Corn Gluten Hydrolysis by Alcalase: Effects of Process Parameters on Hydrolysis, Solubilization and Enzyme Inactivation

D. Kılıç Apar and B. Özbek*
Yıldız Technical University, Department of Chemical Engineering,
Davutpaşa Campus, 34210, Esenler/Istanbul, Turkey

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The aim of this study was to investigate the influences of substrate concentration, enzyme concentration, temperature and pH on hydrolysis and solubilization of corn gluten as well as enzyme stability. The corn gluten was hydrolyzed by Alcalase enzyme (a bacterial protease produced by a selected strain of *Bacillus licheniformis*) that was chosen among five commercial enzymes examined. The optimum process conditions for hydrolysis and solubilization were obtained as 30 g L^{-1} substrate mass concentration, 2.5 mL L^{-1} enzyme concentration, 55 °C and pH 8. Under these conditions, the values of degree of hydrolysis and solubilization were found as 28.4 % and 85.3 % respectively; and enzyme lost its activity by approximatively 74 % at the end of 120 min processing time. Modelling studies were performed to determine the kinetics of hydrolysis, solubilization and enzyme inactivation. The relationship between hydrolysis and solubilization was found linear for all experimental conditions examined. The inactivation energy of Alcalase at the temperature range of 40–55 °C was determined to be 67.86 kJ mol^{-1}.

Key words:
Corn gluten, Alcalase, hydrolysis, solubilization, modelling

Introduction

Over the last decade, the use of plant protein hydrolysates in human nutrition has broadly expanded. They are often used in different nutritional formulations, such as supplementation of drinks to enhance their nutritional and functional properties, or special medical diets.[1] Unlike acid or alkali hydrolysis, enzymic hydrolysis of protein, using selective protease, provides milder process conditions and little or no undesirable side reactions or products. In addition, the final hydrolysate, after being neutralized, contained less salt, and the functionality of the final product can be controlled by the selection of specific enzymes and defining the reaction factors. The main variables determining the result of the reaction are temperature, pH, enzyme to substrate ratio and the reaction time.[2–4]

Most of the fundamental research on food protein hydrolysis is based on soy protein.[3,5–8] Apart from soy bean processing, peas,[9–13] lupin[14,15] and wheat[16–19] are the notable seed protein sources investigated.

Corn gluten which contains 67–71 % proteins is a co-product of corn wet milling.[20] The utilization of corn gluten in food products will be increased by improving the quality of its hydrolysates. In literature, a few studies on preparation of corn gluten hydrolysate have been reported. Hardwick and Glatz[21] investigated the effects of enzyme dosage and meal size on hydrolysis and soluble protein fractions. Suh *et al*.[22] prepared hydrolysates containing ACE inhibitory activity from corn gluten, with various enzymes. Kim *et al.*[2] studied preparation of corn gluten hydrolysate with ACE inhibitory activity and investigate its solubility and moisture sorption, they also reported the effect of heat pretreatment on hydrolysis. In another study by using ultrafiltration membranes, Kim *et al.*[23] investigated the enhancement of ACE inhibitory activity and improvement of the emulsifying and foaming properties of corn gluten hydrolysate.

In the present work, a comprehensive study on hydrolysis and solubility of corn gluten and on residual enzyme activity was performed to investigate the effect of the process conditions such as substrate concentration, enzyme concentration, temperature and pH. The kinetics of hydrolysis, solubilization and enzyme inactivation were examined for all experimental conditions applied.

Materials and methods

Materials: Corn gluten used in this research which contains 62.22 % protein was obtained from Cargill. The commercial enzymes used in this work were Alcalase 2.4L (produced by *Bacillus licheniformis*), Neutrase 0.8L (produced by *Bacillus*

* Author for correspondence:
Fax: +90-212-383 4725; E-mail: bozbek@yildiz.edu.tr

amyloliquefaciens), PTN P-110 (pancreatic Trypsin), Flavouryzme 1000L (produced by *Aspergillus oryzae)*, Protamex (produced by *Bacillus amyloliquefaciens and Bacillus licheniformis*) obtained from Novozymes.

Enzymatic hydrolysis: Hydrolysis experiments were carried out in a 400 mL jacketed reactor with magnetic stirring, pH and temperature control. A measured amount of corn gluten was added to the reactor containing 200 mL of distilled water and allowed to disperse, then the pH and temperature of the reaction solution was set. The experiment was initiated by the addition of enzyme solution. The experiments were carried out at least in duplicate and the reproducibility was within the range of ± 5 %.

Conversion of hydrolysis: The hydrolysis of the reaction was monitored by pH stat method, and the conversion of hydrolysis was computed from the following equation:

$$X_H = \frac{V_B \cdot N_B}{\alpha \cdot M_P \cdot h_{tot}} \quad (1)$$

The degree of dissociation of α-NH_2 groups was computed from the following equation:

$$\alpha = \frac{10^{pH-pK}}{1+10^{pH-pK}} \quad (2)$$

By comparing the pairs of hydrolysis at different pH values (pH_1 and pH_2), for which free amino groups, Leu-NH_2 eqv determined by the TNBS (trinitrobenzene sulfonic acid) reaction, and V_B (base consumptions) are linearly correlated with the slope b, pK was calculated from the following eq. (3):

$$pK = pH_2 + \log(b_1 - b_2) - \log(10^{pH_2 - pH_1}) \cdot (b_2 - b_1) \quad (3)$$

Protein concentration: Soluble protein concentration was determined by *Lowry* method[24] using bovine serum albumin as standard. Assays were carried out in triplicate and their averages were taken. The reproducibility of the measurements was within the range of ± 3 %. The ratio of solubilization was calculated by taking the ratio of soluble protein concentration (background protein free) to the total protein concentration (background protein free).

