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Research Article

Kinetic Models for Glucosamine Production by Acid Hydrolysis of Chitin in Five Mushrooms

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In this paper, glucosamine was produced by acid hydrolysis of five mushrooms. The glucosamine yields were investigated, and the optimum conditions were obtained as follows: acid type, sulfuric acid; acid concentration, 6 M; ratio of raw material to acid volume, 1 : 10; hydrolysis temperature, 100°C; and time, 6 h. Under these conditions, the glucosamine conversion from chitin content reached up to 92%. The results of hydrolysis kinetics indicated that hydrolysis of five mushrooms to glucosamine followed zero-order kinetics. Moreover, the relatively low activation energy for hydrolysis of straw mushroom (18.31 kJ/mol) and the highest glucosamine yield (56.8132 ± 3.5748 mg/g DM, 0.9824 g/g chitin) indicated that hydrolysis of straw mushroom was energy-saving. Thus, sulfuric acid hydrolysis of straw mushroom for glucosamine production should be considered as an efficient process for the future industrial application. However, further study is needed for glucosamine purification.

1. Introduction

Chitin, the well-known natural biopolymer, has been widely applied in food, pharmaceutical, and biotechnological industries owing to its nontoxicity, biodegradability, and biocompatibility [1]. Nowadays, most of the commercial sources of chitin are crab and shrimp shells. However, in nature, chitin occurs not only in the exoskeleton of arthropods but also in the cell walls of fungi and yeast [1–3]. Therefore, fungi can be considered as the potential candidate for the production of chitin. Mushrooms, one of the most known fungi, have been consumed throughout the world for many centuries [4]. Besides their nutritional value, they exhibit therapeutic and medical properties related to preventing various diseases such as cardiovascular diseases, diabetes, and several types of cancer [5–7]. In addition, the chitin content of mushrooms has been reported in the range of 0.3–19.6% DW (dry weight) [3, 8].

Chitin has several applications when converted into its oligomers and monomers, such as glucosamine (2-amino-2-deoxy-D-glucose). In recent years, glucosamine with well-studied efficacy and satisfied safety has been widely used as a dietary supplement for osteoarthritis treatment [9]. It is a well-known fact that the hydrolysis of chitin with concentrated acids yields the formation of glucosamine [1]. For industrial production of glucosamine, concentrated hydrochloric acid is usually employed to hydrolyze chitin [10]. However, this method has low yield (below 65%) [11]. Another concern is the high content of sodium chloride in the finally product may increase the cost of production and the risk of some diseases, such as stroke, heart failure, osteoporosis, stomach cancer, and kidney disease [12]. Alternatively, previous studies have reported that sulfuric acid could be employed for hydrolysis of chitin and other biopolymers [1, 13–15].

According to the literature review, production of glucosamine by acid-catalyzed hydrolysis of chitin in

mushrooms is feasible. However, no study has been conducted to compare the efficiency of different acids for glucosamine production, and little is known about the optimum conditions and kinetics for acid hydrolysis of mushrooms. Therefore, the objectives of this study were to compare the acid-hydrolysis efficiencies of hydrochloric and sulfuric acids, to optimize the hydrolysis conditions, and to investigate the kinetic models.

2. Materials and Methods

2.1. Materials and Chemicals. Five species of edible mushrooms that are widely used for human consumption were selected in this study. These include enoki mushroom (*Flammulina velutipes*) (fresh), oyster mushroom (*Pleurotus ostreatus*) (fresh), straw mushroom (*Volvariella volvacea*) (fresh), shiitake (*Lentinus edodes*) (dried), and wood ear mushroom (*Auricularia auricular*) (dried). The mushrooms were purchased from a local market in Nakhon Ratchasima province, Thailand. The three fresh mushrooms, namely, enoki mushroom, oyster mushroom, straw mushroom, were washed, cut into small pieces and dried in a Gamma 2–16 LSC freeze-drier (Martin Christ GmbH, Osterode am Harz, Germany). In the freeze-drying process, mushroom pieces were frozen at −85°C for 1 h in the freeze-drier chamber, dried at −65°C for 72 h, and then finally dried at 30°C for 24 h. The entire process was carried out at −85°C cold collector temperature and 0.001 mbar chamber pressure. After freeze-drying, five dried mushrooms were ground using an IKA-WERKE M20 universal laboratory miller with a cooling jacket and 4-edged blade and then sieved to obtain a fraction below 40-mesh. The ground mushroom samples were well-sealed in the plastic bags and kept at 4°C throughout the study.

