Korean J. Chem. Eng., **28**(1), 195-202 (2011)
DOI: 10.1007/s11814-010-0316-2

Sesame cake protein hydrolysis by alcalase: Effects of process parameters on hydrolysis, solubilisation, and enzyme inactivation

Elçin Demirhan, Dilek Kılıç Apar, and Belma Özbek†

Department of Chemical Engineering, Davutpaþa Campus, Yıldız Technical University, 34210, Esenler/Istanbul, Turkey
(*Received 22 March 2010 • accepted 30 April 2010*)

Abstract−We investigated the effects of process parameters (substrate concentration, enzyme concentration, temperature and pH) on the hydrolysis and solubilization of sesame cake protein as well as enzyme stability. The sesame cake protein 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 15 g L^{-1} substrate concentration, 3 ml L^{-1} enzyme concentration, 50 °C and pH 8.5. Under these conditions, the values of degree of hydrolysis and solubilization were found as 26.3% and 82.1%, respectively, and enzyme lost its activity by approx. 56% at the end of 120 min processing time. Modeling 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 45-55 °C was determined to be 25544 J mol^{-1}.

Key words: Sesame Cake Protein, Alcalase, Hydrolysis, Solubilization, Modelling

INTRODUCTION

Plant proteins, extracted from abundant and renewable biological resources, play significant roles in human nutrition [1,2]. Enzymatic hydrolysis, a valuable bioprocess, is frequently used to improve functional and nutritional properties of food proteins. Enzymatic proteolysis has been shown to increase solubility, modify foaming and emulsifying properties and to liberate biologically active peptides from certain proteins. Protein hydrolysates are widely used as nutritional supplements, functional ingredients, and flavor enhancers in foods, coffee whiteners, cosmetics, and in the fortification of soft drinks and juices [3-5]. The use of proteolytic enzymes allows selective hydrolysis to produce potentially safer and more defined material. The functionality of the final product can be controlled by the selection of specific enzymes and defining the reaction conditions. The main variables determining the result of the reaction are temperature, pH, enzyme to substrate ratio and the reaction time [6-9].

Most fundamental research on food protein hydrolysis is based on soya protein [8,10,11]; but peas [12-15], lupin [16,17], wheat [18-20] and corn gluten [6,9,21,22] are the other seed protein sources that have been investigated.

Sesame seed has an oil content of between 48 and 55%; as a result, it has become one of the main sources of edible oil. It is also a good source of protein. One of the principal characteristics of this protein is its high methionine and tryptophan content. The sub-product of the oil extraction process is sesame cake, whose protein content can reach 50% depending on the extraction method. Because of the high content and quality of its protein, sesame cake has been used as an animal feed. In fact, the utilization of sesame cake in food products for human consumption will be increased by improving the quality of its hydrolysates [4,23,24]. There are a few studies in literature about the hydrolysis of sesame cake. Perez and Saad [25] investigated the effects of process parameters on hydrolysis of sesame cake hydrolysate. Taha et al. [26] elucidated the optimum conditions for the enzymatic hydrolysis of soybean, sesame seed and rice bran meal protein with two enzymes, papain and bromelain. Bandyopadhyay and Ghosh [4] studied preparation of sesame protein hydrolysates and investigated the improvement of the emulsifying and foaming properties.

In this study, a comprehensive study on hydrolysis and solubility of sesame cake protein and on residual enzyme activity was performed using a commercial protease preparation. The effects of process parameters, such as substrate concentration, enzyme concentration, temperature and pH, on hydrolysis and solubility of sesame cake protein and on residual enzyme activity were investigated. The kinetics of hydrolysis, solubilization and enzyme inactivation were examined for all experimental conditions applied.

MATERIALS AND METHODS

1. Materials

Sesame cake used in this study which contains 37.8% protein was obtained from Necdet Bükey A.Ş. (Izmir, Turkey). The commercial enzymes used in this work were Alcalase 2.4 L (produced by *Bacillus licheniformis*, 2.4 Anson Unit/g=2.8 Anson Unit/ml), Neutrase 0.8 L (produced by *Bacillus amyloliquefaciens*), PTN P-110 (pancreatic Trypsin), Flavouryzme 1,000 L (produced by *Aspergillus oryzae)*, Protamex (produced by *Bacillus amyloliquefaciens* and *Bacillus licheniformis*) obtained from Novozymes.

2. Enzymatic Hydrolysis

Hydrolysis experiments were performed in a 400 ml jacketed reactor with magnetic stirring with pH and temperature control. A

†To whom correspondence should be addressed.
E-mail: bozbek@yildiz.edu.tr

measured amount of sesame cake 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 done at least in duplicate and the reproducibility was within the range of ±5%.

3. 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 [7,22,27]:

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

V_B : base consumption [ml]
N_b : normality of the base [mmol ml^{-1}]
α : average degree of dissociation of the α-NH_2 groups
M_P : mass of protein [g]
$h_{tot.}$: total number of peptide bonds in protein [mmol g^{-1}]
X_H : conversion of hydrolysis

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):

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

4. Protein Concentration

Soluble protein concentration was determined by Lowry method [28] 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 ±5%. The ratio of solubilization was calculated from the ratio of soluble protein concentration to the total protein concentration.

5. Protease Activity

The procedure followed to determine the activity of Alcalase was described by Lamas et al. [29] for measuring the proteolytic activity of pure cardosin A, and consists of slight modifications of the method initially proposed by Tomarelli et al. [30] 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 were 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.

