CHAPTER-III
CONVERSION OF ALLIIN TO
AL LICIN
CONVERSION OF ALLIIN TO ALICIN

Natural products have provided useful templates as leads to establish chemical structures as well as to understand chemical transformations. A garlic amino acid, alliin (S-allyl-L-cysteine sulfoxide), undergo enzymatic transformations on crushing garlic. It rearranges spontaneously to form allicin (diallyl thiosulfinate). Mechanism of which is discussed in chapter 1.

The pH of garlic is 6.0. Its enzyme alliinase reported to have isoelectric pH of 4.0 by Stoll and Seebeck\(^1\), 6.2 by Kazaryan and Goryachenkova\(^2\) and 4.9 by Jansen and Mueller\(^3\). Stoll and Seebeck used cell free garlic enzyme solution and observed fairly constant activity of this enzyme in the extract form in pH range 5-8, which declined abruptly with drop in pH to 4.0 and increase in pH to 9.0. These workers reported 37 °C as optimum temperature for activity of this enzyme. Mazelis and Crews\(^4\) obtained a six fold purification of enzyme using protamine and ammonium sulfate as precipitating agent followed by fractionation on G-200 Sephadex and observed optimum pH for activity of this purified preparation of alliinase with S-methyl-L-cysteine sulfoxide as substrate, to be 6.5. Nock and Mazelis\(^5\) reported optimum pH of 6.0 for activity of purified form of this enzyme prepared by them. Yu and Wu\(^6\) also reported that enzymatic formation of allicin from alliin was favoured around pH 6.5. Sun and Gao\(^7\) reported optimum pH of 6.4 for activity of a purified preparation of alliinase from garlic juice prepared
Lawson and Hughes reported effect of pH, time and temperature on formation of allicin and other thiosulfinates from garlic powder and garlic cloves. These workers observed that all dipropenyl (allicin, 1-propenylallyl, and allyl-1-propenyl) were formed at an optimum pH of 4.5-5.0. The methyl propenyl thiosulfinates (allyl methyl + methyl allyl and 1-propenyl methyl + methyl-1-propenyl) and dimethyl thiosulfinate were optimally formed at pH 6.5-7.0 and 5.5, respectively. Below pH 3.6, no thiosulfinates were formed. These workers also opined that alliinase could be completely and irreversibly inhibited by the acidic conditions found in stomach as neutralization of garlic previously incubated at pH < 3 failed to restore thiosulfinate generation from it. These workers further observed that the dipropenyl thiosulfinates were completely formed in 0.3 min at 37 °C, while the methyl thiosulfinates were not completely formed until 3.5 min. Allyl-1-propenyl thiosulfinate was most rapidly formed and the most unstable thiosulfinate. The stability of dipropenyl thiosulfinate was improved at pH ≤ 4.5.

Kazaryan and Goryachenkova obtained pure preparation of alliinase and found its molecular weight as 1,30,000. It consists of two subunits. Approximate six molecules of firmly bound pyridoxal phosphate were determined per mol. of purest enzyme (four equivalents were apparently bound non specifically outside the active site). Nock and Mazelis determined molecular weight of their enzyme preparation as 85,000 by gel filtration. The molecule consisted of two equal subunits of 42,000 daltons. Chemical and spectral studies demonstrated it to
have 2 moles of pyridoxal-5-phosphate per alliinase molecule. Nock and Mazelis\textsuperscript{9} further suggested that garlic holoenzyme, alliinase, has dimeric structure and is a glycoprotein. Jansen and Mueller\textsuperscript{3} characterised the enzyme protein by relative molecular mass of 1,08,000 and found it consisting of two equal subunits. These reports indicate that alliinase occurs in either isoenzymic forms or varies in its characteristics depending upon the variety or place of origin of garlic studied.