N-(9H-Fluoren-2-ylmethoxycarbonyloxy) succinimide (FMOC-Su) (98% purity) and D-(+)-glucosamine hydrochloride (minimum 99% purity) were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Triethylamine, trifluoroacetic acid, and acetonitrile were of HPLC grade while all other chemicals used were of analytical grade.

2.2. Mushroom Hydrolysis. Acid hydrolysis of mushrooms was performed with a Gerhardt Kjeldatherm digestion unit equipped with a digital temperature controller (Gerhardt GmbH & Co. KG, Königswinter, Germany) according to the method described in our previous study [16]. Firstly, the efficiency of hydrochloric acid and sulfuric acid for glucosamine production was investigated. Based on the previous reported conditions of hydrochloric acid hydrolysis, the parameters selected for the comparison were as follows: ratio of raw material to acid volume (1 : 10, w/v), acid concentration (6 M), hydrolysis temperature (80°C), and hydrolysis time (8 h) [17]. Mean values of glucosamine yield from all acid hydrolysis conditions were compared. Further study was conducted to determine the optimum condition for sulfuric acid hydrolysis conditions of the mushrooms. One Variable at a Time (OVAT) was used to select the optimum conditions for sulfuric acid hydrolysis of mushrooms. Several experimental parameters such as acid concentration (1, 2, 4, 6, and 9 M), ratio of raw material, and acid volume (w/v, 1 : 5, 1 : 10; 1 : 15, 1 : 20, 1 : 25, 1 : 30 and 1 : 35), hydrolysis temperature (40, 60, 80, 90, 100, and 110°C), and hydrolysis time (0.5, 1, 2, 3, 4, 6, 10, 12, 15, 18, 24, 36, and 48 h) were examined. Aliquots were periodically taken from the reaction mixture, cooled at 4°C, and neutralized with calcium carbonate. The neutralized hydrolysate was used for glucosamine quantitative determination.

2.3. Analytical Methods. The crude chitin content of mushroom samples was measured based on the residual mass after extraction. The extraction procedure was adapted from the study of Ifuku et al. [2]. Ground samples were deproteinized by 2% NaOH for 24 h at 90°C and demineralized by 2 M HCl for 48 h at room temperature. After washing with deionized water for 5 times, the residue was dried at 70°C in a vacuum oven for 24 h. The measurements were repeated 3 times. The average values of chitin content were reported and used for the calculation of glucosamine yield and conversion.

Glucosamine was determined by high-performance liquid chromatography (HPLC) according to the AOAC official method 2005.01 with minor modification [16, 18, 19]. Briefly, 0.75 μL trimethylamine was added to 1 mL sample solution and the mixture was allowed to react for at least 12 h at room temperature. Subsequently, a 100 μL portion was mixed with 0.5 mL 15 mM FMOC-Su and reacted in the sonicator water bath at 50°C for 30 min. After that, 4 mL mixture of mobile phases A (water containing 0.05% trifluoroacetic acid, pH 2.4)/B (acetonitrile) (1/1, v/v) was added. Chromatographic separation was conducted on a ZORBAZ ODS C18 column (5 μm particle size, 250 × 4.6 mm I. D.), employing a C18 precolumn guard cartridge. The column temperature was maintained at 30°C. Detection was performed at a wavelength (λ) of 265 nm with an analytical time of 17 min. Samples were eluted with the gradient mode at a flow rate of 0.8 mL/min. The mobile phase consisted of A (0.05% trifluoroacetic acid in DI water, pH 2.4)/B (acetonitrile), which was programmed with a flow rate of 0.8 mL/min as follows: 0–6 min, 30% B; 6–11 min, 30–100% B; 11–15 min, 100–30% B; 15–17 min, 30% B. A calibration curve was prepared from glucosamine chloride standards (20–120 μg/mL).

The glucosamine yield is defined as

$$\text{glucosamine yield (mg/g DM)} = \frac{M_g}{M_S}, \quad (1)$$

where M_g represents the mass of glucosamine in the mushroom hydrolysate and M_S represents the mass of mushroom sample.

The glucosamine conversion is defined as

$$\text{glucosamine conversion (\%)} = \frac{M_g \times 0.973}{M_c} \times 100\%, \quad (2)$$

where M_g represents the mass of glucosamine in the mushroom hydrolysate, M_c represents the mass of chitin in

the mushroom sample, and 0.973 is the conversion factor from glucosamine to chitin.

