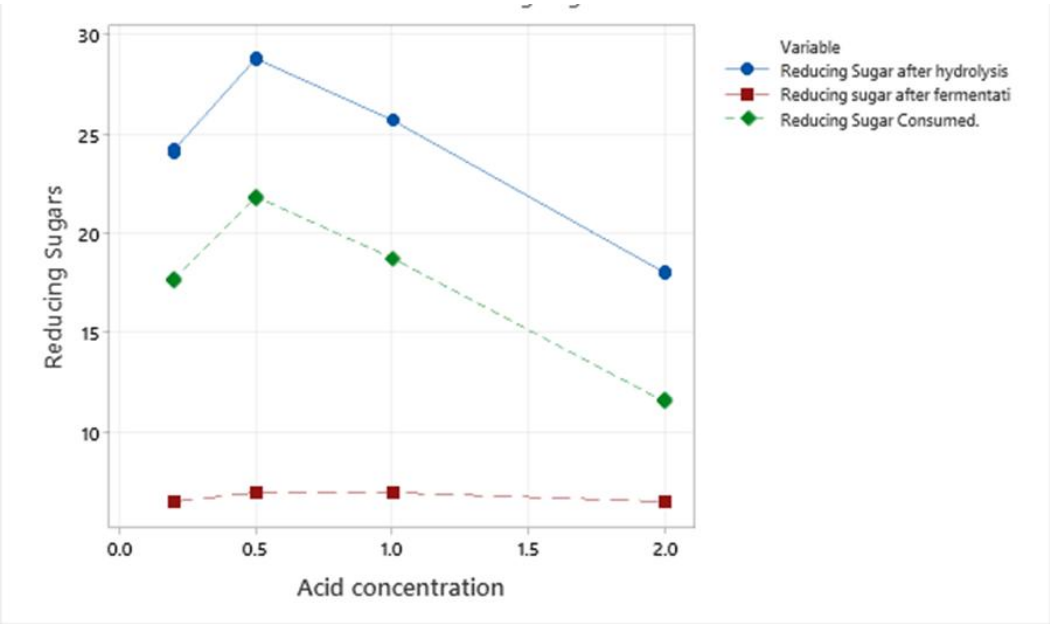

Figure 1: Reducing Sugars by Acid Concentrations

Appropriate one-way comparison of groups, either One-way ANOVA or Kruskal-Wallis test was used to test if there significant differences in reducing sugars and pH after hydrolysis, after fermentation and the amount of reducing sugars consumed based on acid concentration. One-way comparison of groups was used to test whether acid concentration had a significant effect on the amount of reducing sugar after hydrolysis, reducing after fermentation, PH after fermentation and reducing sugars consumed by comparing the amounts of these variables across different acid concentrations (0.2 M, 0.5 M, 1.0 M and 2.0 M under the following null hypothesis:

H_0: Acid concentration has no effect on the variables- reducing sugar after hydrolysis, reducing after fermentation, PH after fermentation and reducing sugars consumed (all means are equal).

Before running the appropriate one-way comparison of groups, normality test was carried out using the Kolmogorov-Smirnov test. This was important because one-way ANOVA assumes that the data should be normally distributed while Kruskal-Wallis Test does not assume normality. Under Kolmogorov-Smirnov test, data is assumed to be normally distributed if the p-value > 0.05; under which one-way ANOVA can be adopted; otherwise, Kruskal-Wallis Test is adopted. The Kolmogorov-Smirnov test for normality results are as shown in Table 2. From the Kolmogorov-Smirnov results, all the p-values are less than 0.05. Therefore, normality assumption is violated for all the variables making Kruskal-Wallis Test appropriate in testing whether there are statistically significant differences in the variables by acid concentration.

Table 2: Normality Assumption Test for Reducing Sugars and pH

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Reducing sugar after hydrolysis	0.243	12	0.048	0.842	12	0.029
Reducing sugar after fermentation	0.330	12	0.001	0.669	12	0.000
pH after fermentation	0.328	12	0.001	0.721	12	0.001
Reducing sugar consumed	0.266	12	0.019	0.831	12	0.022
a. Lilliefors Significance Correction						

The results for the Kruskal-Wallis test are as shown in Table 3. For reducing sugars after hydrolysis, p-value = 0.014. This implied that there are statistically significant differences in the amount of reducing sugar after hydrolysis by HCl acid concentration. The p-values for reducing sugars after fermentation show that p-value = 0.015; an indication that there are statistically significant differences in amount of reducing sugars after fermentation by HCl acid concentration. For pH after fermentation, p-value = 0.012; therefore, there are statistically significant differences in pH after fermentation based on HCl acid concentration. The findings further show that p-value = 0.014 for reducing sugar consumed; this implies that there are statistically significant differences in the amount of reducing sugar consumed by HCl acid concentration.