6. 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, σ (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 sesame cake protein was performed with five commercial enzyme preparations (Alcalase, Neutrase, Flavourzyme, Protamex and PTN) in order to choose the 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 liter and approximately 0.35 AU units enzyme per gram of protein for each experiment. The results are given in Fig. 1. As can be seen, Alcalase and Protamex have stronger capability for hydrolysis compared to other enzyme preparations. On the other hand, at the end of 60 min of processing time, the base consumption for Alcalase and Protamex was 16.12 and 12.96 mmol L^{-1}, respectively. Therefore, Alcalase was chosen as proper enzyme preparation for further studies.

To investigate the effect of substrate concentration on hydrolysis and solubility of sesame cake protein and stability of Alcalase, experi-

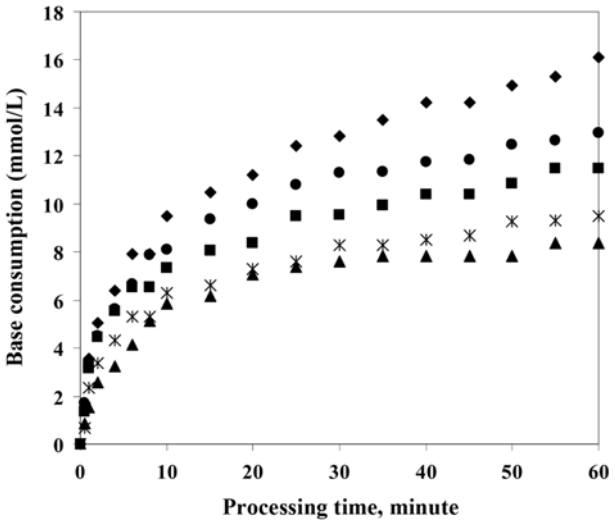

Fig. 1. Base consumption for various commercial enzyme preparations (0.2 mol L^{-1} KOH) vs. processing time, (▲ Flavourzyme,⋊ PTN, ◆ Alcalase, ● Protamex, ■ Neutrase), Experiments were performed at 50 °C, pH 7, 20 g L^{-1} substrate concentration with 0.35 AU of enzyme per g substrate.

ments were conducted at various substrate concentrations ranging between 10 and 30 g L^{-1} protein. Results are given in Figs. 2(a)-(c). As can be seen from Figs. 2(a) and 2(b), typical hydrolysis and solubilization curves were obtained in which the rates of hydrolysis and solubilization decrease sensibly with time after an initial rapid phase. The decreases observed in the rates could be attributed to one of the following phenomena: (a) the decrease in concentration of peptide bonds susceptible to hydrolysis by Alcalase, (b) with an approach to steady state as the degree of hydrolysis and solubilization tends toward a limit value which decreases with the initial substrate concentration, (c) possible inhibition of the enzyme caused by the hydrolysis products, and (d) enzyme inactivation [10, 22,27].

The effect of substrate concentration on enzyme stability is shown in Fig. 2(c). For all initial substrate concentration experiments, the residual enzyme activity values decreased sharply in 10 minutes, and then stabilized approximately at 60% until the end of the process, so that it may be concluded that strong substrate inactivation existed in 10 minutes. Same result was also obtained by Apar and Özbek [22] for the hydrolysis of corn gluten by Alcalase. However, the inactivation level seems to be the same for all substrate concentrations used, indicating that 10 g L^{-1} protein or the amount of any other content of sesame cake that caused denaturation is excess for inactivation, and using substrate concentrations above this value does not cause further inactivation. For these reasons, control experiments were performed to investigate the stability behavior of enzyme in sesame cake-free water and phosphate buffer solution and results presented in Fig. 3. As it can be seen, 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 does not depend on substrate inactivation at initial substrate concentration experiments; and considering the results given in Fig. 3, it can be concluded that after approx. 10 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 concentration could be depend on (a) the limitation of the reaction due to saturation of the enzyme with the

