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Effect of Acid Types, Acid Concentration and Time on 5-Hydroxymethylfurfural (HMF) Production.

Adewumi, Chizoma Nwakego[1], Achugasim, Ozioma[2], Ogali, Regina Enyidiya[2], Akaranta Onyewuchi[2].

[1]Department of Pure and Applied Chemistry, Veritas University, Abuja, Nigeria.

[2]Department of Pure and Industrial Chemistry, University of Port Harcourt. Nigeria.

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Abstract: 5-Hydroxymethylfurfural (HMF) is a vital renewable organic compound found useful as a platform chemical in bio-refining, polymer precursors and other chemical intermediates. HMF is produced via acid-catalyzed dehydration of diverse carbohydrates. The study aimed to investigate the effect of different acid types: sulphuric (H_2SO_4), hydrochloric (HCl) and nitric (HNO_3), at acid concentrations (0.25-0.75 $moldm^{-3}$) and time (15-60 minutes) on HMF production. UV-Visible spectrophotometric and high-performance liquid chromatographic (HPLC) were the two analytical techniques employed to evaluate HMF production in all conditions of analysis. It was observed that the catalytic action of the acids towards HMF formation is in the following decreasing trend HCl > HNO_3 > H_2SO_4. The highest HMF produced (0.255 mg/l) was achieved at 0.75 $moldm^{-3}$ HCl at 60 minutes hydrolysis time. Increasing acid concentration and time was found to increase HMF production with HCl. However, the yield decreased after 45 minutes hydrolysis time for HNO_3 and H_2SO_4 hydrolyzed samples. Therefore, the knowledge obtained from this work will help in designing HMF production process for optimum industrial production.

Keywords: 5-Hydroxymethyfurfural, acid type, acid concentrations, UV-Visible spectrophotometer, HPLC.

I. Introduction

5-Hydroxymethylfurfural (HMF) is a vital renewable organic compound (with aldehyde functional group) used for production of fine chemicals, petroleum-derived commodities, polymer precursors and other chemical intermediates. It has been found useful as a platform chemical in bio-refining because it serves as a precursor for the production of biofuels and numerous high-volume plastics [1-3]. HMF can be produced via acid-catalyzed dehydration of diverse carbohydrates such as glucose, fructose, sucrose and cellulose or as an intermediate product in the Maillard reaction, [3-5]. In the browning process in milk through Maillard reaction, HMF is obtained by degradation of lactulosyllysine through 1,2 enolization [6,7].

Recently, studies have been carried out on quantification and distribution of HMF in food, honey, beverages in order to prevent possible toxicological effects (carcinogenicity, genotoxicity, cytotoxicity and mutagenicity) in the body or fermentation medium [8-10]. HMF is presumed to be an irritant to the eyes, skin, upper respiratory tract and mucous membranes. Human exposures to it can occur via inhalation, ingestion or skin absorption. Recent finding shows that 5HMF might act as an initiator as well as promoter of colon cancer in rats [11]. The furan aldehyde (furfural and HMF) has been found to inhibit the growth of yeast and decreases ethanol productivity and yield [12]. Taherzadeh *et al*. [13], reported that furans decrease fermentability by reducing the activities of several yeast enzymes such as aldehyde dehydrogenase, alcohol dehydrogenase and pyruvate dehydrogenase

