

Statistical Modelling of a Preliminary Process for Depolymerisation of Cassava Non-starch Carbohydrate Using Organic Acids and Salt

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ABSTRACT: A preliminary study on statistical modelling of a process for depolymerisation of cassava non-starch carbohydrate using halide salt assisted phosphoric and pyruvic acids were accomplished. The effects of three independent variables namely; acid concentration, potassium iodide salt and duration were studied using the central composite rotatable design on hydrolysis of the cassava non-starch carbohydrate. Hydrolysate marker, reducing sugar was evaluated as a measure of depolymerisation, consequently used as response in the study. Equations for predicting the response were developed and adequacy confirmed using analysis of variance and residual assessment. The predictive model provides the potentials of using phosphoric acid and pyruvic acid as depolymerisation agent for cassava non-starch carbohydrate in the presence of potassium iodide salt. This would open a new opportunity for depolymerisation of cassava non-starch carbohydrate that is usually used for low premium animal feed or left as dung resulting to environmental pollution.

Keywords: *Cassava, Depolymerisation, Hydrolysate Marker, Non-starch-carbohydrate, Salt-assisted acids.*

Introduction

Conversion of lignocellulose to fermentable sugars is one of the most important steps for bioconversion of lignocellulosic biomass to bio-based chemicals (Zhao *et al.*, 2012). Dilute acid hydrolysis has been considered as one of the most promising methods to convert glycan into fermentable sugars or pretreat biomass to increase enzymatic digestibility of cellulose (Sun and Chang, 2002; Mosier *et al.*, 2005). The most widely used and tested approaches are based on the use of dilute sulphuric acid (Zhang *et al.*, 2011).

However, this approach has several shortcomings. First, sulphuric acid produces toxic fumes. Second, after neutralization, it is typically necessary to desalinate the syrup using high cost ion exchange resins. Third, undesirable by-products are produced even when syrups of an average Dextrose Equivalent are produced because free

glucose is converted to dehydration products such as hydroxymethyl furfural, levulinic and formic acids, which can inhibit microbial growth in a subsequent fermentation step (Liu *et al.*, 2004). Fourth, despite extensive washing, residual granular protein and fat could react with sulphuric acid to produce coloured by products such as bitter maillard compounds that should be eliminated leading to additional cost.

Fontana *et al.* (2008) postulated that the use of phosphoric acid rather than sulphuric acid or hydrochloric acid presents several advantages. Firstly, phosphoric acid is non-volatile, therefore safer to handle. Secondly, by product formation is reduced. Thirdly, when concentrated sugar syrup (40% of solid) are diluted on addition to foods or beverages, the phosphoric acid concentration falls to values as low as 0.01 to 0.05% giving a pleasant taste of acidity at pH value of about 3.0. More, importantly, if the hydrolysate is to be used in a subsequent

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fermentation step there is no need to eliminate the phosphoric acid. Nevertheless, neutralization with ammonia leads to formation of ammonium phosphate, a supplement for growth.

Similarly, Gamez *et al.* (2004) had previously hydrolyzed sugarcane bagasse using phosphoric acid at atmospheric pressure with view to produce fermentable sugar solutions. In another group of works, Zuwei *et al.* (2001) elicited that addition of salts to solvents can increase their conductivity and has a dramatic influence on their rate of heating. The advantages of using phosphoric acid for depolymerisation couple with the influence of halide salts in enhancing rate of heating lead to the conceptual study of statistical modelling of a process for depolymerisation of cassava non starch carbohydrates.

The objective of this study was to accomplish statistical modelling of a process for the depolymerisation of cassava non-starch carbohydrate using phosphoric acid and pyruvic acid in the presence of potassium iodide.

Materials and Methods

- Non-Starch Carbohydrate Isolation

After cassava mash was sieved using muslin cloth to eliminate starch and the residues were screened in 2L of water using sieves with opening ranging from 50µm to 90µm. The recovered non-starch-carbohydrate (NSC) residues were thoroughly rinsed using water till run-off water was clear colourless. The sample fractions obtained were dried using tray dryer, then milled using Laboratory blender. These were packaged for subsequent treatment and analysis. The protocol was adapted from, Baik and Dalgetty (2003).