Kazarayan et al.\textsuperscript{10} studied the effect of various inhibitors on the activity of highly purified alliinase. Hydroxylamine and its O-substituted derivatives were found to be reversible inhibitors. It was also inhibited by D- and L- cycloserine; the L- form being more effective. Among the substrate tested, \( \beta \)-cyano-L-alanine was more effective: specific and irreversible inhibitor. Alliinase was reported to be inhibited by aminothiol but exhibited low sensitivity towards thiol reagents. Jansen et al.\textsuperscript{11} characterized purified alliinase under addition of different substrates, inhibitors, and reducing agents by kinetic and spectral data. Hydroxylamine sulfate (50 µM) inhibited alliinase activity by approximately 90%. Rotenone revealed similar effect at concentration of 10 nM. Spectral studies also confirmed the existence of an unknown flavin coenzyme with purified alliinase enzyme\textsuperscript{11}. Mitsui\textsuperscript{12} reported enhancement of activities of yeast protease, bacterial lipase, malt amylase and E. coli enzyme by addition of garlic powder or its extract with water, ethanol, methanol, acetic acid or acetone.
This chapter pertains to the conversion of alliin to allicin which has been studied in detail in this research. Alliin, on its decomposition, gives pyruvic acid as one of the products. Therefore, the rate of conversion of alliin to allicin has been studied by estimating pyruvic acid at different time intervals as described in chapter II. The standard plot of (absorbance at 455 nm) versus concentration of sodium pyruvate was established after optimizing concentrations of the reagents.

3.1 Setting of Optimum Conditions for the Method

The effect of variation of concentration of the reagents employed, viz., salicyldehyde as well as solutions of sodium hydroxide and sodium pyruvate in this analytical method has been studied against distilled water to achieve the optimum conditions for colour development and its spectroscopic measurement. The results of the effects of variation of sodium hydroxide, salicylaldehyde and temperature are given in Tables 3.1, 3.2 and 3.3, respectively. It was observed that in a total 50 ml reaction-mix, an aliquot of 15 ml of 25% sodium hydroxide was required for getting the maximum absorbance at 455 nm (Table 3.1). It was also observed that an increasing amount of salicylaldehyde from 0.05 ml to 0.7 ml in the reaction-mix increases its absorbance; however, the Beer’s law was observed to be valid in this analytical procedure for upto about 0.2 ml of salicylaldehyde (Fig. 3.1). Therefore, the pyruvic acid in the experimental samples was measured using 15 ml of 25% sodium hydroxide and 0.2 ml of salicylaldehyde throughout this work.
Table 3.1: Effect of sodium hydroxide concentration on determination of sodium pyruvate; Temperature = 30°C; Total volume of reaction - mix = 50 ml.

<table>
<thead>
<tr>
<th>Sodium pyruvate (gm/l) x 10^5</th>
<th>NaOH (25%) (ml.)</th>
<th>Salicylaldehyde * (ml.)</th>
<th>Absorbance at 455 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5</td>
<td>0.2</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.2</td>
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</tr>
<tr>
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<td>0.90</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.2</td>
<td>0.85</td>
</tr>
</tbody>
</table>

*Density = 1.164 - 1.167 gm/ml; refractive index = 1.5730 - 1.5740 at 20°C.
Table 3.2: Effect of Salicylaldehyde on the determination of sodium pyruvate; Temperature = 30°C; Total volume of reaction - mix = 50 ml.

<table>
<thead>
<tr>
<th>Sodium pyruvate (gm/ltr) x 10^5</th>
<th>NaOH(25%) (ml.)</th>
<th>Salicylaldehyde (ml.)</th>
<th>Absorbance at 455 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
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<td>0.05</td>
<td>0.20</td>
</tr>
<tr>
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<td>15</td>
<td>0.10</td>
<td>0.38</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.20</td>
<td>0.74</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
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<td>1.05</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.50</td>
<td>1.15</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.70</td>
<td>1.25</td>
</tr>
</tbody>
</table>
Table 3.3: Effect of temperature on the determination of Sodium pyruvate; Total volume of reaction = mix = 50 ml.