2.4. Kinetic Models. For kinetics study, 10 g ground mushroom sample was hydrolyzed following the addition of 100 mL 6 M sulfuric acid and heat (90, 100, or 110°C). During the 6 h hydrolysis, the subsamples were taken at 30 min intervals. Due to the difficulty in finding a strict mechanism for hydrolysis reactions, it is practical to use simplified models for the determination of kinetics [13]. EXCEL was employed for fitting the mathematical reaction models (zero-, first-, and second-order kinetics) to the experimental data. For a simple zero-order reaction, a plot of $[A]$ versus time would be linear, for a simple first-order reaction, a plot of $\ln[A]$ versus time would be linear, and for a second-order reaction, a plot of $1/[A]$ would be linear [20–22].

Arrhenius' equation gives the dependence of the rate constant of a chemical reaction on the absolute temperature, a pre-exponential factor, and other constants of the reaction:

$$k = A_e^{-E_a/(RT)}, \quad (3)$$

where k is the rate constant; T is the absolute temperature (in Kelvin); A is the pre-exponential factor, a constant for each chemical reaction that defines the rate due to frequency of collisions in the correct orientation; E_a is the activation energy for the reaction (in J/mol or kJ/mol); and R is the universal gas constant, 8.3144598 J/mol/K.

3. Results and Discussion

3.1. Effects of Acid Hydrolysis Parameters on Glucosamine Yield. In order to investigate the hydrolysis efficiency of hydrochloric and sulfuric acids for glucosamine production, the glucosamine yields of five mushrooms by hydrochloric acid and sulfuric acid hydrolysis were compared. The glucosamine yields obtained from sulfuric acid hydrolysis were numerically higher than the values from hydrochloric acid (2.09–4.05 times) (Figure 1). To test the hypothesis that glucosamine yield from sulfuric acid hydrolysis ($n = 6$, $M = 27.9988$, SD = 24.5809) were statistically higher than that from hydrochloric acid hydrolysis ($n = 6$, $M = 6.7367$, SD = 2.8095), an independent t test was conducted. The assumption of homogeneity of variances was tested and satisfied via Levene's F test, $F(10) = 4.335$, $p = 0.064$. The glucosamine yield from sulfuric acid hydrolysis was statistically significant higher than that from hydrochloric acid hydrolysis, $t(10) = 2.105$, $p = 0.031$ (one-tailed). This indicates that sulfuric acid has stronger ability than hydrochloric acid to convert chitin into glucosamine from five mushrooms. These findings are similar to the previous studies that sulfuric acid is more efficient for polymer degradation [14, 23, 24]. In the study of Lavarack et al. [23], sulfuric acid was found to be more active for the formation of xylose from sugarcane bagasse hemicellulose compared to hydrochloric acid. Similarly, Meinita et al. [14] found that sulfuric acid was more active at hydrolyzing *Kappaphycus alvarezii*. Conversely, Herrera et al. [25] reported that hydrochloric acid was more powerful as the catalyst in the hydrolysis of sorghum straw than sulfuric acid. Therefore, the raw material type played an important role in the efficiency of acid hydrolysis. The better catalytic effect of sulfuric acid than hydrochloric acid for the production of glucosamine from the same mushroom sample could be explained by that the molar amount of hydrogen ions in sulfuric acid is twice that of hydrochloric acid. Ravens [26] explained that the hydrolysis of poly (ethylene terephthalate) (PET) is principally determined by the solubility of PET in hydrochloric acid. Similarly, in this study, the quicker dissolution rate and higher solubility of mushroom samples in sulfuric acid may lead to higher glucosamine yield from sulfuric acid hydrolysis.

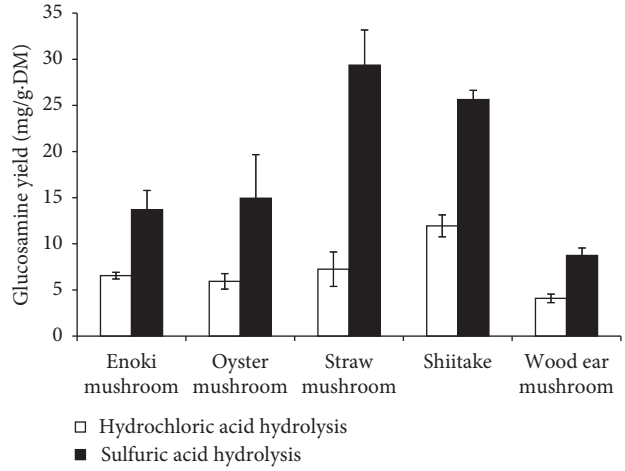

Figure 1: Effects of hydrochloric and sulfuric acids on glucosamine yield. Hydrolysis conditions: ratio of raw material to acid volume (1 : 10, w/v), 6 M acid, 80°C, and 8 h.