- Depolymerisation Procedure

Depolymerisation procedure was adapted from the method of Fontana *et al.* (2008). Simply, the solid (g) to solvent (mL) was 3:100 and aqueous phosphoric and pyruvic

acids were added independently to give final pH values from 1.80 to 3.76 and potassium iodide was added appropriately in accordance scheme shown in Table 1. The suspensions were heated using pressure pot for the stated holding time.

- Measurement of Reducing Sugar

The concentration of soluble reducing sugars were measured using a 3, 5 dinitrosalicylic acid (DNS) method (Chaplin, 1986). Immediately after heating, the solution was diluted with water 10 times and 0.2mL of the diluted sample was transferred to a test tube where it was mixed with 2mL of the DNS reagent. After mixing it with a vortex, the samples were heated in the water bath at 100 °C for 10 min followed by rapid cooling in iced water. The optical densities of the samples were measured at 570nm using a (Bausch & Lomb Rochester NY, USA) spectrophotometer. Glucose was used for the preparation of a standard curve.

- Statistical analysis

The central composite orthogonal designed was analysed as replated by Cochran and Cox (1957). Each of the X-matrix was multiplied by the Y-column (response) to obtain corresponding sums of products that is 0y to 13y for X₀ to X₁ X₃. Consequently, the coefficients b₀ to b₁₃ were calculate as:

$$b_0 = 0.166338(0y) - 0.056791 \sum (iij) \quad (1)$$

$$b_i = 0.073224(iy) \quad (2)$$

$$b_{ii} = 0.062500(iiy) + 0.006889 \sum (iij) - 0.056791(0y) \quad (3)$$

$$b_{ij} = 0.125000(ijy) \quad (4)$$

The quadratic model was fitted using the regression coefficients and the predicted response calculated for each of the observed values. The model was observed for adequacy by subjection to analysis of variance and residual analysis.

- Statistical analysis using Paired t-test

Two-sample t-test was conducted on the 14 observations. The experiments were carried out of the hydrolysates obtained from cassava non-starch non-starch carbohydrate using phosphoric acid and pyruvic acid under homogeneous condition, although the experimental condition of each pair change from one pair to another as shown.

The appropriate test statistic employed is given

$$t_{cal} = \frac{\bar{D}}{S_D/\sqrt{n}} \quad (5)$$

$$\bar{D} = \frac{\sum_{i=1}^n D_i}{n} \quad (6)$$

$$S_D^2 = \frac{\sum_{i=1}^n D_i^2 - [(\sum_{i=1}^n D_i)^2/n]}{n-1} \quad (7)$$

$\bar{D}_i$ = sample mean of difference of each pair of observation

S_D^2 = variance of sample difference

There is no significant difference between the pair samples of the treatment if $t_{cal} \leq t_{Tab}$ at 5% (Snedecor, 1956).

Results and Discussion

Depolymerisation is mandatory for a Table 2.

carbohydrate substrate to be useful for fermentation or microbial utilization or preparation of sugars/sweeteners or other green chemical. Depolymerisation of cassava non-starch carbohydrate was accomplished using phosphoric acid in similitude to depolymerisation of starch and sugar bagasse using phosphoric acid as reported earlier by some workers notably by Fontana *et al.* (2008) and Gamez *et al.* (2004). In addition, pyruvic acid was used in this study because it is food grade without need for purification of its hydrolysate. Sharing safe handling properties like phosphoric acid. The profound depolymerisation index is carbohydrate hydrolysates (sugars) measured in terms of reducing sugar. The central composite orthogonal design to fit the polynomial model of the depolymerisation of cassava non-starch carbohydrate was accomplished as elicited by Cochran and Cox (1957). The computed sums of products and regression coefficients to fit the model are shown in

Table 1. Process variables used in the central composite rotatable design (k = 3)

Independent variables	Code	Levels				
		-1.682	-1	0	1	1.682
Acid (%)	X ₁	0.02	0.04	0.08	0.16	0.32
Salt (mL)	X ₂	0.1	0.2	0.4	0.6	1
Time (h)	X ₃	1	2	3	4	5

Molar concentration of salt = 0.15M

Table 2. Regression coefficients for the quadratic model of phosphoric and pyruvic acids depolymerised non-starch carbohydrate