Table 3: Kruskal-Wallis Test for Differences in Reducing Sugars and pH

	Reducing sugar after hydrolysis	Reducing sugar after fermentation	pH after fermentation	Reducing sugar consumed
Kruskal-Wallis H	10.645	10.429	11.000	10.645
df	3	3	3	3
Asymp. Sig.	0.014	0.015	0.012	0.014
a. Kruskal Wallis Test				
b. Grouping Variable: HCl Acid Concentration				

Kruskal-Wallis test in Table 3 does not specify which groups significantly differ. As a result, post-hoc tests are conducted to identify which groups have a statistically significant difference. In this study, Mann-Whitney Tests were conducted and the results are as tabulated in Table 4. The pairwise comparisons for reducing sugar after hydrolysis show that there were statistically significant differences in reducing sugars for all HCl concentration pairs (all p-values were less than 0.05). For reducing sugars after fermentation, no significant difference was observed in reducing sugar after fermentation for HCl concentration pairs 0.2 M * 2.0 M (p-value = 1.000) and 0.5 M * 1.0 M (p-value = 0.317). For pH after fermentation, statistically significant differences were observed for all HCl concentration pairwise comparison (all p-values were less than 0.05). Similarly, statistically significant differences were observed for reducing sugar consumed for all HCl concentration pairwise comparisons (all p-values were less than 0.05).

Table 4: Pairwise Post-Hoc Analysis

HCl Categories		Reducing sugar after hydrolysis	Reducing sugar after fermentation	pH after fermentation	Reducing sugar consumed
0.2 M * 0.5 M	Asymp. Sig. (2-tailed)	0.043	0.034	0.025	0.043
0.2 M * 1.0 M	Asymp. Sig. (2-tailed)	0.034	0.025	0.025	0.034
0.2 M * 2.0 M	Asymp. Sig. (2-tailed)	0.043	1.000	0.025	0.043
0.5 M * 1.0 M	Asymp. Sig. (2-tailed)	0.034	0.317	.025	0.034
0.5 M * 2.0 M	Asymp. Sig. (2-tailed)	0.043	0.034	0.025	0.043
1.0 M * 2.0 M	Asymp. Sig. (2-tailed)	0.034	0.025	1.000	0.034
a. Grouping Variable: HCl Acid Concentration					

IV. Discussion

The findings showed that there were statistically significant differences in the amount of reducing sugars after hydrolysis for the different acid concentrations. Pairwise comparison results showed that the significant differences were observed for all paired comparisons of the different acid concentrations. A scrutiny of the data revealed that 0.5 M HCl had the highest amount of reducing sugars after hydrolysis. The findings are in alignment with a study by [2] who established that optimal reducing sugars after hydrolysis were found at concentrations of 0-4% while maintaining low temperatures ranging between 20-40^{0}C. The study further demonstrated that use of weak acids for hydrolysis can only be optimal at high temperature ranges of 100-250^{0}C. In another experiment by [3], HCL was used for hydrolysis during production of bioethanol from coconut leaflets. Overall, HCl concentration had a significant effect on the amount of reducing sugars; findings which are in concurrence with our study. In a study by [4], *Saccharomyces cerevisiae* was utilized for bioethanol production from sweet potato peel waste using HCl acid hydrolysis at different concentration and at 121 ^{0}C. The study established that fermentable sugar quantities significantly increased with HCl concentration with optimal acid concentration achieved at 0.5M; findings which agree with our study's results. Beyond 0.5M the amount of reducing sugars reduced; an indication that highly concentrated acids break down starch to fermentable sugars at temperatures that are lower.

