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Kinetic model for the quantitative determination of starch in large number of biological samples

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ABSTRACT

The starch quantification was achieved through the controlled kinetic model of acid hydrolysis of potato starch at pH 3.0. Potato starch (weight average granule diameter, 40.0 µm) was hydrolyzed in a batch reactor using 0.001M HCl (pH 3.0) solution. The conversion of starch into reducing sugar glucose was recorded separately, at different temperatures (343K, 353K and 363K) as a function of time. Maximum (37.92%) conversion of starch to glucose (in 90 minutes) was obtained at 363K. Reaction follows first-order rate equation with respect to starch concentration. Based on the rate constant (k) and half-life ($t_{1/2}$) of acid hydrolysis, unknown samples were quantified for their starch content as per the derived kinetic model. The method was found suitable for quantification of large number of biological samples of similar type in lesser time.

Key words: Starch quantification; Controlled kinetic model; Biological samples; Half-life.

INTRODUCTION

Starch is a complex polysaccharide consisting of a large number of glucose units joined by glycoside bonds [1, 2]. It is the main storage food of the plants, and the key ingredient of most cereal seeds (maize, wheat and rice), tubers (potato) and roots (tapioca). Starch hydrolysis [3] is expressed as

$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} + n\,H_2O \longrightarrow \underset{\text{D-glucose}}{n\,C_6H_{12}O_6}\ .$$

The hydrolysis of starch in our body is controlled through complex catalytic mechanism. However, the acid hydrolysis is the well known method of converting starch into glucose units in the laboratory [4]. In 1842, Starch hydrolysis was first practiced in USA on commercial scale [5]. Kinetics of hydrolysis of sorghum molasses was derived by Khan et al., 1980 from experimentally determined hydrolysis rate constants as function of the acid concentration and temperature [6]. Chemically modified starches through hydrolysis process have also been reported [7]. The reaction conditions were optimized by Yankov et al., 1986 for the starch hydrolysis through thermo stable α- amylase [8]. Now, microbial amylases are widely used in starch hydrolysis in industries [9]. Physico-chemical and functional properties of *Canavalia ensiformis* starch hydrolyzed with HCl were studied by Betancur and Chel in 1997 [10]. Kinetics of acid hydrolysis of cellulose was first described by Xiang et al., in 2003 as two pseudo-homogeneous consecutive First-order reactions [11]. Later on, starch was hydrolyzed to produce mono- and oligosaccharides under hydrothermal conditions with and without carbon dioxide (CO_2) in a small batch reactor [12].

The acid hydrolysis of starch is a set of different parallel first order reactions which are dependent upon pH and temperature [13]. The reaction rate of starch hydrolysis is also varied with particle size of starch granules. Small particle size of starch granules enhances the rate of reaction due to increase in surface area. This study is aimed to investigate the kinetic behaviour of acid hydrolysis of potato starch in controlled optimized conditions, and to

quantify the starch content in large number of unknown potato starch samples using rate constant k on given temperature.

MATERIALS AND METHODS

All the chemicals used, were of analytical grade. Analysis of commercially available potato starch (weight average granule diameter, 40.0µm) was carried out at pH 3.0. Fehling solution was prepared by making two solution, first one was prepared by dissolving 34.64×10^{-3} Kg of crystallized $CuSO_4.5H_2O$ and 2-3 ml concentrated H_2SO_4 in water (1: l) and other by dissolving 175×10^{-3} Kg of Potassium sodium tartarate (Rochelle salt) and 50×10^{-3} Kg of NaOH (97% pure) in water (1: l). An equal volume of each solution was mixed to obtain the Fehling solution for the titrimetric estimation of glucose.

Experimental Method

Acid hydrolysis of starch was performed in a conical shaped glass reactor with thermometer well and sample trapping pocket. A water condenser (standard B 24 joint) was fitted on the mouth of reactor to avoid loss of HCl and water vapour. This reactor was immersed in a thermostatic water bath. This whole reactor system was housed on a magnetic stirrer operated at a fixed rpm throughout the reaction for all sets of experiments.