Different analytical methods have been developed to determine the quantity of furan compounds (HMF and furfural) in hydrolysate and food samples. The UV-Visible spectrophotometric method was found to be fast and cheap but scarcely sensitive and specific, whereas, the high-performance liquid chromatographic (HPLC) method was found to be slow and expensive but more accurate [3,6,10,14-16]. But the UV–VIS Spectrophotometry is considered a green methodology because it involves the use of inexpensive instrumentation, and does not use much toxic solvents as chromatographic methods do [9,17,18]. HMF and furfural have their characteristic absorbance band at 284 nm and 277 nm, respectively, and hence can be determined precisely by UV-Visible-spectral method only if one of them is present in the medium without the interference of the other contaminants [6,13,15]. Nevertheless, in acid hydrolysis process, both are present in the hydrolysate or sugar syrup and their accurate determination become a major challenge. Conversely, it was observed that a major spectral interference species arises in the quantification of HMF and other hydrolysis by-products; however, the interferences were alleviated through double-wavelength technique or simultaneous measurement of the spectra by absorption treatment of charcoal [16] or before and after reduction with sodium borohydride [15], two-phase reaction system with HCl in the presence of DMSO and poly(1-vinyl-2-pyrrolidine)(PVP [19], salting-out technique with different organic solvents [3].

Thus, in this work, the effect of different acid types, acid concentrations and time on HMF production from starch was studied using UV-Visible spectrophotometric method. To verify the data, obtain from the UV-spectra, further precision study was carried out using HPLC method.

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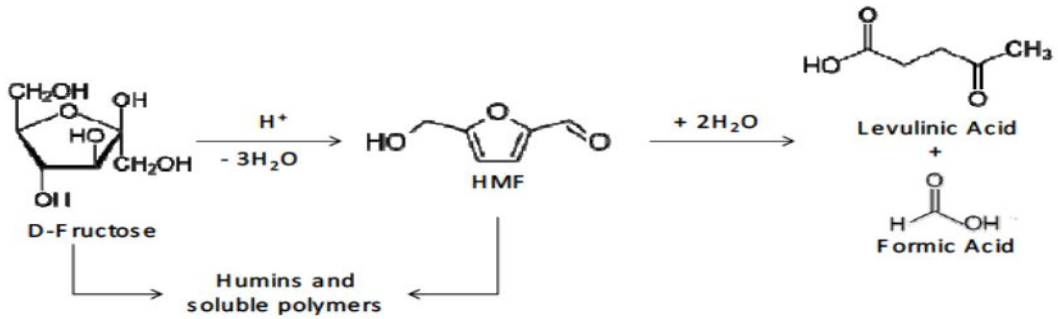

Figure 1: Reaction scheme for HMF production via sugars dehydration (Gomes et al., 2015).

II. Materials and Methods

Reagents and Samples

All reagents used were of analytical grade. A standard stock solution containing 100 mg/L 5-hydroxymethylfurfural was purchased from Sigma-Aldrich, Germany and used to prepare the working standard solutions (0.06 - 0.12 mg/L). Sulfuric (H_2SO_4), Hydrochloric (HCl) and Nitric (HNO_3) acids were purchased from BDH chemicals, England. Acetonitrile (HPLC grade) and ultrapure water was obtained from biotechnology laboratory of Covenant University Ota, Ogun state, while starch samples used for the analysis was obtained from Pure and Industrial Chemistry Laboratory, University of Port Harcourt, Rivers state, Nigeria.

Acid hydrolysis of the starch

Acid hydrolysis of the starch was carried out in an autoclave (Techmel and Techmel U.S.A. model TT-280A) in accordance with the method of Gupta *et al*. [20]. Starch slurry was prepared in a 1: 20 substrates to liquid ratio in 250 ml flask, the mixture was then transferred into the hydrolysing vessel. The operating condition factored into are: Different acid types (H_2SO_4, HCl and HNO_3), acid concentration (0.25, 0.5, 0.75 $molL^{-1}$), Temperature: 121^oC and Time (15, 30, 45 and 60 minutes). At the end of the hydrolysis, the solution was filtered using filter paper and the resultant hydrolysate analysed for HMF concentration.

Analysis of 5-Hydroxymethyl Furfural (HMF) by spectrophotometric method.