Protease activity: The procedure followed to determine the activity of Alcalase was described by Lamas *et al.*[25] for measuring the proteolytic activity of pure cardosin *A*, and consist of slight modifications of the method initially proposed by Tomarelli *et al.*[26] that involves digestion of azocasein; Quantification of the proteolytic activity was based on the amount of peptides released, as monitored via spectrophotometric measurements of absorbance at 440 nm. Enzyme activities prior to hydrolysis process were also determined as the initial activities. In calculations, these activities determined as 100 % activity. Activities at any operational conditions (*A*) were then obtained as the percentage values of the initial activities. Assays were carried out in triplicates and their averages were taken.

Computational work

The software package MATLAB 5.0 was used in the numerical calculations. The parameters were evaluated by the nonlinear least squares method of Marquardt–Levenberg until minimal error was achieved between experimental and calculated values. The residual (SSR) is defined as the sum of the squares of the differences between experimental and calculated data and is given by

$$SSR = \sum_{n=1}^{N_d} (\gamma_n^{obs} - \gamma_n^{cal})^2$$

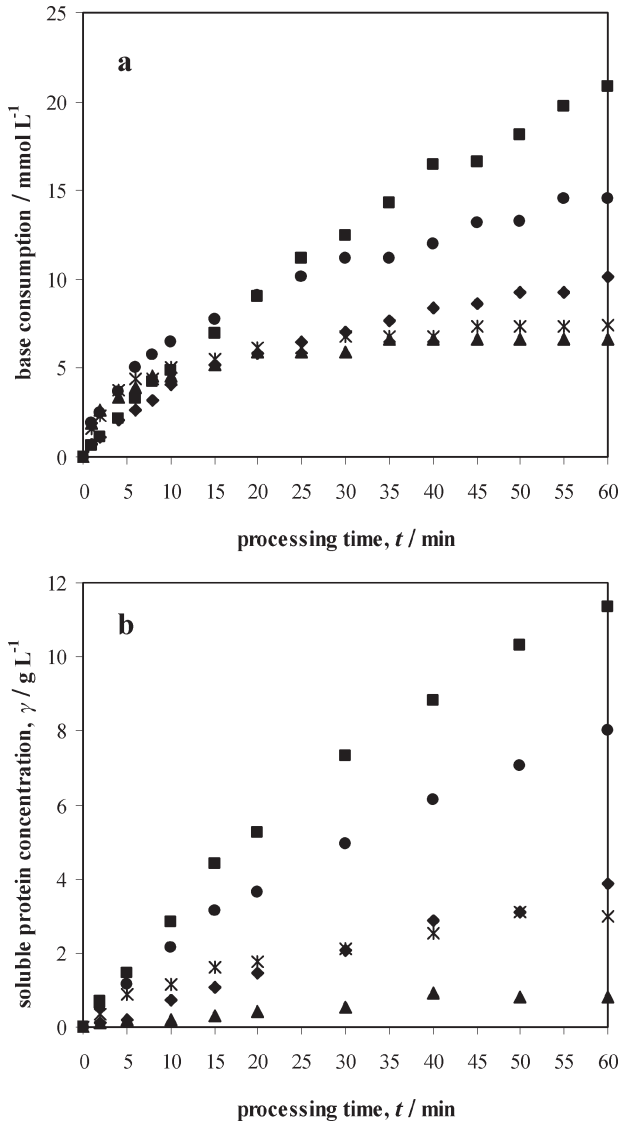
where n is the observation number and N_d is the total number of observations. The estimated variance of the error (population variance) is calculated by the SSR at its minimum divided by its degrees of freedom:

$$\sigma^2 \approx s^2 = \frac{(SSR)_{\min}}{(n-p)}$$

where p is the number of parameters and s^2 is the variance. The standard error, e (the estimated standard deviation) is calculated by taking the square root of the estimated variance of the error.