Potato starch (starch content 2.0×10^{-3} Kg) was charged into clean reactor. Distilled water (400 ml), heated to a fixed reaction temperature, and was poured into it with simultaneously addition of dilute HCl. A mercury glass thermometer (scale 10-150°C) was inserted into contents of reactor. The reaction mixture was given proper stirring to maintain the reaction medium in suspended state. Contents of reactor attained desired temperature within 10 minutes. Reactions were conducted for initial concentration of starch 5.0Kg/m^3 at pH 3.0 and temperature 343-363K. After desired hydrolysis, insoluble protein, fat and some inorganic components present initially in starch were separated by filtration. Reaction mixture (10 ml) was drawn out to analyze the hydrolyzed starch in terms of glucose by titrimetric method [13].

RESULTS AND DISCUSSION

The acid hydrolysis of starch is dependent on various factors, including the type of substrate, extractability of starch, the pH of the solution, reaction temperature and particle size of starch granules. This process has complex reaction mechanism. If the reaction is carried out through controlled reaction parameters, it follows first order reaction kinetics. The Integrated form of first order kinetic equation is given below

$k= 1/t \ln (a/a-x)$

Where, a = initial concentration of starch in Kg/m^3; (a-x) = concentration of starch in Kg/m^3 at time t; and k = rate constant.

This equation is well adapted for acid hydrolysis of starch, if particle sizes, pH of solution and reaction temperature are fixed throughout the reaction.

Initial concentration of starch present in reaction mixture is 5.0 Kg/m^3. First series of experiments of acid hydrolysis of starch was conducted at 353K and pH 3.0. The study of kinetic parameters indicates that acid hydrolysis of starch follows First-order reaction kinetics (Table 1).

Table 1. Kinetic parameters of acid hydrolysis of starch at 353K

S. No.	Time (t) in minute	Initial concentration of starch Kg/m^3 (a)	Concentration of starch at time t Kg/m^3 (a-x)	a/a-x	ln (a/a-x)
1	0	5.0	5	1	0
2	10	5.0	4.859	1.029018	0.0286
3	20	5.0	4.726	1.057977	0.0564
4	30	5.0	4.591	1.089087	0.0853
5	40	5.0	4.46	1.121076	0.1143
6	50	5.0	4.339	1.152339	0.1418
7	60	5.0	4.21	1.187648	0.172
8	70	5.0	4.108	1.217137	0.1965
9	80	5.0	3.971	1.259129	0.2304
10	90	5.0	3.889	1.285678	0.2513

Rate constant obtained from this data is 2.8×10^{-3} min^{-1} at 353K. Other experiments were done using 5.0 Kg/m^3 starch

and dilute HCl maintaining pH 3.0 at 343K and 363K to see the effect of temperature on the reaction kinetics of starch hydrolysis (Table 2).

Table 2. Time dependent acid hydrolysis of starch at different temperatures and pH 3.0

S. No	Time (t) (min)	Experiment 1 (T=343K) Starch concentration (Kg/m^3)			Experiment 2 (T=353K) Starch concentration (Kg/m^3)			Experiment 3 (T=363K) Starch concentration (Kg/m^3)		
		Unreacted	Hydrolyzed	Conversion%	Unreacted	Hydrolyzed	Conversion%	Unreacted	Hydrolyzed	Conversion%
1	0	5.000	0.000	0.00	5.000	0.000	0.00	5.000	0.000	0.00
2	10	4.898	0.102	2.04	4.859	0.141	2.82	4.764	0.236	4.72
3	20	4.805	0.195	3.90	4.726	0.274	5.48	4.544	0.456	9.12
4	30	4.712	0.288	5.76	4.591	0.409	8.18	4.326	0.674	13.48
5	40	4.624	0.376	7.52	4.46	0.54	10.80	4.098	0.902	18.04
6	50	4.555	0.445	8.90	4.339	0.661	13.22	3.905	1.095	21.90
7	60	4.452	0.548	10.96	4.21	0.79	15.80	3.692	1.308	26.16
8	70	4.362	0.638	12.76	4.108	0.892	17.84	3.496	1.504	30.08
9	80	4.258	0.742	14.84	3.971	1.029	20.58	3.277	1.723	34.46
10	90	4.184	0.816	16.32	3.889	1.111	22.22	3.104	1.896	37.92

Rate constants were obtained as 2.0×10^{-3}, 2.8×10^{-3} and 5.2×10^{-3} min^{-1}, respectively at 343K, 353K and 363K (Fig. 1).