Spectrophotometric determinations were carried out in 1 cm quartz cells in a single beam spectrophotometer (Metash UV-5200 spectrophotometer) . Three working solutions of HMF; 0.06, 0.09 and 0.12 mg/l were prepared from a stock solution of 100 mg/l and their absorbance values were obtained from a UV-Visible spectrophotometer at the wavelength of 284 nm. To determine the unknown concentration of each hydrolyzed sample, a standard calibration curve of absorbance against concentration of standard HMF solutions was plotted. To each hydrolyzed sample, 3 ml of hydrolysate (after treating with activated carbon) was analyzed for its HMF concentration using UV-spectrophotometer and the absorbance was obtained at 284 nm. The concentration of each sample was obtained from the plot of absorbance against standard HMF concentration.

Determination of 5-Hydroxymethyl Furfural (HMF) by high performance liquid chromatography (HPLC) method.

The method of Adewumi *et al*. [21] was used to obtain the HMF concentration in the starch (1g) samples hydrolysed with different concentrations of sulphuric acid (0.25-0.75 M) at different time range (15-60 munites). HPLC (Agilent 1290 infinity LC System) equipped with photo diode array detector (DAD- G4212A), binary pump (G4220A), auto sampler (G4226A) and Agilent column (BEH C18, 150 mm × 2.1 mm, 1.7µm, 80^oC) and a Chem-station software (Agilent Technologies) was used to analyse the samples and determine the concentration of HMF. The mobile phase was made up of water/acetonitrile (70/30%) mixture at a flow rate of 0.250 $mlmin^{-1}$ at pressure of 1200 bar. Twenty microliter (20.00 µL) of both the standard HMF and the sample (hydrolysate) were injected into the column and analysis was run for 15 minutes and the spectra recorded at a wavelength of 284 nm. The HMF spectra of the samples were identified by comparing the spectra of HMF standard based on the retention time.

III. Results and Discussion

Effect of acid type on 5-Hydroxymethylfurfural (HMF) production

Tables 1 illustrate the effects of acid types (H_2SO_4, HCl, HNO_3) at different concentrations and time on HMF production from acid hydrolysis of the starch samples. HMF is an organic compound (with furan basic unit) formed from the degradation of sugars (hexoses) at high hydrolysis conditions of acid concentration, temperature and time [11,22]. The effects of pH and temperature on HMF production have been studied and reported that, increasing temperature and reducing pH increases HMF production [11]. The catalytic activities of three acid types (H_2SO_4, HCl and HNO_3) were investigated to determine the trend of glucose degradation to HMF at different acid concentrations of 0.25-0.75 M and 15-60 minutes hydrolysis time at 120 ^oC. It was observed that the catalytic action of the acids towards HMF formation is in the following decreasing trend HCl > HNO_3 > H_2SO_4. The highest HMF yield of 0.255 mg/l was achieved at 0.75 M and 60 minutes hydrolysis time for HCl hydrolyzed sample. Conversely, the highest HMF yield

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of 0.144 and 0.126mg/l was achieved at 45 minutes hydrolysis time for HNO_3 and H_2SO_4 hydrolyzed samples and yield decreased as time increased to 60 minutes.

The basic strength of the dissociated anions within the solution was attributed to the selectivity of HMF and its yield [23]. HCl dissociates completely in water, providing a high concentration of H^+ ions that efficiently catalyze the dehydration of hexose sugars (glucose, fructose) into HMF. Recent studies have shown that regardless of the acid type or nature (acidic strength) in acid hydrolysis, only hydrogen ion (H^+) activity is mainly responsible for glucose degradation while HMF production is dependent on the selectivity of the dissociated anions [3,23]. The activity of the anions toward HMF production was found to be in the order $Cl^- > SO_4^{2-} > PO_4^{3-}$[23,24]. Cl^- ions are more effective in stabilizing the reaction intermediates than NO_3^- or SO_4^{2-}. Hence, the trend: $Cl^- > NO_3^- > SO_4^{2-}$, which matches the observed acid performance: $HCl > HNO_3 > H_2SO_4$. The trend in degradation activity of the acids obtained in this study is in excellent agreement with the work of Roman-Leskov *et al.* [19] in which at low pH of 1.5, the HMF formation from different acid type followed the trend $H_3PO_4 > HCl > H_2SO_4$. On the contrary, Takeuchi *et al.* [24] obtained a different trend of $H_3PO_4 > H_2SO_4 > HCl$.