Results and discussion

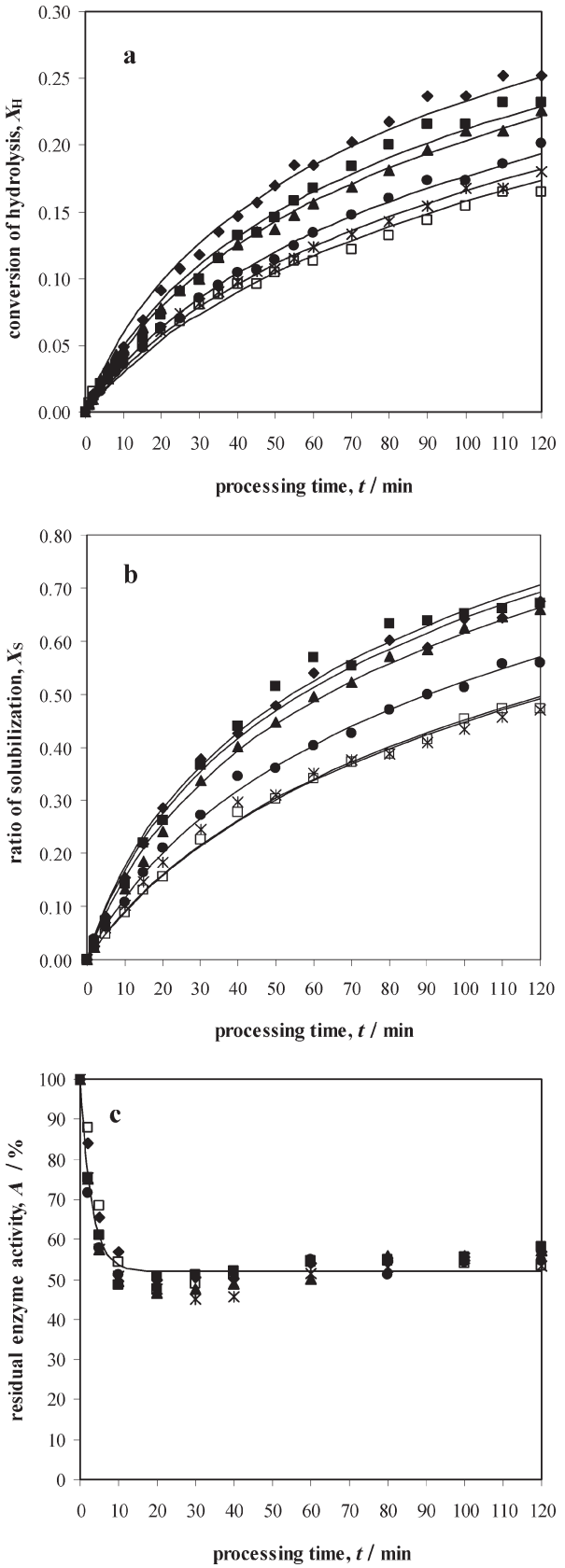
A general survey of enzymatic hydrolysis and solubilization of corn gluten was performed with five commercial enzyme preparations (Alcalase, Neutrase, Flavourzyme, Protamex and PTN) in order to choose appropriate enzyme. The experiments were performed under common operating conditions considering the optimal temperature and pH range of each enzyme; temperature 50 °C, pH 7. The reaction solutions contained 20 g protein per litre and approximately 0.35 AU units enzyme per gram of protein for each experiment. The results were given in Figs. 1a and 1b. As can be seen from these figures, Alcalase and Protamex have stronger capability for hydrolysis and solubilization compared to other enzyme preparations. On the other hand, at the end of 60 min of processing time, the base consumption and soluble protein amount for Alcalase and Protamex were 20.83 and 14.54 mmol L^{-1}; 11.35 and 8.01 g L^{-1}, respectively.



F i g . 1 – *For various commercial enzyme preparations a) Base consumption (0.4 mol L^{-1} KOH) vs. processing time, b) Soluble protein concentration vs. processing time, (▲ Flavourzyme, ✱ PTN, ◆ Neutrase, ● Protamex, ■ Alcalase). Experiments were performed at 50 °C, pH 7, 20 g L^{-1} substrate mass concentration with 0.35 AU of enzyme per g substrate.*

Therefore, Alcalase was chosen as proper enzyme preparation for further studies.

In order to investigate the effect of substrate mass concentration on hydrolysis and solubility of corn gluten and stability of Alcalase, experiments were conducted at various substrate mass concentrations ranging between 10 and 60 g L^{-1} protein. Results were given in Figs. 2a-c. As seen from the Figs. 2a and 2b, typical hydrolysis and solubilization curves obtained in which the degree of hydrolysis and solubilization profile are characterized by an initial fast rate followed by rapid decrease in rate. The decreases observed in the rates could be attributed to one of the following phenomena: (a) the decrease in concentration of peptide bonds sus-



F i g . 2 – *At various substrate mass concentration; a) Conversion of hydrolysis vs. processing time, b) Ratio of solubilization vs. processing time, c) Residual enzyme activity, % vs. processing time, (◆ 10 g L^{-1}, ■ 20 g L^{-1}, ▲ 30 g L^{-1}, ● 40 g L^{-1}, ✱ 50 g L^{-1}, □ 60 g L^{-1}, – kinetic models). Experiments were performed at 50 °C, pH 7 with 2.5 mL L^{-1} enzyme concentration.*

ceptible to hydrolysis by Alcalase, (b) with an approach to steady state as the degree of hydrolysis and solubilization tend toward a limit value which decrease with the initial substrate concentration, (c) possible inhibition of the enzyme caused by the hydrolysis products, (d) enzyme inactivation.[5,27]

Fig. 2c shows the effect of substrate mass concentration on enzyme stability. As seen from this figure, for all initial substrate concentration experiments, the residual enzyme activity values decreased sharply in ten minutes, and then stabilized approximately at the value of 55 % until the end of the process, so that it may be concluded that strong substrate inactivation existed in ten minutes. But, inactivation level seems to same for all substrate mass concentrations used indicating that γ = 10 g L^{-1} protein or the amount of any other content of gluten that caused denaturation is excess for inactivation and using substrate mass concentrations above this value does not cause further inactivation. For these reasons, the control experiments were performed to investigate the stability behavior of enzyme in gluten free water and phosphate buffer solution and results were presented in Fig. 3. As it can be seen from this figure, in the case of water and phosphate buffer solution, the residual enzyme activity values gradually decreased with respect to time. Hence, the sharp decrease in 10 min in initial substrate mass concentration experiments does not depend on substrate inactivation; and considering the results given in Fig. 3, it can be concluded that after approximatively 20 min of processing time, the substrate and/or the hydrolysis products have also a stabilizing effect on the activity. As a result, as there is no inactivation due to substrate, the decrease in the hydrolysis and solubilization with initial substrate mass concentration could be depend on: (a) the limitation of the reaction due to saturation of the enzyme with the substrate, (b) reducing water activity due to increasing the substrate amount, (c) mass transfer limitations as a result of increased viscosity, (d) reversible enzyme inhibition that caused by substrate and/or products.