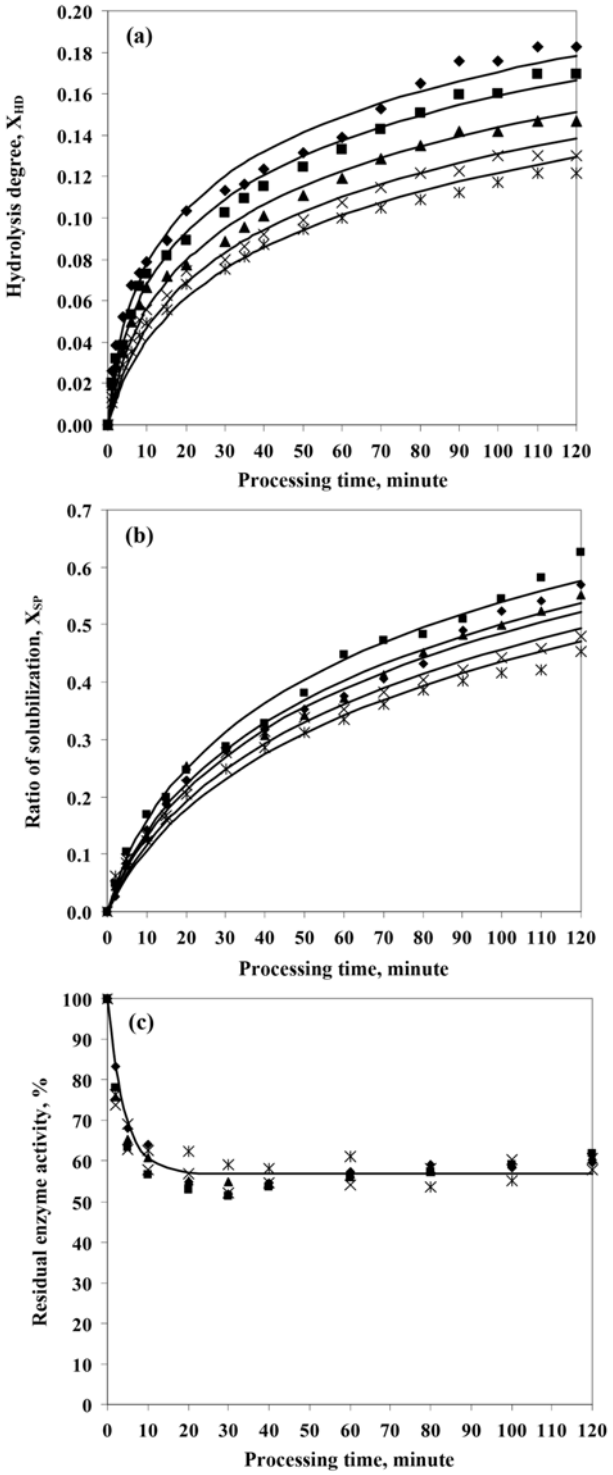

Fig. 2. At various substrate concentration; (a) Degree of hydrolysis vs. processing time, (b) Ratio of solubilization vs. processing time (c) Residual enzyme activity % vs. processing time, (◆ 10 g L^{-1}, ■ 15 g L^{-1}, ▲ 20 g L^{-1}, × 25 g L^{-1}, ✕ 30 g L^{-1}, — kinetic models), Experiments were performed at 50 °C, pH 7 with 2.5 ml L^{-1} enzyme concentration.

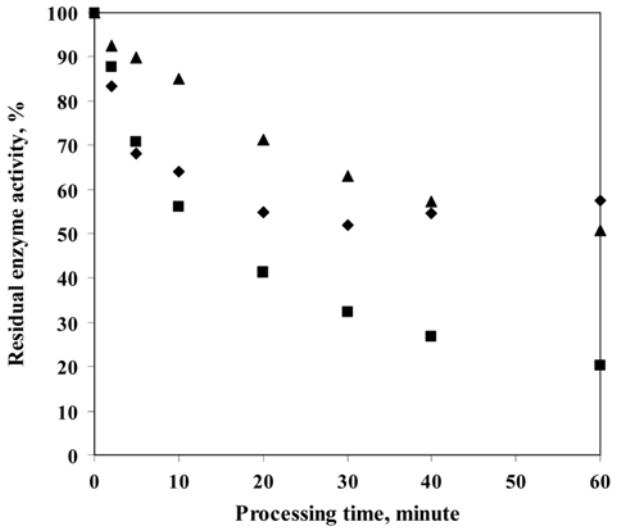

Fig. 3. Control experiments for enzyme stability, T=50 °C, pH= 7, γ_E=2.5 ml L^{-1} (■ in water, ▲ in phosphate buffer solution, ◆ in 1% substrate+water mix.).

substrate, (b) reducing water activity due to increasing the substrate amount, (c) mass transfer limitations as a result of increased viscosity, and (d) reversible enzyme inhibition that caused by substrate and/or products.