In addition, unlike sulfuric acid, which can lead to sulfonation and char formation, HCl tends to cause fewer side reactions or degradation of HMF into levulinic acid and formic acid, especially within optimized time frames

Table1. Effect of acid types on HMF (mg/l) production at different acid concentrations and time

PARAMETERS	Acid conc ($moldm^{-3}$)	Hydrolysis Time (mins)	Acid Types		
			H_2SO_4	**HNO_3**	**HCl**
	0.25	15	0.06	0.109	0.12
		30	0.071	0.116	0.124
		45	0.079	0.116	0.139
		60	0.072	0.113	0.15
	0.50	15	0.081	0.114	0.081
		30	0.084	0.127	0.084
		45	0.111	0.136	0.111
		60	0.107	0.133	0.107
	0.75	15	0.097	0.116	0.143
		30	0.101	0.131	0.21
		45	0.126	0.144	0.218
		60	0.113	0.137	0.255

Effects of acid concentration and time on HMF production

Time and acid concentration are some of the reaction parameters that affect the yield of not only sugars but also sugar degradation products such as HMF and furfural [22,25]. It was observed (Figures 2-4) that an increase in concentration (from 0.25-0.75 M) and time (from 15-60 minutes) favored HMF formation and yield for starch samples hydrolyzed with HCl. However, for samples hydrolyzed with H_2SO_4 and HNO_3, HMF formation increased as time increased from 15 – 45 minutes but yield decreased as time is further increased to 60 minutes. The decrease in HMF yield at longer time may be attributed to the breakdown of HMF to levulinic acid (LA) and formic acid (FA) as presented in Figure 1 [3]. Report has it that the time course of the quantity of FA is similar to that of LA and that they are always formed in a molar ratio of 1:1 [25]. The trajectory of HMF formation with time when hydrolyzed with HCl is in line with the report of Tasic *et al.* [26]. The observation obtained in this study is similar to others in literature as presented in Table 3

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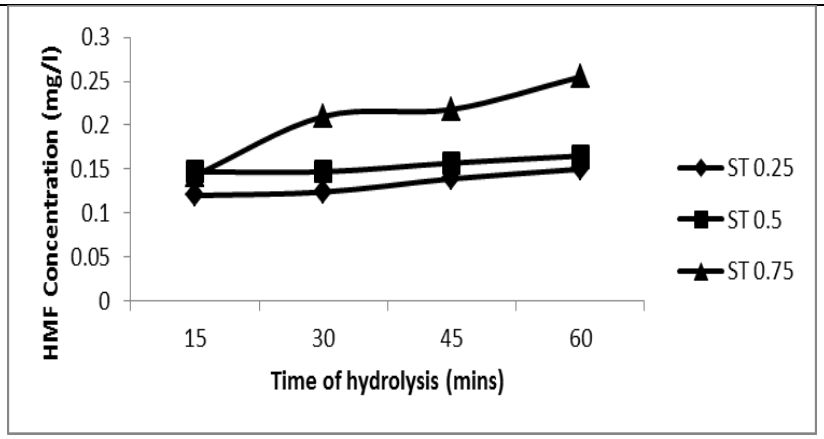