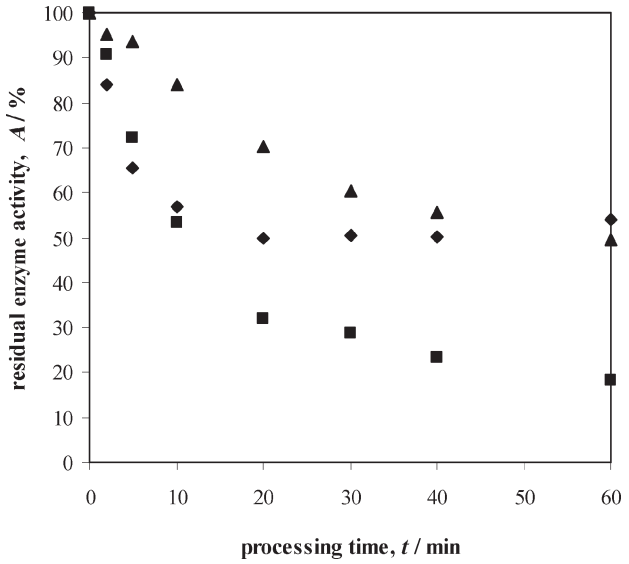


Fig. 3 – *Control experiments for enzyme stability, T = 50 °C, pH 7, γ_E = 2.5 mL L^{-1} (▲ in phosphate buffer solution, ■ in water, ◆ in 1 % substrate + water mix.)*

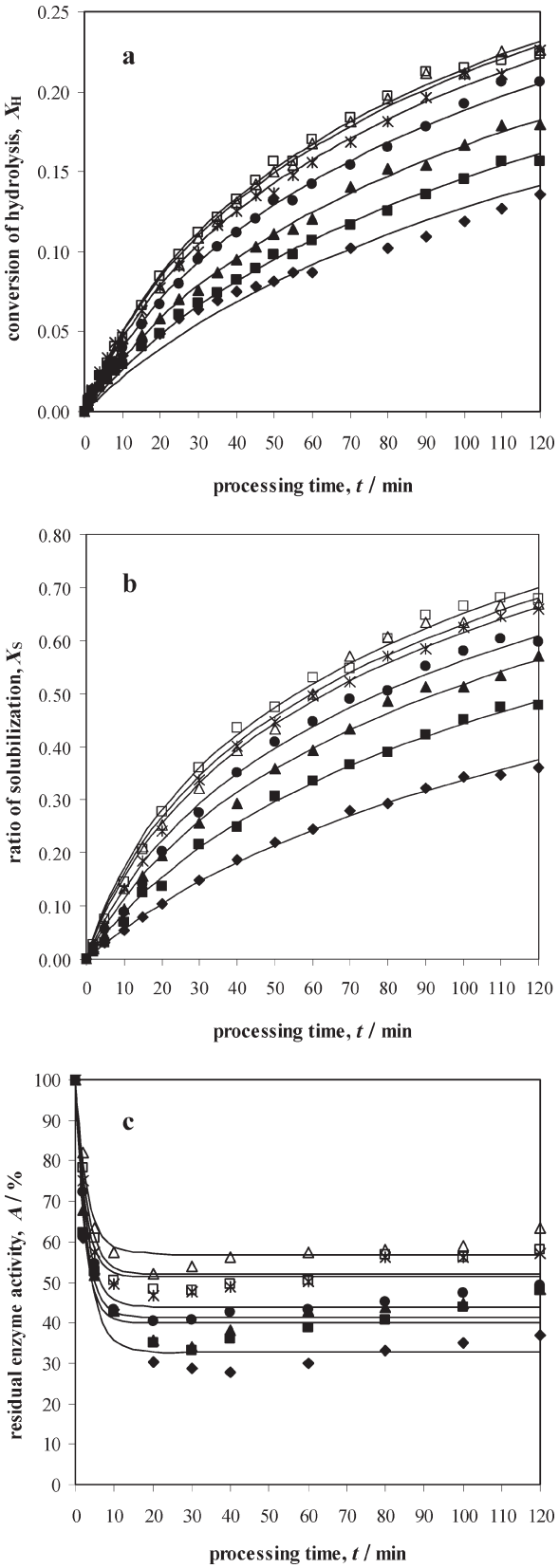
Effect of enzyme volume concentration on hydrolysis and solubility of corn gluten and stability of Alcalase was investigated by using enzyme volume concentrations in the range of 0.5–3.5 mL L^{-1}, and the results were given in Figs. 4a-c. By increasing the enzyme concentration up to 2.5 mL L^{-1}, both degree of hydrolysis (Fig. 4a) and solubilization (Fig. 4b) increased due to the increase on the reaction rate. However, after this enzyme volume concentration, the rate of these increases was diminished, and the values of degree of hydrolysis and solubilization obtained were found nearly similar. This result could be explained by the saturation of the substrate with enzyme.

Fig. 4c shows the stability behavior of Alcalase with respect to time at various enzyme concentrations. As it can be seen from this figure, the residual enzyme activity values again decreased sharply in ten minutes and then stabilized. However, the enzyme stability increased with increasing the enzyme amount.