Sum of products	Hydrolysate		Regression coefficient	Hydrolysate	
	Phosphoric acid	Pyruvic acid		Phosphoric acid	Pyruvic acid
Oy	218.97	188.47	b ₀	17.46221883	16.11383338
Y	25.06	27.99	b ₁	1.83499	2.0495376
2y	13.53	16.20	b ₂	0.99072072	1.1862288
3y	27.16	22.65	b ₃	1.98876384	1.6585236
11y	115.97	93.93	b ₁₁	-2.88736984	-2.98459385
22y	117.33	90.43	b ₂₂	-2.80236984	-3.20334385
33y	100.57	83.92	b ₃₃	-3.84986984	-3.61021885
12y	7.61	6.87	b ₁₂	0.95125	0.85875
13y	3.37	3.77	b ₁₃	0.42125	0.47125
23y	5.21	3.77	b ₂₃	0.65125	0.47125
Σ (ii)y	333.87	268.28			

As stated earlier depolymerisation of non-starch carbohydrate was accomplished by phosphoric acid, similar to earlier report by Fontana *et al.* (2008) and Gamez *et al.* (2004) that worked on depolymerisation of starch and sugar bagasse respectively. However the amount of hydrolysate (reducing sugar) was lower in comparison to the sugar-hydrolysate from starch. The low hydrolysates of cassava non-starch carbohydrate could be explained in terms of high structural crystallinity of non-starch carbohydrate which consist mainly hemicelluloses, cellulose and lignin in comparison to pure starch. Therefore, the quadratic model takes the form:

$$\begin{aligned} \text{Ph}^{\text{AH}} = & 17.46222 + 1.83499X_1 + 0.99072X_2 + 1.98876X_3 - 2.88737X_1^2 - \\ & 2.802369X_2^2 - 3.8498698X_3^2 + 0.95125X_1X_2 + 0.42125X_1X_3 + \\ & 0.65125X_2X_3 \end{aligned} \quad (8)$$

The predicted cassava non-starch carbohydrate hydrolysate accomplished using phosphoric acid for each of the experimental run and their respective residual are shown in Table 3. Examination of the residuals suggests that the fitted model was reasonably adequate. The assertion was confirmed on model testing. Added to this, the analysis of variance to test the fitness of the model is presented in Table 4. The first and second order terms were significant as shown by the higher calculated F-ratio in comparison with the tabulated values. However, since the calculated F-ratio for the lack of fit was lower than the tabulated value, consequently adequacy of the fitted model is affirmed.

Also, pyruvic acid – caused hydrolysate was also examined. The quadratic model takes the form:

$$\begin{aligned} \text{Py}^{\text{AH}} = & 16.113833 + 2.0495398X_1 + 1.1862288X_2 + 1.653524X_3 - \\ & 2.98459385X_1^2 - 3.20334X_2^2 - 3.610219X_3^2 + \\ & 0.85875X_1X_2 + 0.47125X_1X_3 + 0.47125X_2X_3 \end{aligned} \quad (9).$$

The predicted cassava non-starch carbohydrate hydrolysate accomplished using pyruvic acid for each of the experimental run and their respective residual are shown in Table 3. As logically explained for the first hydrolysates, examination of the residuals suggests that the fitted model (9) was reasonably adequate. This claim was confirmed on model testing. Added to this, the analysis of variance to test the fitness of the model is presented in Table 4.

The first and second order terms were significant as shown by the higher calculated F-ratio in comparison with the tabulated values. Since the calculated F-ratio for the lack of fit was lower than the tabulated value, hence adequacy of the fitted model is affirmed.

An insight to the comparative hydrolytic potentials of phosphoric acid and pyruvic acid on cassava non-starch carbohydrate can be gained by taking a cursory look on Table 5. Analysis showed that there was no statistical difference between the hydrolysates obtained from the two acids used in this study. It is important to note that the fact that pyruvate, a salt of pyruvic acid is a metabolic product suggests that pyruvic acid stands preferred for depolymerisation of carbohydrate for preparation of food chemicals than phosphoric acid.

Conclusion

The potentials of using halide salt assisted phosphoric acid and pyruvic acid as an agent for depolymerisation of cassava non-starch carbohydrate were demonstrated. Predictive model was developed for the process and adequacy of the model was confirmed using analysis of variance and residual assessment. This study provides insight to the possibility of depolymerising cassava non-starch

carbohydrate which could serve as a feed stock for fermentation and production of green chemicals.