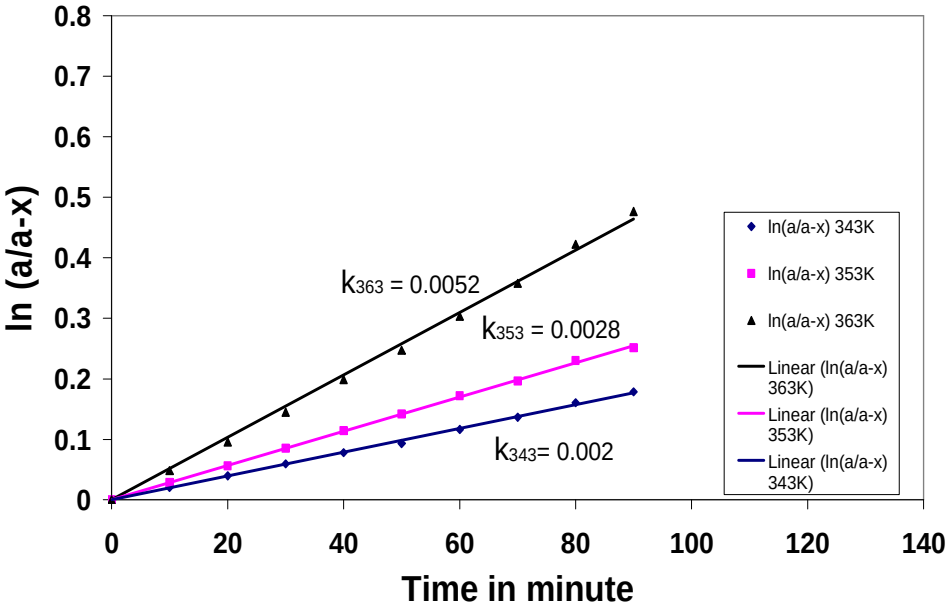

Fig. 1. The effect of temperature on the reaction rate of acid hydrolysis of starch (Initial concentration a = 5.0 Kg/m^3) at pH 3.0. Values of rate constant k are given in min^{-1}

Table 3. Time dependent acid hydrolysis of starch at different concentrations
(Temperature 353K and pH 3.0)

S. No.	Time (t) (min)	Experiment 4 Starch concentration (Kg/m^3)			Experiment 5 Starch concentration (Kg/m^3)		
		Unreacted	Hydrolyzed	Conversion%	Unreacted	Hydrolyzed	Conversion%
1	0	2	0	0	8	0	0
2	10	1.945	0.055	2.75	7.762	0.238	2.97
3	20	1.894	0.106	5.3	7.548	0.452	5.65
4	30	1.84	0.16	8	7.334	0.666	8.32
5	40	1.787	0.213	10.65	7.12	0.88	11
6	50	1.739	0.261	13.05	6.925	1.075	13.43
7	60	1.689	0.311	15.55	6.712	1.288	16.1
8	70	1.647	0.353	17.65	6.549	1.451	18.14
9	80	1.591	0.409	20.45	6.339	1.661	20.76
10	90	1.569	0.431	21.55	6.198	1.802	22.52

The accuracy of the model was verified by experimental data, which are not used for developing kinetic model. For this

reason, set of experimental data were generated by taking concentration of starch (a = 2.0 and 8.0 Kg/m^3) at 353K and pH 3.0 (Table 3).

Thus, experimental best-fit value of rate constant, k is 2.8×10^{-3} min^{-1}. This will also confirm that kinetics of hydrolysis reaction is independent of initial concentration of starch at a particular temperature (Fig. 2).