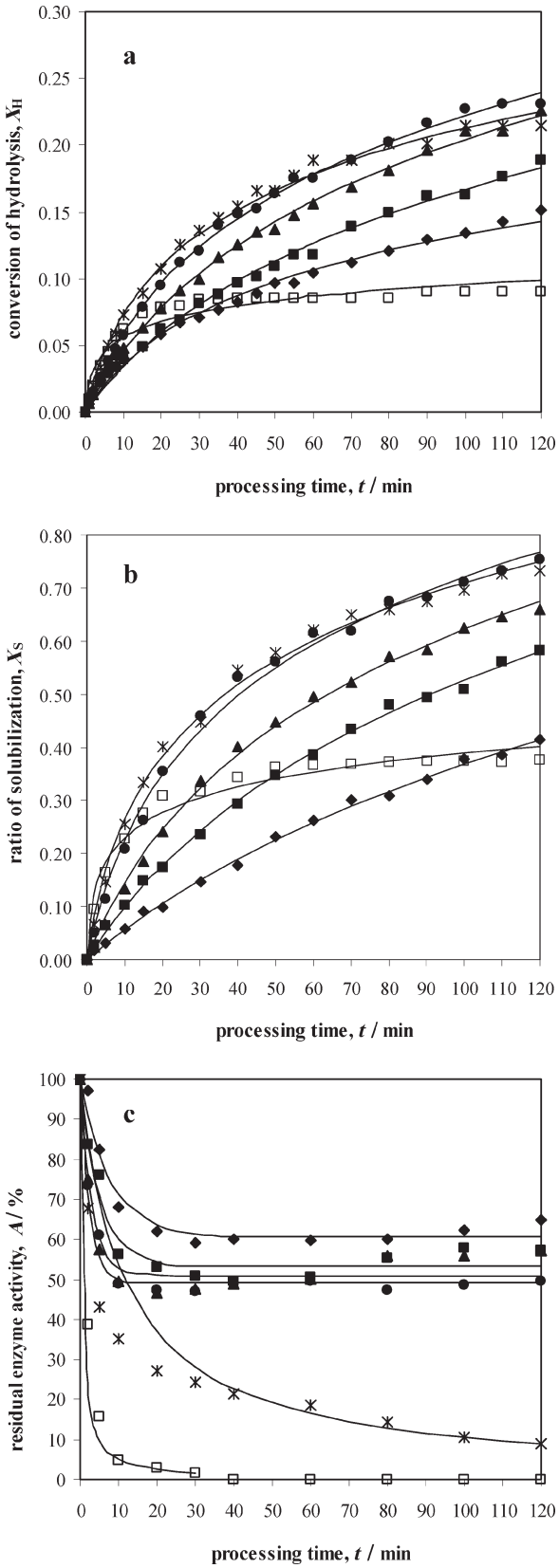
The influence of temperature on hydrolysis and solubility of corn gluten were presented in Figs. 5a and 5b. The optimum temperature for hydrolysis and solubilization of corn gluten was found to be 55 °C as the highest degree of hydrolysis and solubilization obtained at this temperature. At 60 °C, the degree of hydrolysis and solubilization values were slightly higher than the values obtained at 55 °C at only during the first 60 min of the reaction; then, the values obtained for 60 °C were situated below those values obtained at 55 °C. At 65 °C, the degree of hydrolysis and solubilization values became constant after 30 min as the enzyme completely inactivated.

Fig. 5c shows the effect of temperature on enzyme stability. As seen from this figure, in the range of 40-55 °C, there is a slight enzyme inactivation with respect to temperature. In this range, the enzyme lost its activity sharply in ten minutes; and then, it maintained its stability until the end of the process. The irreversible enzyme inactivation became stronger at 60 °C, and enzyme completely inactivated at 65 °C in 30 min.

The change in conformation of the whole substrate protein, as well of the enzyme itself effected by changes in pH may influence the access of a protease to particular pairs of amino acid residues



F i g . 4 – *At various enzyme concentration; a) Conversion of hydrolysis vs. processing time, b) Ratio of solubilization vs. processing time, c) Residual enzyme actitiy % vs. processing time, (◆ 0.5 mL L^{-1} , ■ 1 mL L^{-1}, ▲ 1.5 mL L^{-1}, ● 2 mL L^{-1}, ∗ 2.5 mL L^{-1}, □ 3 mL L^{-1}, △ 3.5 mL L^{-1}, – kinetic models). Experiments were performed at 50 °C, pH 7, 30 g L^{-1} of substrate mass concentration.*



F i g . 5 – *At various temperature values; a) Conversion of hydrolysis vs. processing time, b) Ratio of solubilization vs. processing time, c) Residual enzyme activity % vs. processing time, (◆ 40 °C, ■ 45 °C, ▲ 50 °C, ● 55 °C, ∗ 60 °C, □ 65 °C, – kinetic models). Experiments were performed at pH 7, 30 g L^{-1} of substrate mass concentration with 2.5 mL L^{-1} enzyme concentration.*

within the substrate structure.[28] Hence, in order to investigate the effect of pH on hydrolysis and solubility of corn gluten and stability of Alcalase, experiments were conducted at various pH values ranging from 6.5 to 8.5. Results show that, the optimum pH value for hydrolysis and solubilization is 8 since the degree of hydrolysis and solubilization increased as pH increased up to this value (Figs. 6a and 6b). The degree of hydrolysis and solubilization values obtained at pH 8.5 were found nearly similar with those obtained at pH 8. Fig. 6c shows the stability behavior of Alcalase with respect to time at various pH values. As seen from this figure, in the range of pH 6.5–7.5, there is a slight enzyme inactivation due to the increase on pH; but after the pH value of 7.5, the enzyme inactivation became stronger.