Fig.2. Amount of HMF produced at different HCl concentrations and time

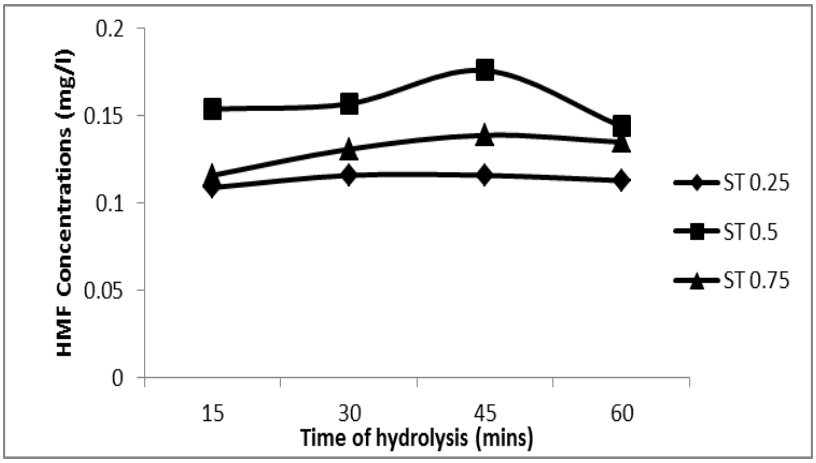

Fig. 3. Amount of HMF production at different HNO_3 acid concentrations and time

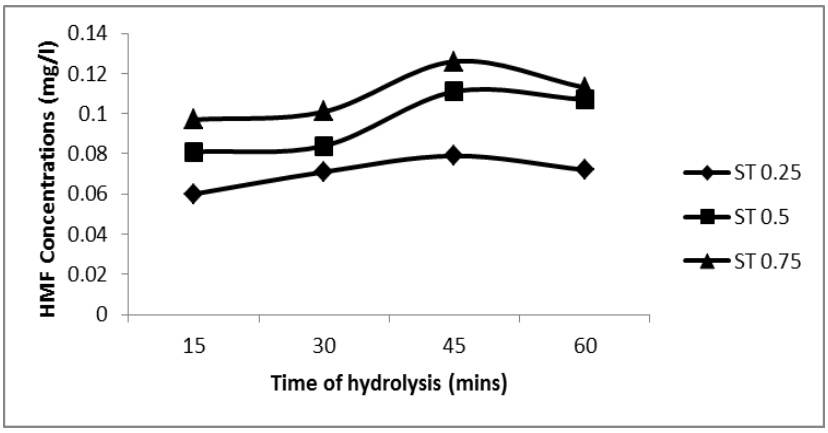

Fig.4. Amount of HMF production at different H_2SO_4 acid concentrations and time

In order to validate the effect of time and acid concentration on HMF production, the starch was hydrolyzed with different concentrations of H_2SO_4 at different time intervals and an experimental procedure was carried out at 284 nm using high performance liquid chromatography (HPLC). The result obtained (Table 2, Figiure 5) is in excellent agreement with those obtained from spectrophotometric analysis (Figure 2) for samples hydrolyzed with sulphuric acid. At 0.25 M acid concentration, HMF yield increased as time increased from 15-60 minutes. However, for 0.5 M concentration, HMF yield decreased after 45 minutes hydrolysis while the reverse was the case for 0.75 M concentration in which yield decreased as time increases. The highest HMF production of 86.57% was achieved at 0.75 M acid concentration at 15 minutes hydrolysis time (Table 2). The observed reduction in HMF yields especially at 0.75 M concentration over time may be attributed to HMF degradation to FA and LA. This observation is in line with the work of Peng *et al.* (2010) who reported the highest HMF yield at 40 minutes hydrolysis time; further increase of time to 200 minutes resulted to decrease in the yield of HMF but rapid increase in the yield of FA and LA. Therefore, in designing

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any industrial production process (either for biofuel or polymer production), the product choice (glucose, HMF or ethanol) will determine the type of acid or concentration and time that would be utilized in order to obtain optimum yield.

Table 2. Results of HPLC analysis of HMF concentrations obtained from H_2SO_4 hydrolyzed starch samples.