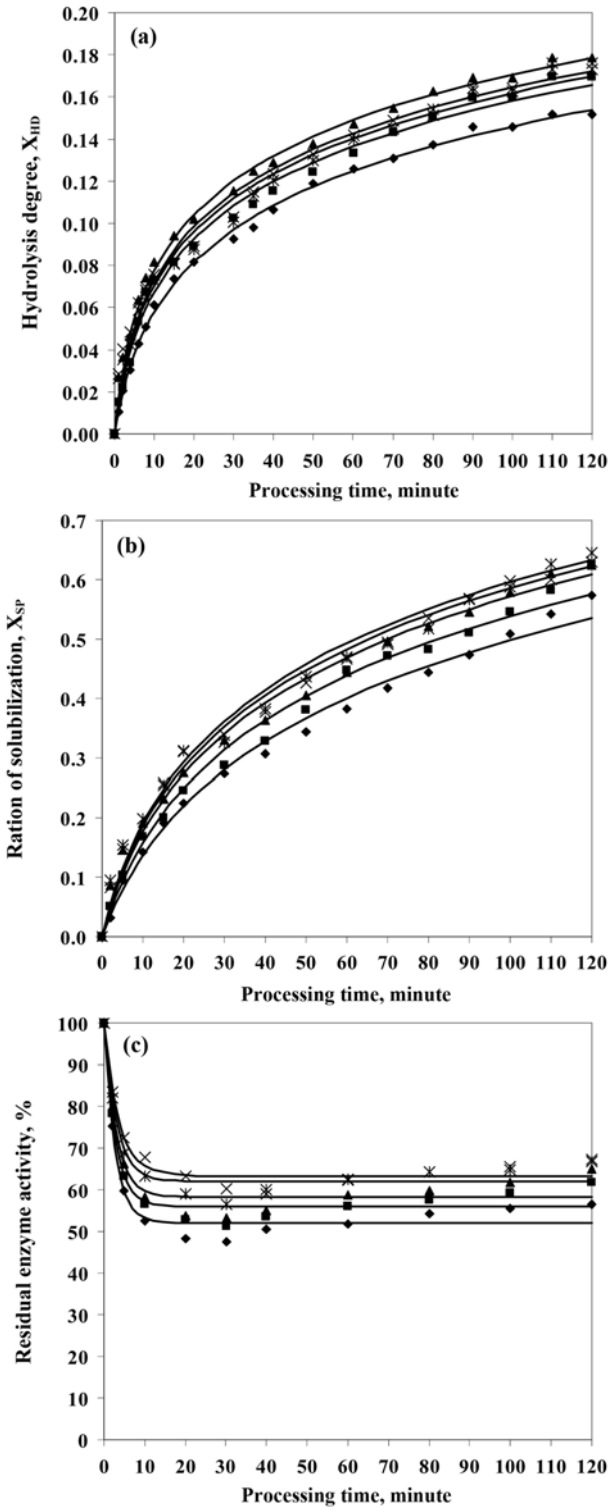

Fig. 4. At various enzyme concentration; (a) Degree of hydrolysis vs. processing time, (b) Ratio of solubilization vs. processing time, (c) Residual enzyme activity % vs. processing time, (◆ 2 ml L^{-1} , ■ 2.5 ml L^{-1}, ▲ 3 ml L^{-1}, × 3.5 ml L^{-1}, ✕4 ml L^{-1}, — kinetic models), Experiments were performed at 50 °C, pH 7, 15 g L^{-1} of substrate concentration.

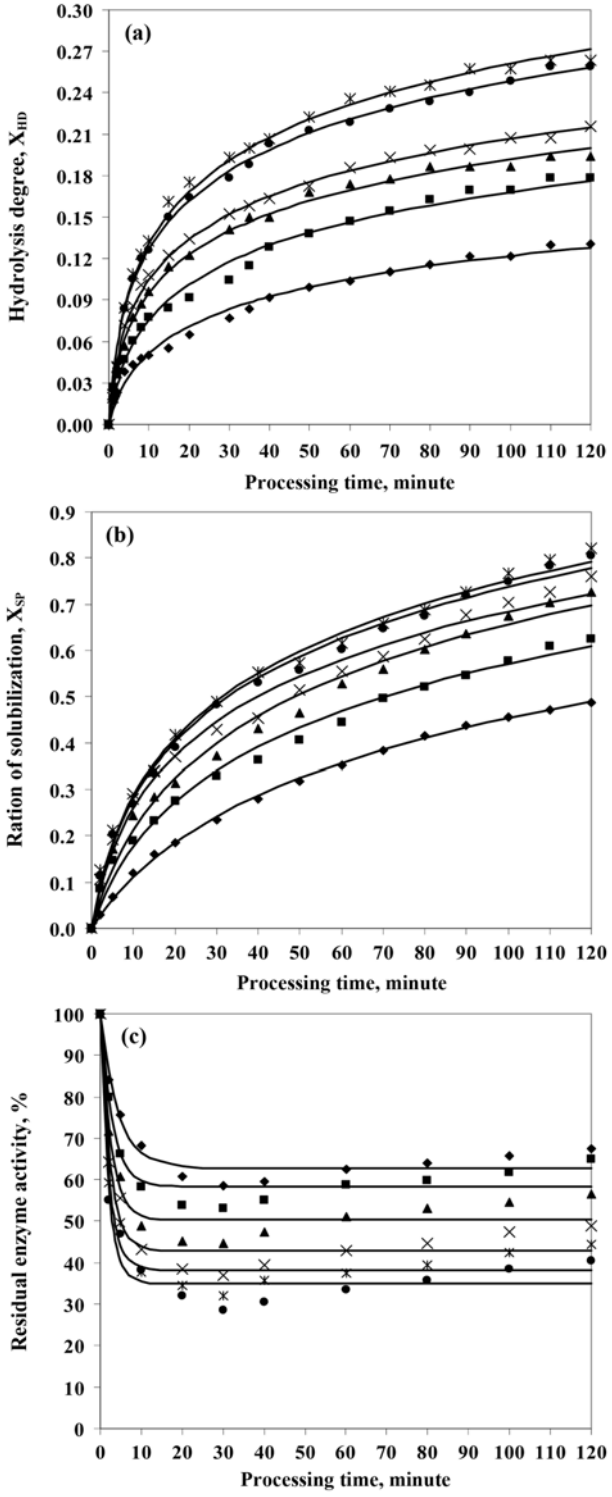

Fig. 5. At various pH values; (a) Degree of hydrolysis vs. processing time, (b) Ratio of solubilization vs. processing time, (c) Residual enzyme activity % vs. processing time, (◆ pH 6.5, ■ pH 7, ▲ pH 7.5, × pH 8, ✕ pH 8.5, ● pH 9, — kinetic models) Experiments were performed at 50 °C, 15 g L^{-1} of substrate concentration with 3 ml L^{-1} enzyme concentration.

The effect of enzyme concentration on hydrolysis and solubility of sesame cake protein and stability of Alcalase was investigated by using enzyme concentrations in the range of 2-4 ml L^{-1}, and the results given in Figs. 4(a)-(c). The degree of hydrolysis (Fig. 4(a)) and solubilization (Fig. 4(b)) was increased as the enzyme concentration increased, due to the increase on the reaction rate. However, after enzyme concentration of 3 ml L^{-1}, the rate of these increases was diminished, and the values of degree of hydrolysis and solublisation obtained were found nearly similar. This result could be explained by the saturation of the substrate with enzyme.