Comparison of the values of degree of hydrolysis and solubilization obtained in this study with those from the data in the literature is difficult since the authors used different enzymes and substrates in their research as well as experimental conditions applied were different such as temperature, pH and etc. However, the proportional relation between the degree of hydrolysis and enzyme/substrate ratio were recorded for the hydrolysis of pea protein by Trypsin[9] and by Alcalase,[13] for the hydrolysis of whey protein by MKC Protease 660L and Alcalase 0.6L,[27] for the hydrolysis of lean meat protein by Alcalase[29] and for the hydrolysis of hemoglobin by Alcalase.[30] On the other hand, the proportional relation between solubilization and enzyme/substrate ratio were recorded for the hydrolysis of soybean by a protease produced by *Penicillium duponti* K 1104[5] and for the hydrolysis of whey protein by trypsin.[31] Also, the optimum temperature and pH value obtained in this study for hydrolysis of corn gluten is consistent with the optimum pH and temperature range given for Alcalase.[3]

Modelling studies

In general, the reaction rates of enzymatic hydrolysis characterized according to Michaelis-Menten kinetic models.[5,27,29,32,33] However, there are also some studies that represent the kinetics with the empirical models[9,31] and simple exponential equations.[13,27,30] In the present study, evaluation of the results show that the kinetics of hydrolysis and solubilization for all experiments performed could be represented by the following kinetic equation:

$$\frac{\mathrm{d}X}{\mathrm{d}t} = a \cdot \exp(-b \cdot X) \qquad (4)$$

where X denotes conversion of hydrolysis or ratio of solubilization; and a and b are the parameters

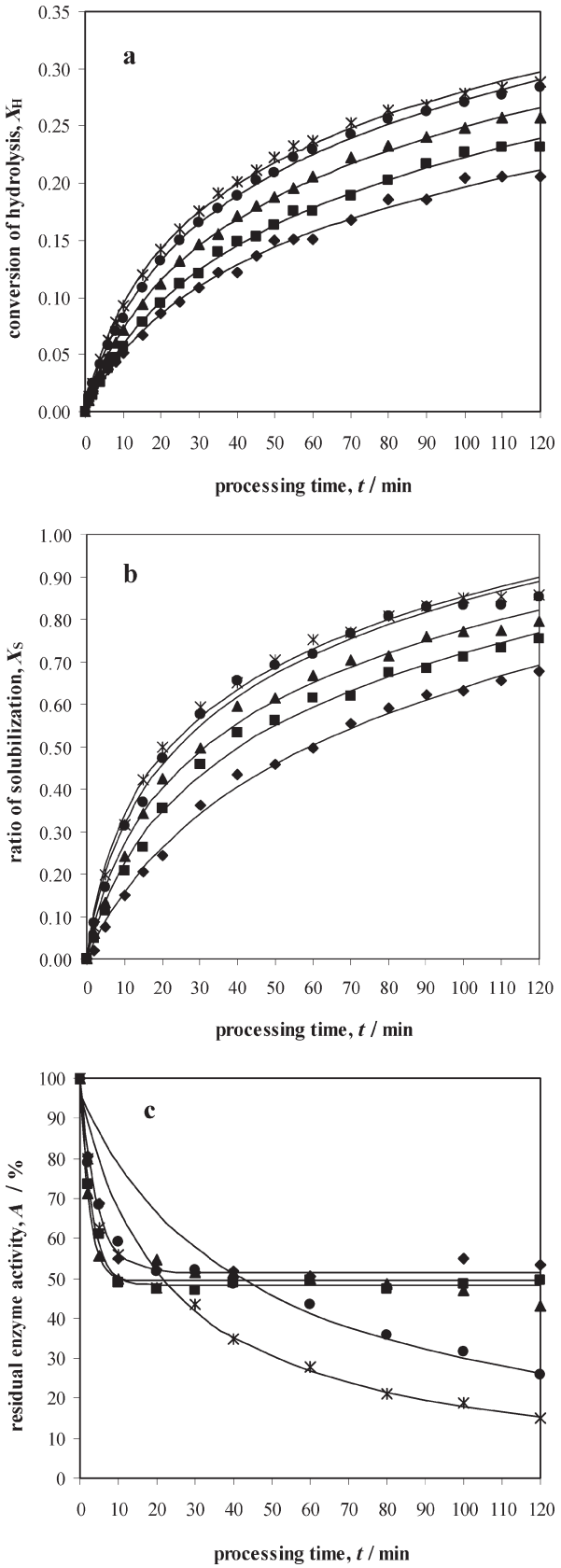


Fig. 6 – *At various pH values; a) Conversion of hydrolysis vs. processing time, b) Ratio of solubilization vs. processing time, c) Residual enzyme activity % vs. processing time, (◆ pH 6.5, ■ pH 7, ▲ pH 7.5, ● pH 8, ✱ pH 8.5, – kinetic models). Experiments were performed at 55 °C, 30 g L^{-1} of substrate mass concentration with 2.5 mL L^{-1} enzyme concentration.*

have different values for the different experiments. The constants, standard error (*e*) and R^2 statistic values for eq. (4) were estimated for all experiments performed and given in Table 1. In agreement with previous studies,[13,27,30] parameter *b* did not change with the initial substrate or enzyme concentration and can be considered independent from these variables and constant in the operating conditions with an average value of 8.86 for hydrolysis and 3.33 for solubilization, while the parameter *a* increased when the initial enzyme concentration increased, and decreased when the initial substrate mass concentration increased. On the other hand, both parameters *a* and *b* changed with the change in temperature and pH.