To obtain the maximum glucosamine yield, it was of interest to investigate the effects of different hydrolysis conditions on glucosamine yields. The concentration of sulfuric acid plays important role in the hydrolysis efficiency, and too high or too low acid concentration can decrease the glucosamine yield. As shown in Figure 2(a), the glucosamine yield increased when the sulfuric acid concentration was increased from 1 to 6 M. As discussed before, hydrogen ions played an important role during hydrolysis. The molar amount of hydrogen ions increased with the increasing acid concentration; therefore, the glucosamine yield increased with the increasing acid concentration. A further increase in sulfuric acid concentration (6 to 9 M) did not enhance the yield of glucosamine indicating that the molar amount of hydrogen ions reached saturation in the hydrolysis system. On the other hand, there is no significant difference between different ratios of raw material to acid volume (Figure 2(b)), which means the selected minimum acid volume (5 mL) already provides enough solvent volume and catalytic hydrogen ions for the reaction. Hydrolysis temperature had an important impact on glucosamine yield because that temperature affected the catalytic ability of sulfuric acid. The glucosamine yield was increased when the hydrolysis temperature increased. When the temperature was increased from 90°C to 110°C, there was no significant improvement of

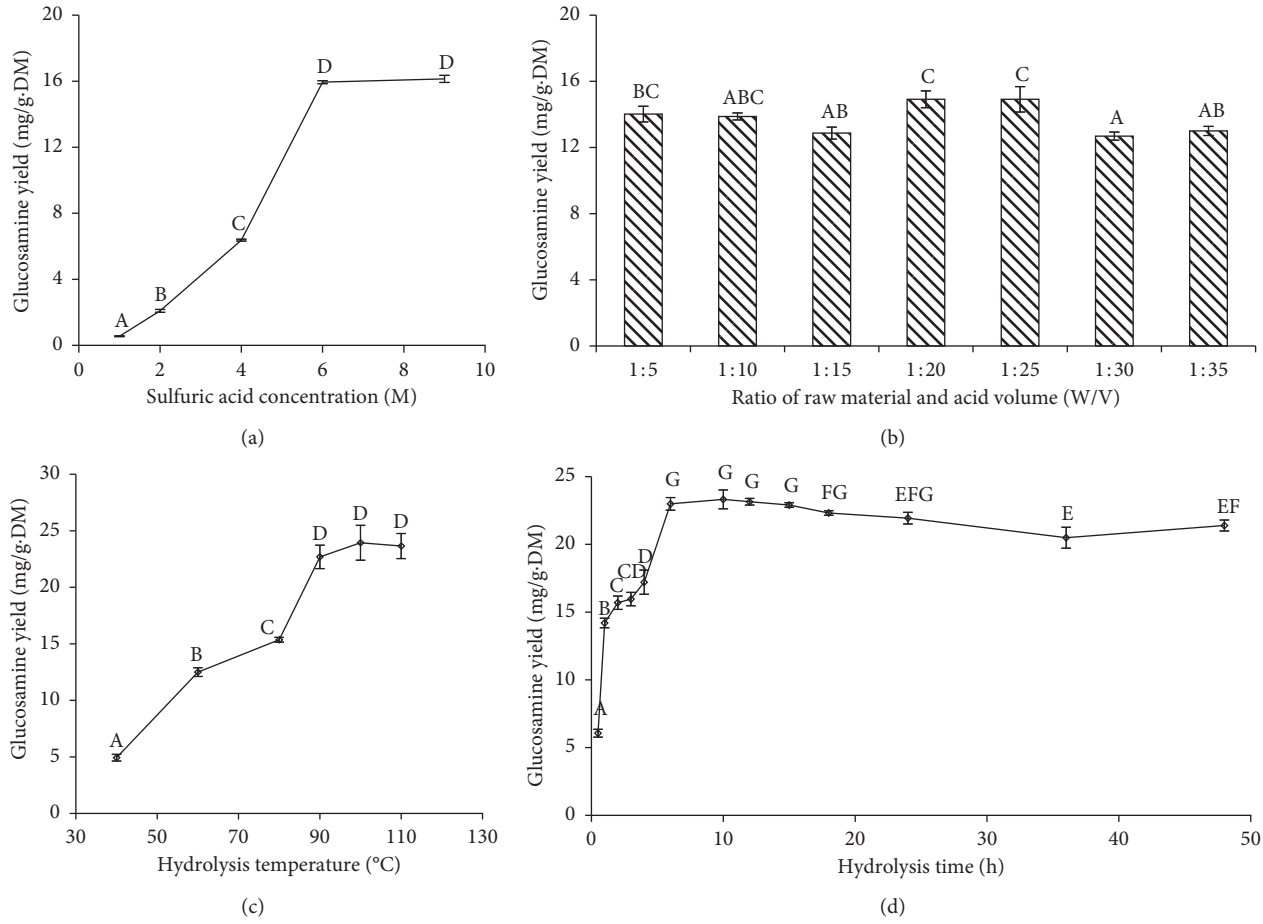

Figure 2: Effects of sulfuric acid concentration, ratio of raw material to acid volume, hydrolysis temperature, and time on glucosamine yield. (a) Sulfuric acid concentration; (b) ratio of raw material to acid volume; (c) hydrolysis temperature; (d) hydrolysis time. Shared letters indicate means that are not significantly different ($p < 0.05$) by one-way ANOVA with post hoc Tukey's HSD.

glucosamine yield (Figure 2(c)). The enhancement of glucosamine yield at higher temperatures could be attributed to the increase of catalytic efficiency with the increase of temperature. On the other hand, the elevated temperatures softened the cell wall matrices of chitin, proteins, and glucans and allowed the acid to hydrolyze chitin to form glucosamine [2, 23]. To investigate the effect of hydrolysis time on glucosamine yield, the glucosamine yield was monitored for 48 h. Because there were abundant reaction substrates in the solution, the reaction rate was faster; as the reaction progressed, the chitin amount became less and less and the reaction rate was lower. Therefore, there was an obvious increase within 1 h and a relatively slow increase from 1 to 6 h. It was identified that the acid hydrolysis was finished in 6 h, after which no significant change of the glucosamine yield was found (Figure 2(d)). Therefore, the optimum hydrolysis time was judged at 6 h.