Table 3. Residual analysis of cassava non starch carbohydrate *hydrolysate (reducing sugar)

Experimental Run	Phosphoric acid Hydrolysate			Pyruvic acid Hydrolysate		
	Observed	Predicted	Residual	Observed	Predicted	Residual
1.	4.19	5.1319	-0.9419	3.11	3.2230	-0.112
2.	7.50	6.0569	1.4431	5.94	6.3793	-0.4393
3.	5.00	3.904	1.096	4.69	2.9300	1.76
4.	9.00	8.638	0.362	7.80	7.8090	-0.009
5.	7.50	6.964	0.500	5.94	4.6547	1.2853
6.	9.38	9.5746	-0.1946	7.50	7.9788	-0.4788
7.	7.80	8.3459	-0.5459	6.25	6.2520	-0.002
8.	16.60	14.7609	1.8391	14.4	13.0110	1.389
9.	6.56	6.207	0.353	3.10	4.2200	-1.12
10.	10.76	12.38	-1.62	10.44	11.1178	-0.6778
11.	7.80	7.8676	-0.0676	4.50	5.0564	-0.5564
12.	10.00	11.20	-1.200	7.80	9.0468	-1.2468
13.	2.50	3.23	-0.73	2.00	3.1100	-1.11
14.	9.38	9.92	-0.54	8.00	8.6900	-0.6900
15.	15.00	17.4622	-2.4622	18.00	16.11383338	1.8862
16.	20.00	17.4622	2.5378	15.00	16.11383338	-1.11383338
17.	18.00	17.4622	0.5378	15.00	16.11383338	-1.11383338
18.	18.00	17.4622	0.5378	16.00	16.11383338	-0.113833
19.	16.00	17.4622	-1.4622	18.00	16.11383338	1.8862
20.	18.00	17.4622	0.5378	15.00	16.11383338	-1.1138

*mg glucose/mL, Residual = observed value- predicted value.

Table 4. Analysis of Variance (ANOVA) for the predictive model equations

Acid Dependent	Statistical term variable	DF	SS	MS	F – ratio		
					Calculated	Tabulated	
						5%	1%
Phosphoric acid	First order	3	113.404	37.80	12.194	5.41	12.06
	Second order	6	387.529	64.59	20.84	4.95	10.67
	Lack of fit	5	13.50	2.70	0.87	5.05	10.97
	Error	5	15.50	3.10			
	Total	19	529.933				
Pyruvic acid	First order	3	114.152	38.05	17.56	5.41	12.06
	Second order	6	397.389	66.23	30.56	4.95	10.67
	Lack of fit	5	14.0206	2.804	1.295	5.05	10.97
	Error	5	10.8334	2.167			
	Total	19	536.395				

DF = degree of freedom, SS = sum of square, MS = mean square

DF = Degree of Freedom SS = Sum of Square MS = Mean Square

Table 5. Comparative statistical assessment of halide salt assisted phosphoric and pyruvic acids depolymerised cassava non-starch * hydrolysates

Experimental Run	Phosphoric Acid	Pyruvic Acid	Difference	d ²	t _{cal.0.05}	t _{tab0.05}
1	4.19	3.11	1.08	1.1664	0.9665	2.160
2	7.50	5.94	1.56	2.4336		
3	5.00	4.69	0.31	0.0961		
4	9.00	7.80	1.2	1.44		
5	7.50	5.94	1.56	2.4336		
6	9.38	7.50	1.88	3.5344		
7	7.80	6.25	1.55	2.4025		
8	16.60	14.4	2.2	4.84		
9	6.56	3.10	3.46	11.9716		
10	10.76	10.44	0.32	0.1024		
11	7.80	4.50	3.30	10.89		
12	10.00	7.80	2.20	4.84		
13	2.50	2.00	0.50	0.25		
14	9.38	8.00	1.38	1.9044		
Total	113.97	91.47	22.50	48.305		
Mean	8.1407	6.5336	1.607			

*mg glucose/mL, n=14, Difference of mean ($\bar{D}$) = 1.6071, S^2_D = 0.9342

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