The relationship between the hydrolysis and solubilization were also investigated by evaluation of the experimental results. And, it is found that the solubility increased in a linear relationship with the degree of hydrolysis. As the proportionality obtained between the hydrolysis and solubility was almost constant for all conditions examined, a general linear equation (eq. (5)) was obtained to represent the relationship between the hydrolysis and solubilization with R^2 statistic value of 0.9955 and standard error (*e*) of 0.0064. The linear relationship be-

Table 1 – *Estimated constants, standard error (e) and R^2 statistic values for eq. (4)*

γ_S / g L^{-1}	Hydrolysis				Solubilization			
	a	*b*	*e*	R^2	*a*	*b*	*e*	R^2
10	0.0078	8.86	0.0070	0.9967	0.0225	3.33	0.0170	0.9974
20	0.0062	8.86	0.0076	0.9954	0.0237	3.33	0.0242	0.9951
30	0.0057	8.86	0.0032	0.9990	0.0202	3.33	0.0130	0.9984
40	0.0043	8.86	0.0032	0.9987	0.0142	3.33	0.0100	0.9986
50	0.0039	8.86	0.0039	0.9976	0.0105	3.33	0.0189	0.9929
60	0.0034	8.86	0.0068	0.9911	0.0103	3.33	0.0088	0.9986
γ_E / mL L^{-1}								
0.5	0.0023	8.86	0.0091	0.9728	0.0062	3.33	0.0074	0.9983
1.0	0.0030	8.86	0.0027	0.9986	0.0101	3.33	0.0096	0.9984
1.5	0.0038	8.86	0.0026	0.9991	0.0138	3.33	0.0121	0.9981
2.0	0.0048	8.86	0.0031	0.9989	0.0165	3.33	0.0198	0.9952
2.5	0.0057	8.86	0.0032	0.9990	0.0202	3.33	0.0130	0.9984
3.0	0.0064	8.86	0.0034	0.9989	0.0232	3.33	0.0159	0.9979
3.5	0.0062	8.86	0.0046	0.9982	0.0215	3.33	0.0214	0.9961
T / °C								
40	0.0049	16.49	0.0045	0.9952	0.0060	2.49	0.0071	0.9988
45	0.0040	9.36	0.0043	0.9974	0.0113	2.62	0.0084	0.9992
50	0.0057	8.86	0.0032	0.9990	0.0202	3.33	0.0130	0.9984
55	0.0080	9.80	0.0032	0.9992	0.0349	3.63	0.0196	0.9973
60	0.0127	13.67	0.0048	0.9979	0.0507	4.45	0.0161	0.9980
65	0.0430	57.66	0.0072	0.9676	0.1748	14.18	0.0196	0.9863
pH								
6.5	0.0071	11.10	0.0041	0.9982	0.0203	3.11	0.0146	0.9983
7.0	0.0080	9.80	0.0032	0.9992	0.0349	3.63	0.0196	0.9973
7.5	0.0107	9.79	0.0030	0.9994	0.0474	3.79	0.0224	0.9968
8.0	0.0134	9.65	0.0033	0.9994	0.0619	3.79	0.0245	0.9967
8.5	0.0159	10.12	0.0039	0.9992	0.0712	3.95	0.0223	0.9972

tween the degree of hydrolysis and solubility was also stated by Soral-Smietana *et al.*[12] for hydrolysis of pea protein by trypsin and by Bombara *et al.*[19] for the modification of wheat flour with protease.

$$X_H = 0.3239 \cdot X_S - 0.00011 \quad (5)$$

Evaluation of the enzyme deactivation data show that for all substrate and enzyme concentration experiments and in 45–55 °C temperature and 6.5–7.5 pH range the deactivation of Alcalase could be represented by a single step unimolecular non-first-order enzyme deactivation model (eq. (6)) given by Sadana and Henley[34] who reported that the single step inactivation leads to a final state that does exhibit some residual activity. On the other hand, the enzyme inactivation data obtained after the temperature of 55 °C and pH value of 7.5 were fitted to the second order inactivation equation (eq. (7)). Above these temperature and pH values, the experimental results obtained confirmed that the enzyme inactivation became stronger (Figs. 4c and 5c) which indicated that there is a shift of the inactivation mechanism (from the single step unimolecular non-first-order enzyme deactivation model to the second order inactivation model).

$$A = (100 - a_1) \exp(-k_D \cdot t) + a_1 \quad (6)$$

$$1/A = 1/A_0 + k \cdot t \quad (7)$$

In eqs. (6) and (7); A is residual enzyme activity (percentage values after hydrolysis), a_1 is the ratio of the specific activity of the final state to the initial state, k_D and k are the degradation coefficients (min^{-1}). The estimated constants, standard error (e) and R^2 statistic values for eqs. (6) and (7) were given in Table 2.

Table 2 – *Estimated constants, standard error (e) and R^2 statistic values for eqs. (6) and (7)*