Under the optimum conditions, namely, dry mushroom sample, ratio of raw material to acid volume, 1 : 10; sulfuric acid concentration, 6 M; hydrolysis temperature, 100°C; and time, 6 h, the hydrolysis of five mushrooms were conducted and the conversions and glucosamine yields were calculated (Table 1).

Values in 3th column followed by different superscript letters indicate significant differences ($p < 0.05$) between samples when analyzed by one-way ANOVA and post hoc Tukey's HSD test.

Among the five mushrooms used in this study, the results from one-way ANOVA with post hoc Tukey's HSD test revealed that straw mushroom had the highest glucosamine yield (56.8132 ± 3.1671 mg/g DM). It indicated that straw mushroom had the highest chitin content among these five mushrooms (Table 1), and it could be considered as a better source of chitin for the production of glucosamine. Moreover, the relatively high conversion in five tested mushrooms (greater than 95%) implied sulfuric acid hydrolysis was an effective method for glucosamine production (Figure 1 and Table 1). The choice of suitable acid and control of hydrolysis conditions are crucial for the production of glucosamine. Our results indicated that the chitin content of mushroom was a crucial factor for glucosamine yield. As compared to crustacean shells, the traditional and current commercial source of glucosamine, mushrooms exhibited lower glucosamine yields which are attributed to the difference in chitin contents of raw materials. However, the supply of crustacean shells can be limited and potentially toxic due to heavy metal and pesticide

Table 1: The chitin content, glucosamine yield, and conversion of five mushrooms under optimum conditions*.

Mushroom	Chitin content (mg/g DM)	Glucosamine yield (mg/g DM)	Glucosamine yield (g/g chitin)	Conversion (%)
Enoki mushroom	26.43 ± 1.2061	25.2615 ± 1.2479[b]	0.9558	93.00
Oyster mushroom	27.03 ± 0.0070	26.9934 ± 0.4778[b]	0.9986	97.17
Straw mushroom	57.83 ± 2.3227	56.8132 ± 3.5748[d]	0.9824	95.59
Shiitake	38.52 ± 1.7264	37.4460 ± 1.1173[c]	0.9721	94.59
Wood ear mushroom	12.96 ± 1.0074	12.3593 ± 1.1899[a]	0.9536	92.79

*Hydrolysis conditions: dry mushroom sample; ratio of raw material to acid volume, 1 : 10; sulfuric acid concentration, 6 M; hydrolysis temperature, 100°C; and time, 6 h.

contamination like typical seafood fish [27, 28]. Furthermore, the production of glucosamine from crustacean shells usually needs extra pretreatment including deproteinization, demineralization, and depigmentation [29]. On the other hand, mushrooms can be organically cultivated throughout the year in a short period without geographical and seasonal restrictions. Additionally, mushrooms are relatively consistent in composition and are not associated with inorganic materials; thus, no demineralization treatment is required and the heavy metal hazard can be avoided [3]. Moreover, glucosamine from mushrooms is suitable for vegetarians and shellfish-allergic consumers. Therefore, mushrooms' potential as an economical and low-cost source of glucosamine should be encouraged.