Code	Acid conc ($moldm^{-3}$)	Hydrolysis Time (mins)	Retention Time (mins)	Area (mAU*s)	Area (%)
1	0.25	15	12.332	16073	35.98
2		30	12.333	71167	69.42
3		45	12.316	63649	75.30
4		60	12.324	71731	81.51
5	0.50	15	12.327	63189	78.81
6		30	12.343	56950	81.19
7		45	12.326	87511	82.25
8		60	12.351	91655	80.64
9	0.75	15	12.335	74664	86.57
10		30	12.338	83990	80.06
12		45	12.343	90435	79.65
11		60	12.322	55327	47.65

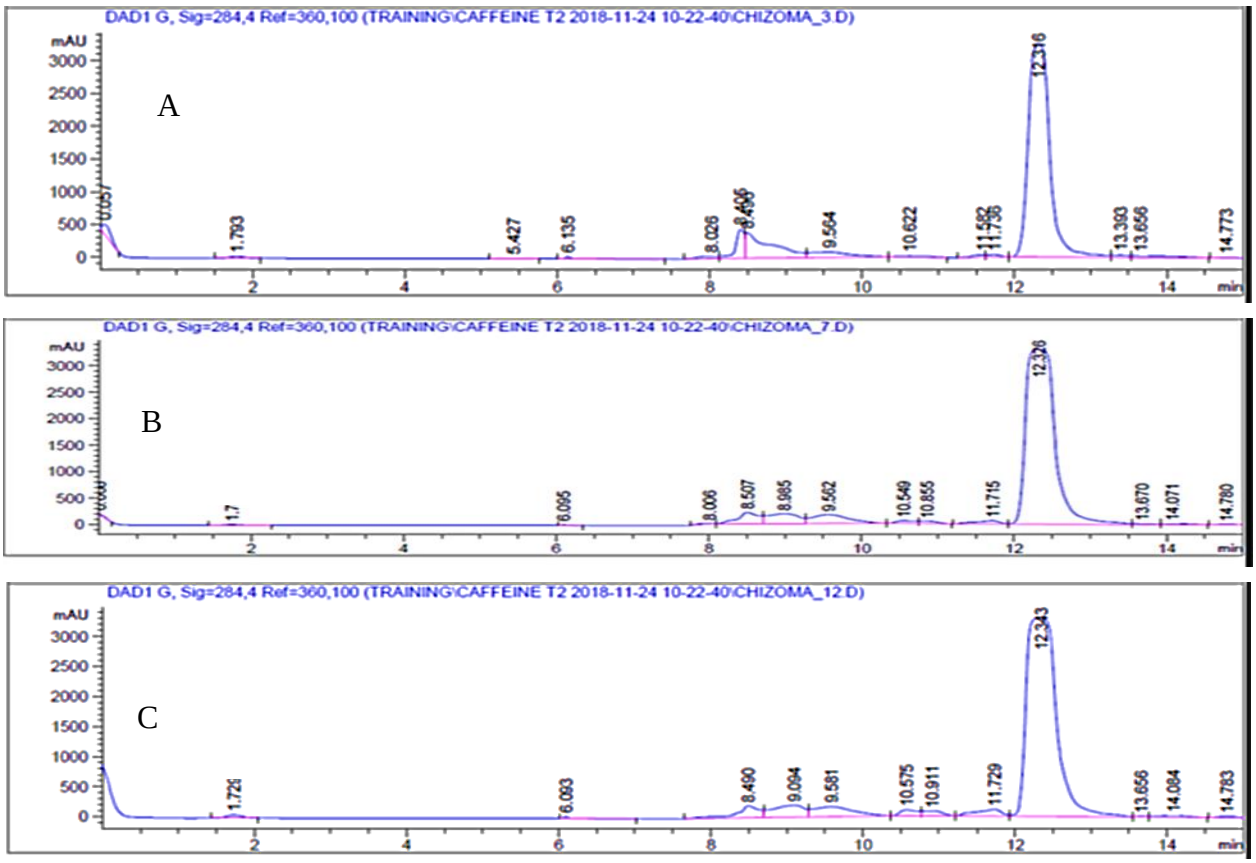

Fig 5. Representative HMF chromatogram of different acid concentrations of (a) 0.25M, (b) 0.5M and (c) 0.75M hydrolyzed at 45 minutes.