	Equations	e	R^2
γ_S / g L^{-1}			
10–60 g L^{-1}	$A = (100–51.96) \exp(–0.3395\ t) + 51.96$	3.0034	0.9834
γ_E / mL L^{-1}			
0.5	$A = (100–32.71) \exp(–0.3111\ t) + 32.71$	5.0198	0.9751
1.0	$A = (100–39.92) \exp(–0.4146\ t) + 39.92$	4.9194	0.9696
1.5	$A = (100–41.18) \exp(–0.3714\ t) + 41.18$	4.2837	0.9768
2.0	$A = (100–43.85) \exp(–0.3441\ t) + 43.85$	2.8824	0.9886
2.5	$A = (100–50.90) \exp(–0.3261\ t) + 50.90$	3.8517	0.9700
3.0	$A = (100–52.05) \exp(–0.3322\ t) + 52.05$	3.7504	0.9747
3.5	$A = (100–56.91) \exp(–0.3244\ t) + 56.91$	3.2520	0.9768
T / °C			
40	$A = (100–60.58) \exp(–0.1304\ t) + 60.58$	2.9719	0.9830
45	$A = (100–53.32) \exp(–0.1953\ t) + 55.32$	3.3460	0.9791
50	$A = (100–50.90) \exp(–0.3261\ t) + 50.90$	3.8517	0.9700
55	$A = (100–49.04) \exp(–0.4125\ t) + 49.04$	2.1478	0.9915
60	$1/A = 1/97.94 + 0.00084\ t$	0.0064	0.9802
65*	$1/A = 1/103.01 + 0.01903\ t$	0.0385	0.9867
pH			
6.5	$A = (100–51.52) \exp(–0.2329\ t) + 51.52$	2.0905	0.9925
7.0	$A = (100–49.04) \exp(–0.4125\ t) + 49.04$	2.1478	0.9915
7.5	$A = (100–49.36) \exp(–0.4213\ t) + 49.36$	3.0489	0.9837
8.0	$1/A = 1/96.01 + 0.00023\ t$	0.0024	0.9593
8.5	$1/A = 1/96.90 + 0.00045\ t$	0.0020	0.9940

* For 0–30 min

The inactivation energy of Alcalase for the temperature range of 40–55 °C can be determined by using the Arrhenius relationship:

$$\ln k_D = \ln k_{D_0} - E_D/RT \qquad (8)$$

The plot of ln k_D vs. T^{-1} given in Fig. 7. As seen from this figure, the data accurately fit to eq. (8). The value E_D was estimated as 67.86 kJ mol^{-1} with the standard error (e) and R^2 statistic values of 0.0798 and 0.9920, respectively. This result differs from that determined by Marquez and Vazquez[30] who reported that the inactivation energy for Alcalase is 25.35 kJ mol^{-1} and by Adler-Nissen[3] who suggested an average value of 271 kJ mol^{-1}.

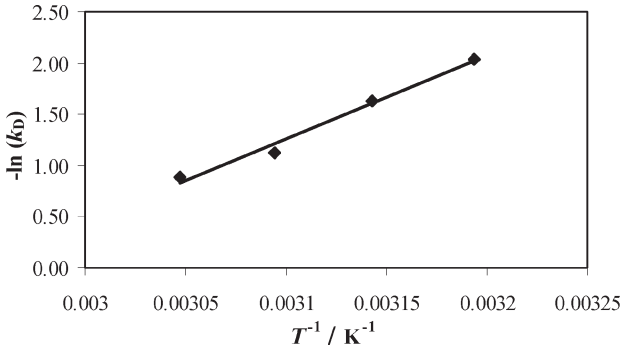


Fig. 7 – *Arrhenius plot for enzymatic inactivation determination*

Conclusions

In order to choose appropriate enzyme, corn gluten hydrolysis and solubilization experiments were performed with five commercial enzyme preparations (Alcalase, Neutrase, Flavourzyme, Protamex and PTN). It was found that Alcalase has highest capability for hydrolysis and solubilization compared to other enzyme preparations. Therefore, hydrolysis and solubilization of corn gluten by Alcalase was performed at various substrate mass concentrations, enzyme concentrations, temperatures and pH values. The degree of hydrolysis and solubilization changed proportionally with enzyme/substrate mass ratio. The optimum substrate mass concentration, enzyme concentration, temperature and pH for hydrolysis and solubilization were obtained as 30 g L^{-1}, 2.5 mL L^{-1}, 55 °C and 8, respectively. At these conditions, the values of degree of hydrolysis and solubilization were found as 28.4 % and 85.3 %; and enzyme lost its activity by approximatively 74 % at the end of 120 min processing time. The results show that strong enzyme inactivation exists after the temperature of 55 °C and pH of 7.5.

Mathematical models were proposed to predict the degree of hydrolysis, solubilization and the residual enzyme activity have been confirmed with the experimental results. For each experimental condition, a simple exponential function accurately represented the hydrolysis and solubilization data of corn gluten with respect to time. On the other hand, a general linear equation, that accurately fit the all data obtained from the all experiments, was used to represent the relationship between the hydrolysis and solubilization. The enzyme inactivation; at all substrate and enzyme concentrations used and in 45–55 °C temperature range and in 6.5–7.5 pH range; represented by a single step unimolecular non-first-order enzyme deactivation model. However, above the temperature of 55 °C and pH value of 7.5, the enzyme inactivation was represented with a second order inactivation equation as the inactivation mechanism changed. The inactivation energy of Alcalase for the temperature range of 40–55 °C was determined by using the Arrhenius relationship and found as 67.86 kJ mol^{-1}.

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Nomenclature

A – residual enzyme activity, %

a, b – parameters in eq. (4)

a_1 – ratio of the specific activity of the final state to the initial state, –

E_D – inactivation energy, kJ mol^{-1}

h_{tot} – total number of peptide bonds in protein, mmol g^{-1}

k, k_D – degradation coefficient, min^{-1}

M_p – mass of protein, g

N_b – normality of the base, mmol mL^{-1}

T – temperature

t – processing time, min

V_B – base volume consumption, mL

X_H – conversion of hydrolysis

X_S – ratio of solubilization

α – average degree of dissociation of the α-NH_2 groups

γ_E – enzyme concentration, mL L^{-1}

γ_S – substrate mass concentration, g L^{-1}

e – standard error

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