3.2. Kinetic Models. Due to the difficulty in finding a strict mechanism for hydrolysis reactions, it is usual to use simplified models to determine the kinetics [23, 30]. The initial stage of the hydrolysis was fitted with three reaction models, respectively. $[A]_0$ was calculated based on the determined chitin content of each mushroom, and $[A]_t$ = the difference between original chitin concentration and liberated glucosamine at reaction time t. The kinetic equations and corresponding coefficient of determination (R^2) are listed in Table 2. It can be concluded that most of the hydrolysis of five mushrooms to glucosamine followed the zero-order kinetics. The hydrolysis of straw mushroom, shiitake, and wood ear mushroom were well-fitted with zero-order reaction model. Hydrolysis of enoki mushroom and oyster mushroom at 90°C was the first-order reaction, while that at 100°C and 110°C fitted well with the zero-order model. The coefficient of determination and correlation coefficients are all above 0.9 indicating agreements between the experimental data and the kinetic models are good.

As shown in Table 2, using 6 M sulfuric acid, the effect of temperature on glucosamine yield was investigated, and the temperature efficients (Q_{10}), which represented the relative change in glucosamine production rate as a result of increasing the temperature by 10°C, are listed in Table 3. The Q_{10} factors between 90°C and 100°C were higher than the values between 100°C and 110°C which showed that the glucosamine yields and the reaction rate were increased drastically when the temperature increased from 90°C to 100°C, especially enoki mushroom and oyster mushroom. The results indicate that when the temperature is higher than 100°C, the change in temperature would not have significant effect on glucosamine yield.

According to Arrhenius' equation, a plot of the natural logarithm of the rate constants as a function of the inverse of the absolute temperature (Arrhenius plot) can be drawn and the activation energy required for glucosamine conversion can be calculated from the slope of the line. The rate constant k and activation energy (E_a) for glucosamine were calculated and summarized in Table 3. The activation energies were in the range of 6.64–131.00 kJ/mol. Hydrolysis of shiitake exhibited the lowest activation energy, which may indicate that shiitake was easier to hydrolyze than other four mushrooms under the sulfuric acid hydrolysis conditions.

The activation energies for sulfuric acid hydrolysis of three mushrooms, namely, straw mushroom, shiitake, and wood ear mushroom, were lower than the activation energy of 78 kJ/mol of chitosan reported by Yan and Evenocheck [31]. An activation energy value of 131.00 kJ/mol has been determined for hydrolysis of oyster mushroom, showing a relative difficult tendency for glucosamine to be liberated. However, this value was lower than the activation energies for hydrolysis of chitin in hydrochloric acid [31, 32]. The reason may be that, as mentioned before, sulfuric acid was more effective than hydrochloric acid. Vårum et al. [32] have reported the activation energies for acid hydrolysis of two almost fully de-N-acetylated chitosans were 152.2 and 158.1 kJ/mol, respectively. The activation energies for acid hydrolysis of two partially N-acetylated chitosans were also determined to be 130.4 and 134.3 kJ/mol, respectively. In addition, at the beginning (up to 1.5 h) of the hydrolysis of wood ear mushroom, the reaction rate was almost zero (Figure 3). This phenomenon may be explained by the higher amount of glucans, and the strong bonding strength the chitin-glucan complex exist in wood ear mushroom [33].

3.3. Glucosamine Decomposition. Due to the deamination of glucosamine during acid hydrolysis, it is important to minimize the glucosamine decomposition to obtain an accurate result for kinetics study [1, 21]. To monitor the decomposition, glucosamine standards were weighed into digestion flasks and treated under the optimum hydrolysis condition (100°C, 6 M H_2SO_4). At the end of each half-hour interval, a sample was taken and analyzed for glucosamine content. The results are shown in Figure 3.

The degradation of glucosamine fits a zero-order decomposition model. About 15.01%, 19.86%, and 21.04% of glucosamine were lost at hydrolysis time of 4.5, 6, and 6.5 h, respectively. On the other hand, after 6 h hydrolysis, about 90% chitin in mushroom could be converted into

Table 2: Fitting of experimental results to different reaction models.