Fig. 4(c) shows the stability behavior of Alcalase with respect to time at various enzyme concentrations. The residual enzyme activity values again decreased sharply in 10 minutes and then stabilized. However, the enzyme stability increased with increasing the enzyme concentration.

The change in conformation of the whole substrate protein, as well of the enzyme itself affected by changes in pH may influence the access of a protease to particular pairs of amino acid residues within the substrate structure [31]. Hence, in order to investigate the effect of pH on hydrolysis and solubility of sesame cake protein and stability of Alcalase, experiments were conducted at various pH values ranging from 6.5 to 9. Results show that the optimum pH value for hydrolysis and solublization is 8.5 since the degree of hydrolysis and solubilization increased as pH increased up to this value (Figs. 5(a) and 5(b)). The degree of hydrolysis and solubilization values obtained at pH 9 was found nearly similar with those obtained at pH 8.5. The stability behavior of Alcalase with respect to time at various pH values is shown in Fig. 5(c). As can be seen, in the range of pH 6.5-8, there is a slight enzyme inactivation due to the increase on pH, but after the pH value of 8 the enzyme inactivation became stronger.

The influence of temperature on hydrolysis and solubility of sesame cake protein is presented in Figs. 6(a) and 6(b). The optimum temperature for hydrolysis and solublization of sesame cake protein was found to be 50 ºC as the highest degree of hydrolysis and solubilization obtained at this temperature. At 55 ºC, the degree of hydrolysis and solubilization values was slightly higher than the values obtained at 50 ºC, the values obtained for 55 ºC and 60 ºC were situated below those values obtained at 50 ºC. After 65 ºC, the degree of hydrolysis and solublization values became constant after 40 min as the enzyme completely inactivated.

The effect of temperature on enzyme stability is shown in Fig. 6(c). In the range of 45-55 ºC, there is a slight enzyme inactivation with respect to temperature. In this range, the enzyme lost its activity sharply in 10 minutes; and then maintained its stability until the end of the process. The irreversible enzyme inactivation became stronger at 60 ºC and enzyme completely inactivated at 65 ºC in 40 min.

Comparison of the values for 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 and pH. However, the proportional relation between the degree of hydrolysis and enzyme/substrate ratio was recorded for the hydrolysis of pea protein by Trypsin [13] and by Alcalase [17], for the hydrolysis of whey protein by MKC Protease 660 L and Alcalase 0.6 L [28], for the hydrolysis of lean meat protein by Alcalase [32], for the hydrolysis of hemoglobin by Alcalase [33], and for the hydrolysis of corn gluten by Alcalase [22]. On the other hand, the proportional relation between solubili-

