Research Article / Araştırma Makalesi
pH-STAT CALIBRATION FOR SESAME CAKE PROTEIN HYDROLYSIS BY ALCALASE

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ABSTRACT

In the present study, pH-stat method was calibrated by TNBS and OPA reactions for control of hydrolysis of sesame cake protein by Alcalase. Hydrolysis experiments were conducted in a batch reactor at the temperature of 50°C for various pH values, and at pH 7.5 for the temperatures of 40°C and 60°C. The calibration was characterized by good linear correlations for both methods. The mean pK values of sesame cake protein hydrolysates were obtained as 6.88, 6.65 and 6.42 for 40, 50 and 60°C, respectively.

Keywords: Sesame cake protein, hydrolysis, Alcalase, pH-stat, pK.

1. INTRODUCTION

Plant proteins, extracted from abundant and renewable biological resources, play significant roles in human nutrition [1,2]. Protein hydrolysis has been shown to produce interesting effects on their technological properties such as solubility, foaming, emulsifying and gelation [2,3]. It is possible to improve functional properties of proteins by chemical or enzymatic modifications. However, the use of enzymes provides milder process condition and allows for a selective hydrolysis of protein [4].

Sesame seed is the one of the main sources of edible oil as it has an oil content of between 48 and 55%. It is also a good source of protein. One of the principal characteristics of this protein is its high methionine and tryptophan content [5]. The sub-product of the oil extraction process is sesame cake has a protein content which can reach 50% depending on the

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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 [6,7].

In protein hydrolysis, the key parameter for monitoring the reaction is the degree of hydrolysis. Several methods of monitoring the degree of hydrolysis have been described in the literature, for example pH-stat, osmometry, soluble nitrogen content, thtrinitro-benzene-sulfonic acid (TNBS) and o-phthaldialdehyde (OPA) [8-10].

Determination of the degree of hydrolysis by colorimetric methods is time consuming. The pH-stat is by far the most convenient method and measures the rate of hydrolysis by quantifying the release of protons by titration. By measuring the amount of base added during the hydrolysis, it is possible to measure the rate of hydrolysis. However, to establish the relationship between the base consumption and degree of hydrolysis, it is essential to find out the mean pK value of the amino groups released during the hydrolysis process. In the literature, the calibration of pH-stat with TNBS reaction for hydrolysis of pea protein isolate [11], hydrolysis of casein and whey proteins [12]; and hydrolysis of corn gluten [13] have been studied. However, there has not been any study in the literature about the calibration of pH-stat for the hydrolysis sesame cake protein. Therefore, the aim of the present work was to calibrate the pH-stat by determining the mean pK values of the sesame cake protein hydrolysates at different temperatures and pH values for control of the hydrolysis reaction by Alcalase.

2. MATERIALS AND METHODS

Sesame cake used in this study which contains 37.8 % protein was obtained from Necdet Bükey A.Ş. The enzyme used in this work was Alcalase 2.4 L, a bacterial endo-peptidase produced by Bacillus Licheniformis, obtained from Novozymes.

Hydrolysis experiments were carried out in a 200 ml jacked reactor with magnetic stirring and pH and temperature control. During the hydrolysis the pH was maintained by the addition of 0.2 N KOH. In this study, all experiments were carried out at least in duplicate and the deviation between trials was within ±5%. For determination of the free α-amino groups both TNBS [8] and OPA [10] methods were used. For each sample, the assays were carried out in triplicate and their averages were taken.

2.1. Theory of Calibration

The equation which relates $H_D$ to base consumption is:

$$H_D = B \times N_b \times \frac{1}{\alpha_\alpha} \times \frac{1}{P_M} \times \frac{1}{h_{tot}} \times 100\%$$

(1)

$B$: base consumption in ml (or L), $N_b$: normality of the base $\alpha$: average degree of dissociation of the α-NH groups, $P_M$: mass of protein in g (or Kg), $h_{tot}$: total number of peptide bonds in meq/g protein, $H_D$: hydrolysis degree %

$\alpha^{-1}$ is the calibration factor for the pH-stat, and it is the reciprocal of the degree of dissociation;

$$\alpha = \frac{10^{pH-pK}}{1 + 10^{pH-pK}}$$

(2)

For the calibration of the pH-stat; the amount of base consumed during hydrolysis was recorded. The samples of hydrolysates were taken at timed intervals and L-leucine and L-serine equivalents were determined by TNBS and OPA reactions respectively. After correlation of the base consumption with L-leucine and L-serine equivalents, slopes (b) of the straight lines were
obtained. Then, the pK values were calculated from the following equations given by Adler Nissen (1986):[9]

\[
pK = \frac{pH_2 + \log(b_1 - b_2) - \log(10^{pH_2 - pH_1} \times b_2 - b_1)}{b_1 - b_2}
\]

(3)

\[
pH_2 > pH_1, \quad b_1 \text{ and } b_2 \text{ are the slopes which correspond to } pH_1 \text{ and } pH_2.
\]

\[
pK = pH + \log \left[ \frac{b}{b_0} \times (1 + 10^{pK_0 - pH}) - 1 \right]
\]

(4)

\[b \text{ and } b_0 \text{ measured at same pH.}\]