Mushroom	Temperature (°C)	Reaction kinetic equation		Coefficient of determination (R^2)
Enoki mushroom	90	Zero-order	$[A]_0 - [A] = 0.0774t + 4.588$	0.9569
		First-order	$\ln[A]_0 - \ln[A] = 0.0107t - 0.2763$	**0.9591**
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0026t - 0.1756$	0.6460
	100	*Zero-order*	$[A]_0 - [A] = 0.0764t + 5.7584$	**0.9475**
		First-order	$\ln[A]_0 - \ln[A] = 0.0135t - 0.4136$	0.9358
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0061t - 0.4577$	0.5367
	110	*Zero-order*	$[A]_0 - [A] = 0.0734t + 6.2301$	**0.9623**
		First-order	$\ln[A]_0 - \ln[A] = 0.013t - 0.3832$	0.9273
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0056t - 0.4247$	0.5263
Oyster mushroom	90	Zero-order	$[A]_0 - [A] = 0.0686t + 5.63$	0.9592
		First-order	$\ln[A]_0 - \ln[A] = 0.0076t - 0.0502$	**0.9886**
		Second-order	$(1/[A]) - (1/[A]_0) = 0.001t - 0.044$	0.8856
	100	*Zero-order*	$[A]_0 - [A] = 0.0716t + 7.162$	**0.9380**
		First-order	$\ln[A]_0 - \ln[A] = 0.0126t - 0.2857$	0.7945
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0065t - 0.4364$	0.2831
	110	*Zero-order*	$[A]_0 - [A] = 0.0709t + 8.6716$	**0.8642**
		First-order	$\ln[A]_0 - \ln[A] = 0.0209t - 0.613$	0.6013
		Second-order	$(1/[A]) - (1/[A]_0) = 0.1511t - 8.9392$	0.2163
Straw mushroom	90	*Zero-order*	$[A]_0 - [A] = 0.1178t - 1.4688$	**0.9586**
		First-order	$\ln[A]_0 - \ln[A] = 0.0031t - 0.084$	0.8902
		Second-order	$(1/[A]) - (1/[A]_0) = 9E-05t - 0.0039$	0.7658
	100	*Zero-order*	$[A]_0 - [A] = 0.1532t - 0.3459$	**0.9833**
		First-order	$\ln[A]_0 - \ln[A] = 0.0053t - 0.1745$	0.8779
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0002t - 0.0131$	0.6756
	110	*Zero-order*	$[A]_0 - [A] = 0.1611t + 1.7021$	**0.9803**
		First-order	$\ln[A]_0 - \ln[A] = 0.0069t - 0.2508$	0.8194
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0004t - 0.03$	0.5223
Shiitake	90	*Zero-order*	$[A]_0 - [A] = 0.0988t + 0.5635$	**0.9979**
		First-order	$\ln[A]_0 - \ln[A] = 0.0048t - 0.1109$	0.9707
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0003t - 0.0118$	0.8737
	100	*Zero-order*	$[A]_0 - [A] = 0.107t + 1.5256$	**0.9913**
		First-order	$\ln[A]_0 - \ln[A] = 0.0062t - 0.1703$	0.933
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0004t - 0.0252$	0.7393
	110	*Zero-order*	$[A]_0 - [A] = 0.1107t + 2.0852$	**0.9914**
		First-order	$\ln[A]_0 - \ln[A] = 0.007t - 0.1956$	0.9355
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0006t - 0.0332$	0.7471
Wood ear mushroom	90	*Zero-order*	$[A]_0 - [A] = 0.043t - 3.0324$	**0.9281**
		First-order	$\ln[A]_0 - \ln[A] = 0.0053t - 0.3378$	0.8191
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0009t - 0.0645$	0.6934
	100	*Zero-order*	$[A]_0 - [A] = 0.0567t - 3.1392$	**0.9469**
		First-order	$\ln[A]_0 - \ln[A] = 0.014t - 0.8964$	0.8087
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0128t - 1.0476$	0.4911
	110	*Zero-order*	$[A]_0 - [A] = 0.059t - 2.8705$	**0.9148**
		First-order	$\ln[A]_0 - \ln[A] = 0.018t - 1.0525$	0.8416
		Second-order	$(1/[A]) - (1/[A]_0) = 0.0249t - 1.8992$	0.6206

$[A]_0$ is the initial chitin content of each mushroom and $[A]$ is the chitin content at reaction time t.

glucosamine and a loss of 20% glucosamine might be observed according to the decomposition model. To minimize the glucosamine loss during hydrolysis, shorter hydrolysis time, higher temperature, or higher acid concentration might be employed. However, the decomposition kinetics during the mushroom hydrolysis process can be complicated through the processes of chitin depolymerization and deacetylation, which is followed by the glucosamine generation. The glucosamine decomposition during mushroom hydrolysis does not appear to have a significant impact. According to the decomposition model, 20% of glucosamine was lost after 6 h hydrolysis. However, the glucosamine conversions from five mushrooms were above 92% (Table 1), which were experimentally close to the chitin content of five mushrooms. This demonstrates that the decomposition of glucosamine during mushroom hydrolysis at the optimal conditions should have minimal impact on the accuracy of results.