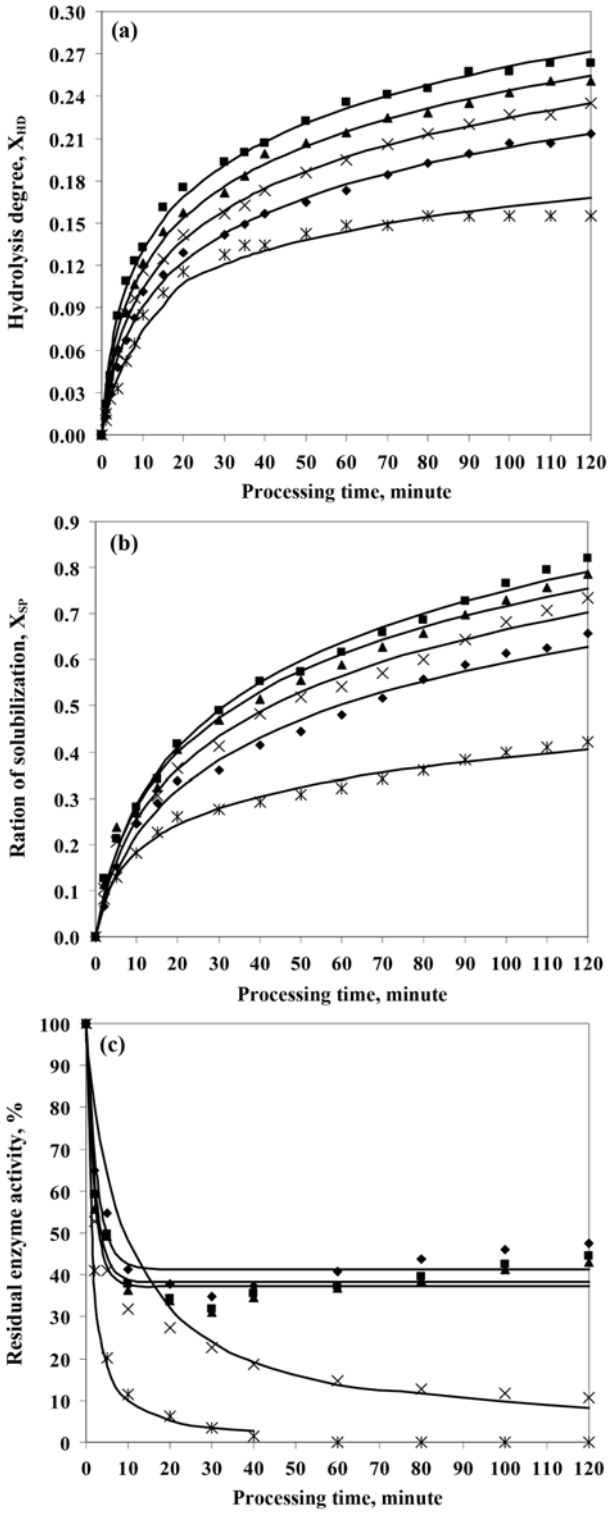

Fig. 6. At various temperature values; (a) Degree of hydrolysis vs. processing time, (b) Ratio of solubilization vs. processing time, (c) Residual enzyme activity % vs. processing time, (◆ 45 ºC, ■ 50 ºC, ▲ 55 ºC, × 60 ºC, ✕ 65 ºC, — kinetic models) Experiments were performed at pH 8.5, 15 g L^{-1} of substrate concentration with 3 ml L^{-1} enzyme concentration.

zation and enzyme/substrate ratio was recorded for the hydrolysis of soybean by a protease produced by *Penicillium duponti* K 1104 [10], for the hydrolysis of whey protein by trypsin [34], and for the hydrolysis of corn gluten by Alcalase [22]. Also, the optimum temperature and pH value obtained in this study for hydrolysis is consistent with the optimum pH and temperature range given for Alcalase [7].

1. Modelling Studies

In general, the reaction rates of enzymatic hydrolysis are characterized according to Michaelis-Menten kinetic models [10,27,35, 36]. However, there are also some studies that represent the kinetics with the empirical models [13,34] and simple exponential equations [17,27,33]. In the present study, evaluation of the results shows that the kinetics of hydrolysis and solubilization for all experiments performed could be represented by the following kinetic equation:

$$\frac{dX}{dt} = a \cdot \exp(-b \cdot X) \quad (4)$$

where X denotes conversion of hydrolysis or ratio of solubilization; and a and b are the parameters that have different values for the different experiments. The constants, standard error (σ) and R^2 statistic values for Eq. (4) were estimated for all experiments performed and given in Table 1. In agreement with previous studies [17,22, 27,33], 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 22.99 for hydrolysis and 4.52 for solubilization. Whereas, the parameter a increased when the initial enzyme concentration increased, and decreased when the initial substrate 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 was 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 0.9871 and standard error (σ) of 0.0191. The linear relationship between the degree of hydrolysis and solubility was also stated by Soral-Smietana et al. [16] for hydrolysis of pea protein by trypsin, by Bombara et al. [37] for the modification of wheat flour with protease and by Apar and Ozbek [22] for hydrolysis of corn gluten by Alcalase.

$$X_{DH} = 0.3048 X_{SP} + 0.0107 \quad (5)$$

Evaluation of the enzyme deactivation data shows that for all substrate, enzyme concentration and pH experiments and in 45-55 °C temperature the deactivation of Alcalase could be represented by a single step unimolecular non-first-order enzyme deactivation model

Table 1. Estimated constants, standard error (σ) and R^2 statistic values for Eq. (4)

	Hydrolysis				Solubilization			
γ_S/g L^{-1}	A	b	s	R^2	a	b	s	R^2
10	0.0216	22.99	0.0069	0.9922	0.0190	4.52	0.0178	0.9953
15	0.0164	22.99	0.0047	0.9959	0.0231	4.52	0.0207	0.9944
20	0.0114	22.99	0.0059	0.9918	0.0186	4.52	0.0161	0.9959
25	0.0084	22.99	0.0060	0.9896	0.0153	4.52	0.0191	0.9923
30	0.0067	22.99	0.0056	0.9896	0.0136	4.52	0.0196	0.9907
γ_E/ml L^{-1}								
2.0	0.0121	22.99	0.0091	0.9959	0.0189	4.52	0.0168	0.9958
2.5	0.0164	22.99	0.0031	0.9959	0.0231	4.52	0.0207	0.9944
3.0	0.0176	22.99	0.0032	0.9939	0.0271	4.52	0.0220	0.9937
3.5	0.0187	22.99	0.0034	0.9918	0.0290	4.52	0.0207	0.9946
4.0	0.0196	22.99	0.0046	0.9941	0.0296	4.52	0.0262	0.9914
pH								
6.5	0.0121	29.57	0.0044	0.9942	0.0144	4.40	0.0052	0.9995
7.0	0.0176	22.99	0.0032	0.9939	0.0271	4.52	0.0220	0.9937
7.5	0.0334	22.59	0.0036	0.9984	0.0342	4.14	0.0259	0.9937
8.0	0.0399	21.61	0.0035	0.9987	0.0509	4.70	0.0268	0.9936
8.5	0.0477	16.86	0.0062	0.9974	0.0562	4.29	0.0213	0.9966
9.0	0.0492	18.11	0.0058	0.9975	0.0552	4.38	0.0223	0.9961
T/°C								
45	0.0239	18.76	0.0045	0.9952	0.0411	5.24	0.0228	0.9941
50	0.0477	16.86	0.0062	0.9974	0.0562	4.29	0.0213	0.9966
55	0.0378	17.15	0.0055	0.9978	0.0576	4.63	0.0231	0.9956
60	0.0298	17.72	0.0076	0.9952	0.0497	4.84	0.0215	0.9956
65	0.0363	28.77	0.0095	0.9844	0.0566	10.58	0.0117	0.9958

(Eq. (6)) given by Sadana and Henley [38], 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 were fitted to the second order inactivation equation (Eq. (7)). Above these temperature values, the experimental results obtained confirmed that the enzyme inactivation became stronger (Figs. 6(c)), 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 (σ) and R^2 statistic values for Eqs. (6) and (7) are given in Table 2.