3. 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

\[
SSR = \sum_{m=1}^{N_d} (c_{m}^{obs} - c_{m}^{cal})^2
\]

where m 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:

\[
\sigma^2 \approx s^2 = \frac{(SSR)_{min}}{(m - p)}
\]

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

4. RESULTS AND DISCUSSION

The mean pK value of the amino groups released during the hydrolysis process was first determined by Adler-Nissen (1986) by comparing the base consumption with the amino groups released. Since then, most researchers in this field have used these results for any substrates [3,13-17]. In the present study, pH-stat method was calibrated by TNBS and OPA reactions for control of hydrolysis of sesame cake protein by Alcalase. The hydrolysis reactions were carried out for 60 minutes at 1\% (w/v) protein concentration with addition of 0.25 \% (v/v) enzyme at 50°C for pH values of 6.5, 7, 7.5, 8 and at the pH value of 7.5 for the temperatures of 40°C and 60°C.

The relationship between consumption of base and content of amino groups determined both by TNBS and OPA reactions were given in Figures 1-2 and Figures 3-4, respectively. As can be seen from these figures, the calibration of pH-stat with TNBS and OPA reactions were characterized by good linear correlations that gave values for the coefficient of determination of greater than 0.9913 and 0.9919; and standard errors lower than 0.0413 and 0.0345, respectively.
Figure 1. Calibration of pH-stat with TNBS reaction at various pH values
(◊ pH 6.5, □ pH 7, Δ pH 7.5, Ж pH 8, — models)

Figure 2. Calibration of pH-stat with TNBS reaction at various temperature values
(◊ 40°C, Δ 50°C, □ 60°C, — models)

Similar high coefficient of determination values were reported in the literature for the calibration of pH-stat with TNBS reaction for hydrolysis of pea protein isolate [11], hydrolysis of casein and whey proteins [12]; and hydrolysis of corn gluten [13]. However poor correlations were reported by Spellman et al. (2003) between the methods of pH-stat, TNBS and OPA for Debitrase HYW20 whey protein hydrolysates.
The extent of the hydrolysis reaction increased with the increase on temperature and pH as the base consumption and amino group concentration increased with pH (Figs 1 and 3) and temperature (Figs 2 and 4). At the end of 60 minutes of processing time; at pH 8 (at T=50°C), 2.01 meqv/g protein base consumption was obtained with respect to 1.54 meqv Leusine-NH\(_2\)/g protein and 1.68 meqv Serine-NH\(_2\)/g protein; and at 60°C (at pH 7.5), 1.6 meqv/g protein base consumption was obtained with respect to 1.5 meqv Leucine-NH\(_2\)/g protein and 1.62 meqv Serine-NH\(_2\)/g protein.
The slopes obtained from the linear correlations of base consumption and concentration of free amino groups; and calculated pK values for sesame cake protein hydrolysates were given in Tables 1 and 2. As can be seen from these tables, the pK values obtained as a result of the calibration performed by TNBS and OPA reactions were found almost same. This result was not surprised as the calibration of pH-stat with both methods was characterized by good linear correlations. Nielsen et al. (2001) and Apar and Özbek (2010) have also reported high correlation between the TNBS and OPA method for soy protein and corn gluten hydrolysates. Hence, considering all the results, the mean pK values of sesame cake protein hydrolysates were obtained as 6.88, 6.65 and 6.42 for 40, 50 and 60°C respectively.

The variation on pK with temperature could be calculated from Gibss-Helmholtz equation. The ionisation enthalpy of the amino group was reported as 45 kJ/mol by Steinhart & Beychok (1964) [18]. By inserting this value in Gibss-Helmholtz equation, it was found that for a change of 10°C, pK will change about 0.23 pH units. In the present study, the difference between the pK values obtained for 40 and 50°C is 0.23 pH units, and the difference between the pK values obtained for 50 and 60°C is 0.23 pH units. These results confirm the reliability of the study.

Table 1. Estimated pK values (by using Equation 3) for the reactions performed at different pH values (T = 50°C)

<table>
<thead>
<tr>
<th>Data for the calibration by TNBS reaction</th>
<th>Data for the calibration by OPA reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 1</td>
<td>pH 2</td>
</tr>
<tr>
<td>6.5</td>
<td>7</td>
</tr>
<tr>
<td>7</td>
<td>7.5</td>
</tr>
<tr>
<td>7.5</td>
<td>8</td>
</tr>
<tr>
<td>6.5</td>
<td>7.5</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>6.5</td>
<td>8</td>
</tr>
<tr>
<td>Mean Value</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Estimated pK values (by using Equation 4) for the reactions performed at different temperatures (pH = 7.5)

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Data for the calibration by TNBS reaction</th>
<th>Data for the calibration by OPA reaction</th>
<th>mean pK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope (b)</td>
<td>pK</td>
<td>Slope (b)</td>
</tr>
<tr>
<td>40°C</td>
<td>0.9313</td>
<td>6.87</td>
<td>1.0327</td>
</tr>
<tr>
<td>50°C</td>
<td>0.8581</td>
<td>6.64</td>
<td>0.9462</td>
</tr>
<tr>
<td>60°C</td>
<td>0.8149</td>
<td>6.41</td>
<td>0.8975</td>
</tr>
</tbody>
</table>
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REFERENCES / KAYNAKLAR