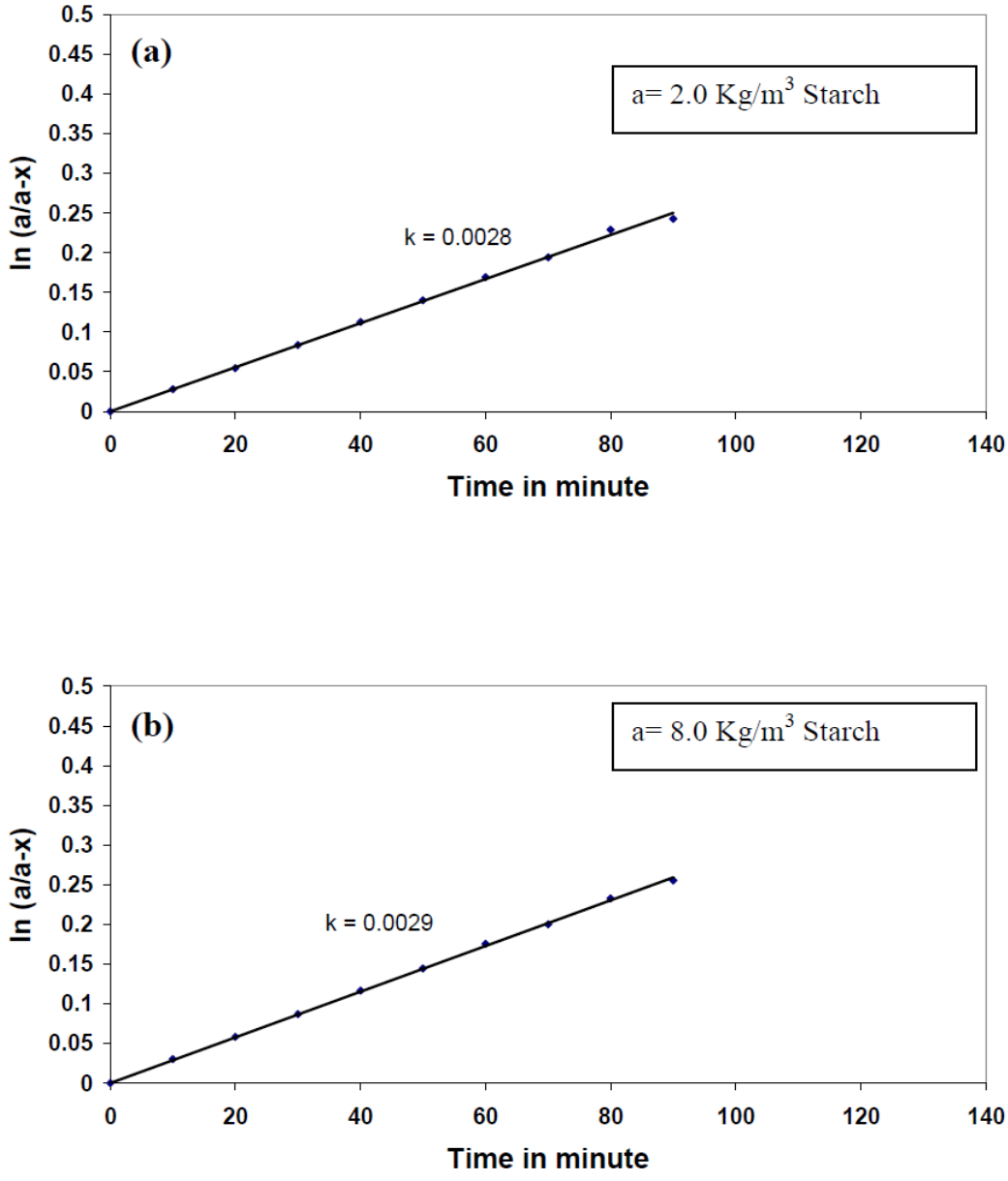

Fig. 2. Acid hydrolysis of starch at different concentrations at 353K and pH 3.0. (a) Initial starch concentration 2.0 Kg/m^3 and (b) Initial starch concentration 8.0 Kg/m^3. Values of rate constant k are given in min^{-1}

At pH 3.0, maximum conversion of starch to reducing sugar was 22.22% (at 353K) and 37.92% (at 363K) after 90 minutes. The quantification of starch was carried out by simply calculating the half-life value of reaction by following expression

Half life ($t_{1/2}$) in minutes = 0.693/k

By calculating the half-life (Table 4) of acid hydrolysis of starch at given temperature and pH, one can easily assume that the 50% starch has been hydrolyzed. The quantification of resulting glucose at this point gives us the half value of starch content. The double of this quantity can be taken as total starch in the unknown potato starch samples.