4. Conclusion

From the results reported in this work, it can be concluded that sulfuric acid was an effective catalyst for glucosamine production. The maximum glucosamine conversion of

Table 3: The kinetics parameters for mushroom hydrolysis.

Raw material	Temperature (°C)	K (1/min)	Q_{10}	E_a (kJ/mol)
Enoki mushroom	90	0.0107		
	100	0.0764	7.1402	113.00
	110	0.0734	0.9607	
Oyster mushroom	90	0.0076		
	100	0.0716	9.4211	131.00
	110	0.0709	0.9902	
Straw mushroom	90	0.1178		
	100	0.1532	1.3005	18.31
	110	0.1611	1.0516	
Shiitake	90	0.0988		
	100	0.107	1.0830	6.64
	110	0.1107	1.0346	
Wood ear mushroom	90	0.043		
	100	0.0567	1.3186	18.51
	110	0.059	1.0406	

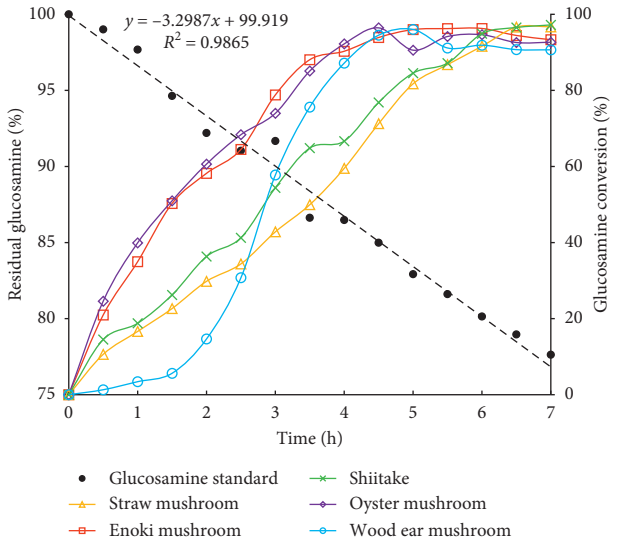

Figure 3: Stability of glucosamine standard and glucosamine conversion from five mushrooms under optimum hydrolysis conditions (100°C, 6 M H_2SO_4).

99.86% was achieved by employing the optimum conditions, which were hydrolysis temperature of 100°C, time of 6 h, and sulfuric acid concentration of 6 M. The hydrolysis kinetics of five mushrooms was studied. The values of activation energy were in the range of 6.64–131.00 kJ/mol. Shiitake was easiest to hydrolyze among the five mushrooms, while oyster mushroom exhibited the most difficult tendency for glucosamine to be liberated. Since the higher amount of glucans and the strong bonding strength the chitin-glucan complex exist in wood ear mushroom, the reaction rate at the beginning (up to 1.5 h) was almost zero. The relatively low activation energy for hydrolysis of straw mushroom (18.31 kJ/mol) and the highest glucosamine yield (56.8132 ± 3.5748 mg/g DM, 0.9824 g/g chitin) indicated that hydrolysis of straw mushroom was energy-saving. Thus, sulfuric acid hydrolysis of straw mushroom for glucosamine production could be considered as an efficient process for the future industrial application, and further study was required to get higher glucosamine purity from suitable purification procedure.

Nomenclature

A:	Pre-exponential factor
ANOVA:	Analysis of variance
AOAC:	Association of Official Analytical Chemists
°C:	Degree Celsius
DI:	Deionized
DW:	Dry weight
E_a:	Activation energy
FMOC-Su:	N-(9H-fluoren-2-ylmethoxycarbonyloxy) succinimide
h:	Hour
HPLC:	High-performance liquid chromatography
I. D.:	Internal diameter
k:	Reaction rate
min:	Minute
OVAT:	One Variable at a Time
Q_{10}:	Temperature efficient
R:	Universal gas constant
R^2:	Coefficient of determination
SD:	Standard deviation
T:	Absolute temperature (in Kelvin)
v:	Volume
w:	Weight.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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