The inactivation energy of Alcalase for the temperature range of 45-55 °C can be determined by using the Arrhenius relationship:

$$\ln k_D=\ln k_{D0}-E_D/RT \quad (8)$$

The plot of $\ln k_D$ vs. T^{-1} is given in Fig. 7. As seen, the data accurately fit to Eq. (8). The value E_D was estimated as 25,544 J mol^{-1} with the standard error (σ) and R^2 statistic values of 0.03021 and 0.9897, respectively. This result is similar to that determined by Marquez and Vazquez [33], who reported that the inactivation energy for hydrolysis of hemoglobin by Alcalase is 25,353 J mol^{-1}.

Table 2. Estimated constants, standard error (σ) and R^2 statistic values for Eqs. (6) and (7)

γ_S/g L^{-1}	Equations	σ	R^2
10-60 g L^{-1}	A=(100−56.78)exp(−0.2408.t)+56.78	2.6382	0.9851
γ_E/ml L^{-1}			
2.0	A=(100−51.96)exp(−0.3647.t)+51.96	2.8352	0.9848
2.5	A=(100−55.90)exp(−0.3526.t)+55.90	3.0596	0.9794
3.0	A=(100−58.25)exp(−0.3277.t)+58.25	3.5832	0.9693
3.5	A=(100−61.92)exp(−0.3113.t)+61.92	2.9791	0.9745
4.0	A=(100−63.30)exp(−0.2748.t)+63.30	2.4985	0.9807
pH			
6.5	A=(100−62.83)exp(−0.2848.t)+62.83	2.9573	0.9747
7.0	A=(100−58.25)exp(−0.3277.t)+58.25	3.5832	0.9693
7.5	A=(100−50.29)exp(−0.3805.t)+50.29	4.1455	0.9696
8.0	A=(100−42.94)exp(−0.4118.t)+42.94	4.3307	0.9738
8.5	A=(100−38.25)exp(−0.4664.t)+38.25	4.2437	0.9781
9.0	A=(100−35.07)exp(−0.5016.t)+35.07	4.4873	0.9776
T/°C			
45	A=(100−41.33)exp(−0.3871.t)+41.33	4.4455	0.9743
50	A=(100−38.25)exp(−0.4664.t)+38.25	4.2437	0.9781
55	A=(100−37.21)exp(−0.5195.t)+37.21	4.7466	0.9732
60	1/A=1/96.15+0.00084.t	0.0084	0.9749
65*	1/A=1/101.52+0.0091.t	0.0089	0.9913

*For 0-40 min

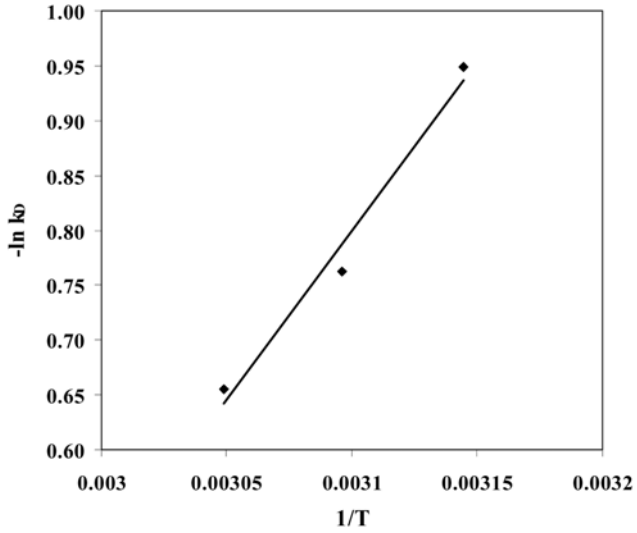

Fig. 7. Arrhenius plot for enzymatic inactivation determination.

CONCLUSIONS

To choose appropriate enzyme, sesame cake protein 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 sesame cake protein by Alcalase was performed at various substrate concentrations, enzyme concentrations, temperatures and pH values. The degree of hydrolysis and solubilization changed proportionally with enzyme/substrate mass ratio. The optimum substrate concentration, enzyme concentration, temperature and pH for hydrolysis and solubilization were obtained as 15 g L^{-1}, 3 ml L^{-1}, 50 °C and 8.5, respectively. At these conditions, the values of degree of hydrolysis and solubilization were found as 26.3% and 82.1%, and enzyme lost its activity by approx. 56% at the end of 120 min processing time.

Mathematical models were proposed to predict the degree of hydrolysis; solubilization and the residual enzyme activity were confirmed with the experimental results. For each experimental condition, a simple exponential function accurately represented the hydrolysis and solubilization data of sesame cake protein 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 substrates, enzyme concentrations and pH values were used and in 45-55 °C temperature range; represented by a single step unimolecular non-first-order enzyme deactivation model. However, above 55 °C, 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 45-55 °C was determined by using the Arrhenius relationship and found as 25,544 J mol^{-1}.

ACKNOWLEDGEMENTS

This research has been supported by Yıldız Technical Univer-

sity Scientific Projects Coordination Department (Project number: 29-07-01-01). Authors gratefully thank Novoyzmes and Necdet Bükey A.Ş. for their support. Elçin Demirhan gratefully acknowledges TUBITAK (Scientific and Technological Research Council of Turkey) for the scholarship.