In general, statistically significant differences were found in reducing sugars after fermentation, reducing sugars consumed and pH after fermentation for the different acid concentrations. All pairwise comparisons for reducing sugars consumed showed significant difference for all paired acid concentrations. The results also showed that 0.5 M HCl concentration had the highest amount of reducing sugars consumed while 0.2 M and 2.0 M HCl concentrations had the lowest reducing sugar after fermentation. The data also reveals that 1.0 M and 2.0 M maintained a higher pH (less acidic). During acid hydrolysis, the concentration of the acid used is one of the factors that can have an effect on the conversion yield. These findings disagree with [6] who assessed how acids pre-treatment affected bioethanol fermentation process using microalgae using sulphuric and acetic acids for hydrolysis at concentrations of 1%, 3%, 5%, 7% and 9%. From the findings, reducing sugar was most consumed for both acids at 5% concentration. The findings in [6] established that the difference in reducing sugars consumed was not significant for both acids at all levels of concentration considered for the experiment. At higher concentrations of 9%, both acids generated least sugar consumed at 16.65 g/L for sulphuric acid and 16.25 g/L for acetic acid; partially agreeing with this study. High acids concentration during pre-treatment and hydrolysis convert monosaccharides into some known inhibitors, for example furfural, which leads to a decline in the amount of reducing sugars to be consumed.

An experiment by [7] optimized acid hydrolysis in bioethanol production using *Pachysolen tannophilus* yeast and *Ulva rigida* as the feedstock while utilizing sulphuric and hydrochloric acids of concentrations 1-10%. From the experimental results, the amount of reducing sugars increased up to acids concentration of 5% for both acids from where it declined thus conforming to the results of our study. For [4], concentration of the acid does not determine reducing sugars and pH after fermentation, which is in disagreement with this study. Reducing sugars depends on the amount of sugar consumed during fermentation and the fermentation conditions. Acid concentration as outlined in [4]only determines reducing sugars amount released for fermentation but not those consumed which is in contrast with our study.

V. Conclusion And Recommendations

Different concentrations of HCl have a significant influence on the amount of reducing sugars released during hydrolysis and those consumed during fermentation. The acidity levels (pH) also varied depending on HCl concentration. The study established that optimal HCl concentration for hydrolysis was 0.5 M since it produced the highest amount of reducing sugars and supported efficient fermentation. The study therefore recommends that excessive high HCl concentration should be avoided during hydrolysis as this may result in reduced sugar availability which has a negative effect on the efficiency of fermentation.

References

[1]. Woźniak A, Kuligowski K, Świerczek L, Cenian A. Review Of Lignocellulosic Biomass Pretreatment Using Physical, Thermal And Chemical Methods For Higher Yields In Bioethanol Production. Sustainability. 2025 Jan 2;17(1):287.

[2]. Kim JW, Mazza G. Optimization Of Phosphoric Acid Catalyzed Fractionation And Enzymatic Digestibility Of Flax Shives. Industrial Crops And Products. 2008 Nov 1;28(3):346-55.

[3]. Shet VB, Bhat M, Naik M, Mascarenhas LN, Goveas LC, Rao CV, Ujwal P, Sandesh K, Aparna A. Acid Hydrolysis Optimization Of Cocoa Pod Shell Using Response Surface Methodology Approach Toward Ethanol Production. Agriculture And Natural Resources. 2018 Dec 1;52(6):581-7.

[4]. Ojewumi ME, Adeeyo OA, Akingbade OM, Babatunde DE, Ayoola AA, Awolu OO, Ojewumi EO, Omodara OJ. Evaluation Of Glucose Syrup Produced From Cassava Hydrolyzed With Malted Grains (Rice, Sorghum & Maize). International Journal Of Pharmaceutical Sciences And Research. 2018;9(8):1000-11.

[5]. Abdulla R, Johnny Q, Jawan R, Sani SA. Agrowastes Of Banana Peels As An Eco-Friendly Feedstock For The Production Of Biofuels Using Immobilized Yeast Cells. Iniop Conference Series: Earth And Environmental Science 2022 Nov 1 (Vol. 1103, No. 1, P. 012022). IOP Publishing.

[6]. Phwan CK, Chew KW, Sebayang AH, Ong HC, Ling TC, Malek MA, Ho YC, Show PL. Effects Of Acids Pre-Treatment On The Microbial Fermentation Process For Bioethanol Production From Microalgae. Biotechnology For Biofuels. 2019 Jul 31;12(1):191.
[7]. El Harchi M, Kachkach FF, El Mtili N. Optimization Of Thermal Acid Hydrolysis For Bioethanol Production From Ulva Rigida With Yeast Pachysolen Tannophilus. South African Journal Of Botany. 2018 Mar 1;115:161-9.
[8]. Okoro TC, Onwumelu DC, Igbinidu-Uwuigbe EP, Ofoegbu DC, Onwuka MK, Nnebeana SE. Determination Of Optimum Conditions For Maximum Bioethanol Yield From The Nigerian Sweet Potato (Ipomoea Batatas (L.) Lam). World Journal Of Advanced Engineering Technology And Sciences. 2022;7(1):075-85.