Table 4: Half-life values of acid hydrolysis of starch at different temperatures

S. No.	Temperature (K)	Rate constant k (min^{-1})	Half life ($t_{1/2}$) in (min)
1	343	$2.0X10^{-3}$	346.5
2	353	$2.8X10^{-3}$	247.5
3	363	$5.2X10^{-3}$	133.2

To validate the model three unknown samples of potato starch (PS-1, PS-2 and PS-3) were treated with 0.001M HCl (pH 3.0) for 247 min 30 s at 353K, and 133 min 12 s at 363K. The starch content at this point represents the half concentration of total starch content. The total starch content was quantified with less than 9.33% relative error through this experiment. The relative error was decreased in the samples having higher quantity of starch (Table 5). The conversion of starch into glucose is not completely achievable and therefore the controlled kinetic model is suitable for starch quantification.

Table 5. Quantification of starch in unknown samples of potato starch using controlled kinetic parameters
(pH 3.0 and particle size 40.0µm)

Sample	Concentration of potato starch Kg/m^3					
	Temperature (353K)			Temperature (363K)		
	Reaction time 247min 30 s			Reaction time 133 min 12 s		
	Starch taken	Calculated	Relative error	Starch taken	Calculated	Relative error
PS-1	2.5	2.65	-6.00	2.5	2.37	5.20
PS-2	1.5	1.64	-9.33	1.5	1.38	8.00
PS-3	6.0	6.15	-2.50	6.0	5.88	2.00

CONCLUSION

The aim of this study was to investigate the kinetics of acid hydrolysis of starch in controlled reaction conditions. The study was carried out for the potato starch (weight average granule diameter, 40.0µm) at pH 3.0. The behaviour of the reaction was observed at 343K, 353K and 363K, and rate constant k was found $2.0X10^{-3}$, $2.8X10^{-3}$ and $5.2X10^{-3}$ min^{-1} at these temperatures. The model was verified using different initial concentrations of starch, for which the rate constant was also found ~$2.8X10^{-3}$ min^{-1} at 353K. Hence, the reaction was thought to be independent from initial concentration of starch. Further, the half-life values for acid hydrolysis were calculated at different temperatures, and the substantial decrease in the half-life of reactions was observed with increase in temperature. The quantification of the glucose at half-life time of the reaction gives 50% of starch content in the given unknown samples, which can simply be doubled for total starch content in the samples. The model is well adequate for the particular type of starch with unique properties, and is operated in controlled kinetic parameters. The large number of unknown biological samples, having similar type of starch content can be quantified in lesser time than the conventional methods.

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REFERENCES

[1] Y. Nakamura, *Plant Sci. Lett.*, **1983**, 30(3), 259-265.
[2] V. Verma, M. S. Avasthi, A. R. Gupta, M. Singh, A. Kushwaha, *Euro. J. Exp. Bio.*, **2011**, 1(3), 107-113.
[3] I. L. Finar, Organic Chemistry, in Carbohydrates, 6th ed., vol 1. Harlow: Addison-Wesley Longman Inc., **1998**, pp. 505-506.
[4] S. You, M. S. Izydorczyk, *Carbohydr. Polym.*, **2007**, 69(3), 489-502.
[5] K. Othmer, Encyclopedia of Chemical Technology, 3rd ed., vol 22, edited by Herman F. et al. USA: Academc press., **1983**, pp. 499-502.
[6] A. R. Khan, R. J. Robinson, Johnson, *J. Food Sci.*, **1980**, 45, 1449-1449.
[7] K. N. Awokoya, L. M. Nwokocha, B. A. Moronkola, D. O. Moronkola, *Der Chemica Sinica*, **2011**, 2(6), 228-244.
[8] D. Yankov, E. Dobreva, V. Beschkov, E. Emanuilova, *Enzyme Microb. Tech.*, **1986**, 8(11), 665-667.
[9] B. Devi, B. G. Unni, S. B. Wann, R. Samanta, *Adv. Appl. Sci. Res.*, **2012**, 3(5), 2739-2744.
[10] A. D. Betancur, G. L. Chel, *J. Agric. Food Chem.*, **1997**, 45, 4237-4241.
[11] Q. Xiang, Y. Y. Lee, P. Pettersson, R. W. Torget, *Appl. Biochem. Biotechnol.*, **2003**, 105, 505-514.

[12] T. Miyazawa, T. Funazukuri, *Biotechnol. Progr.*, **2005**, 21, 1782-1785.
[13] B. Bej, R. K. Basu, S. N. Ash, *J. Sci. Ind. Res.*, **2008**, 67, 295-298.