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 [J 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]
T : temperature, °C [K]
t : processing time [min]
V_B : base volume consumption [ml]
X_{DH} : hydrolysis degree
X_{SP} : ratio of solubilization (soluble protein to total protein)

REFERENCES

1. E. K. Khalid, E. E. Babiker and A. H. El, *Food Chem.*, **82**, 361 (2003).
2. L. Nouri, J. Legrand, Y. Popineau and P. Belleville, *Chem. Eng. J.*, **65**, 187 (1997).
3. M. K. Schmidl, S. L. Taylor and J. A. Nordlee, *Food Technol.*, **48**(10), 77 (1994).
4. K. Bandyopadhyay and S. Ghosh, *J. Agric. Food Chem.*, **50**, 6854 (2002).
5. G. Chabanon, I. Chevalot, X. Framboisier, S. Chenue and I. Marc, *Process Biochem.*, **42**, 1419 (2007).
6. J. M. Kim, J. H. Whang, K. M. Kim, J. H. Koh and H. J. Suh, *Process Biochem.*, **39**, 989 (2004).
7. J. Alder-Nissen, Enzymic hydrolysis of food Proteins. Elsevier Applied Science Publishers, London and New York (1986).
8. J. Alder-Nissen, *J. Agric. Food Chem.*, **24**, 1090 (1976).
9. D. K. Apar and B. Ozbek, *J. Chem. Technol. Biotechnol.*, **82**, 1107 (2007).
10. A. Constantinides and B. Adu-Amankwa, *Biotechnol. Bioeng.*, **22**, 1543 (1980).
11. J. Adler Nissen, US Patent, 4,100,024 (1978).
12. J. Y. Lee, H. D. Lee and C. H. Lee, *Food Res. Int.*, **34**, 217 (2001).
13. M. Karamac, C. Amarowicz and H. Kostyra, *Czech J. Food Sci.*, **20**(1), 1 (2002).
14. M. C. Marquez and V. Fernandez, *Chem. Biochem. Eng. Q.*, **16**, 31 (2002).
15. J. M. Periago, L. M. Vidal, G. Ros, F. Rincon, C. Martinez, G. Lopez, J. Rodrigo and I. Martinez, *Food Chem.*, **63**, 71 (1998).
16. M. Soral-Smietana, R. Swigon, R. Amarowicz and L. Sijtsma, *Nahrung*, **42**, 217 (1998).
17. M. M. C. Moreno and F. V. Cuadrado, *Process Biochem.*, **28**, 481 (1993).
18. A. Raymundo, J. Empis and I. Sousa, *Food Res. Technol.*, **207**(2), 91 (1998).
19. H. Lqari, J. Pedroche, J. Giron-Calle, J. Vioque and F. Millan, *Grasas Y. Aceites*, **56**(2), 135 (2005).
20. S. R. Drago and R. J. Gonzalez, *Innovative Food Sci. Emerg. Technol.*, **1**, 269 (2001).
21. X. Kong, H. Zhou and H. Qian, *Food Chem.*, **101**, 615 (2007).
22. D. K. Apar and B. Ozbek, *Chem. Biochem. Eng. Q.*, **22**(2), 203 (2008).
23. E. M. Escamilla-Silva, S. H. Guzman-Maldonado, A. Cano-Medinal and G. Gonzalez-Alatorre, *J. Sci. Food Agric.*, **83**, 972 (2003).
24. A. Toshihiko Osawa, O. Keiko Nagai and N. Kyoko Shibuya, US Patent, 5,993,795 (1999).
25. C. Perez and R. Saad, *Archivos Latinoamericanos De Nutrition*, **34**(4), 735 (1984).
26. F. S. Taha, M. A. Ibrahim and E. A. El-Zanaty, *Grasa y Aceites*, **53**(3), 267 (2002).
27. P. Gonzalez-Tello, F. Camacho, E. Jurado, M. P. Paez and E. M. Guadix, *Biotechnol. Bioeng.*, **44**, 523 (1994).
28. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
29. E. Lamas, R. Barros, V. Balcao and F. X. Malcata, *Enzyme Microb. Technol.*, **28**, 642 (2001).
30. R. Tomarelli, M. Charney and M. Harding, *J. Lab. Clin. Med.*, **34**, 428 (1949).
31. R. M. Barros and F. X. Malcata, *Food Chem.*, **88**, 351 (2004).
32. G. M. O'Meara and P. A. Munro, *Biotechnol. Bioeng.*, **27**, 861, (1985).
33. M. C. Marquez and M. A. Vazquez, *Process Biochem.*, **35**, 111 (1999).
34. A. Margot, E. Flaschel and A. Renken, *Process Biochem.*, **32**, 217 (1997).
35. R. Sousa, G. P. Lopes, P. W. Tardioli, R. L. C. Giordano, P. I. F. Almeida and R. C. Giordano, *Brazilian J. Chem. Eng.*, **2**, 147 (2004).
36. P. W. Tardioli, R. Sousa, R. C. Giordano and R. L. C. Giordano, *Enzyme Microbial Technol.*, **36**, 555 (2005).
37. N. Bombara, M. C. Anon and A. M. R. Pilosof, *Lebensm.-Wiss. u-Technol.*, **30**, 441 (1997).
38. A. Sadana and J. P. Henley, *Biotechnol. Bioeng.*, 30, 717 (1987).