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Table 3: Comparison of the Present Study with other works on HMF Production

Study / Author(s)	Feedstock	Acid Type	Conditions	Max HMF Yield	Key Findings / Contribution
Adewumi et al. (Present Study)	Starch	HCl, HNO_3, H_2SO_4	0.25–0.75 M, 15–60 min, 121 °C	**0.255 mg/L (HCl, 60 min)**	HCl gave highest yield; Cl^- ions favored HMF production; HMF decreased with time for HNO_3 & H_2SO_4
Zhang et al. [27]	Glucose	HCl + Lewis acid catalyst	140 °C, 30 min	43.7% conversion	Acid-catalyst combo enhanced HMF yield; showed Cl^- advantage
Chi et al. [28]	Cellulose	H_2SO_4, HCl	1 M, 100–140 °C	~0.21 mg/mL	Time-dependent degradation of HMF to LA/FA at high temp; HCl favored shorter reactions
Devi et al. [29]	Mixed lignocellulose	Dilute H_2SO_4	0.5–1.0 M, 30–120 min	28% HMF yield	Sulfuric acid less efficient; high sugar degradation at long times
Peng et al. [30]	Cellulose	Metal chlorides + HCl	160 °C, 40 min	~54% HMF yield	Reported decline in HMF after peak time due to LA/FA formation

Environmental and Industrial Benefits of HMF Production via Acid Hydrolysis

The findings of this study highlight the significant environmental and industrial benefits of acid-catalyzed HMF production from starch.

Industrial benefits: HMF is a versatile precursor for:

- 2,5-furandicarboxylic acid (FDCA): A monomer for bio-based plastics (e.g., polyethylene furanoate),
- Fuel additives, and
- Pharmaceuticals

Environmental Benefits: HCl as a Green Alternative

Among the acids used, HCl showed the highest HMF yield. HCl, in industrial processes, is often preferred over sulfuric acid due to:

- Easier neutralization (e.g., forms NaCl, which is environmentally benign),
- Lower risk of forming persistent byproducts, and
- Potential for recycling and recovery in closed-loop systems.

This makes HCl a safer and cleaner catalytic agent, contributing to reduced environmental toxicity and less corrosive effluent streams.

VI. Conclusion

This work was undertaken to determine the effect of different acid types: sulphuric, hydrochloric and nitric acids at concentrations of (0.25-0.75 $moldm^{-3}$) and time (15-60 minutes) on HMF production from starch using UV-Visible spectrophotometric and high-performance liquid chromatographic (HPLC) methods. It was observed that the catalytic action of the acids towards HMF formation is in the following decreasing trend HCl > HNO_3 > H_2SO_4. The highest HMF yield of 0.255 mg/l was achieved at 0.75 M and 60 minutes hydrolysis time for HCl hydrolyzed sample. It was observed that an increase in concentration (from 0.25-0.75 M) and time (from 15-60 minutes) favored HMF formation and yield for starch samples hydrolyzed with HCl. However, for samples hydrolyzed with H_2SO_4 and HNO_3, HMF formation increased as time increased from 15 – 45 minutes but yield decreased as time is further increased to 60 minutes. Therefore, the knowledge obtained from this work will help in designing HMF production process for optimum industrial production. This study thus, identified HCl as the most effective catalyst and optimizing hydrolysis conditions, would provide a foundation for green, economical, and scalable production pathways. These benefits collectively advance the adoption of renewable feedstocks and sustainable processing methods in chemical manufacturing.

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Declaration of Competing Interest

The authors declared no conflict of interest.

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