<table>
<thead>
<tr>
<th>Sodium pyruvate (gm/l) x 10^5</th>
<th>NaOH (25%) (ml.)</th>
<th>Salicylaldehyde (ml.)</th>
<th>Temperature (°C)</th>
<th>Absorbance at 455 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>15</td>
<td>0.2</td>
<td>20</td>
<td>0.80</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.2</td>
<td>30</td>
<td>0.90</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.2</td>
<td>40</td>
<td>0.85</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.2</td>
<td>50</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.2</td>
<td>75</td>
<td>0.56</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>0.2</td>
<td>100</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Figure 3.1: Effect of Salicylaldehyde on absorbance; [Sodium pyruvate] = $5 \times 10^{-5}$ gm/lt; NaOH (25%) = 15ml; Total volume of reaction - mix = 50 ml; Temperature = 30°C
The determination of rate of decomposition of alliin to allicin in the experimental samples of garlic extract has also been described in chapter II (2.22). The effect of incubation temperature, acetic acid, rectified spirit/ethanol, and pH has been studied on the decomposition or enzymic conversion of alliin to allicin and its results are reported in this chapter.

3.2 Effect of Incubation Temperature

The amounts of pyruvic acid formed in garlic macerates, obtained by macerating de-skinned garlic cloves with distilled water incubated at different temperatures from 30-50 °C, as a function of time, were determined by using 1 ml, 2 ml, and 3 ml filtered extracts are reported in Tables 3.4-3.7.

The plots of [pyruvic acid] versus incubation time at different incubation temperatures are represented graphically in Figures 3.2-3.5. These plots also exhibited a trend similar to those depicted by plots of effects of rectified spirit and pH. The rates were faster initially but decreased for incubation periods exceeding 60 min. Therefore, the rate constants ($K_{obs.}$) for formation of pyruvic acid i.e. rate of decomposition of alliin to allicin have been calculated from the slopes of linear portion of the plots. The values of rate constants are summarised in Table 3.8.
Table 3.4: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in distilled water and incubated for upto 120 minutes at 30°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract = 1 ml 10^3 gms/litre</th>
<th>Garlic extract = 2 ml 10^3 gms/litre</th>
<th>Garlic extract = 3 ml 10^3 gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[Pyruvic acid]</td>
<td>[Pyruvic acid]</td>
<td>[Pyruvic acid]</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.05 1.545</td>
<td>0.10 3.024</td>
<td>0.18 5.390</td>
</tr>
<tr>
<td>30</td>
<td>0.13 3.911</td>
<td>0.25 7.460</td>
<td>0.35 10.418</td>
</tr>
<tr>
<td>45</td>
<td>0.20 5.981</td>
<td>0.42 12.488</td>
<td>0.53 15.742</td>
</tr>
<tr>
<td>60</td>
<td>0.29 8.643</td>
<td>0.56 16.629</td>
<td>0.67 19.882</td>
</tr>
<tr>
<td>75</td>
<td>0.31 9.235</td>
<td>0.60 17.812</td>
<td>0.70 20.770</td>
</tr>
<tr>
<td>90</td>
<td>0.32 9.531</td>
<td>0.62 18.403</td>
<td>0.76 22.544</td>
</tr>
<tr>
<td>120</td>
<td>0.34 10.122</td>
<td>0.63 18.699</td>
<td>0.78 23.136</td>
</tr>
</tbody>
</table>
Table 3.5: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in distilled water and incubated for upto 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract = 1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract = 2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract = 3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00 -</td>
<td>0.00 -</td>
<td>0.00 -</td>
</tr>
<tr>
<td>15</td>
<td>0.13 $3.911$</td>
<td>0.14 $4.207$</td>
<td>0.23 $6.869$</td>
</tr>
<tr>
<td>30</td>
<td>0.26 $7.756$</td>
<td>0.33 $9.826$</td>
<td>0.42 $12.488$</td>
</tr>
<tr>
<td>45</td>
<td>0.35 $10.418$</td>
<td>0.50 $14.854$</td>
<td>0.63 $18.699$</td>
</tr>
<tr>
<td>60</td>
<td>0.50 $14.854$</td>
<td>0.68 $20.178$</td>
<td>0.83 $24.615$</td>
</tr>
<tr>
<td>75</td>
<td>0.54 $16.037$</td>
<td>0.73 $21.657$</td>
<td>0.87 $25.798$</td>
</tr>
<tr>
<td>90</td>
<td>0.55 $16.333$</td>
<td>0.74 $21.953$</td>
<td>0.90 $26.685$</td>
</tr>
<tr>
<td>120</td>
<td>0.56 $16.629$</td>
<td>0.76 $22.544$</td>
<td>0.92 $27.276$</td>
</tr>
</tbody>
</table>
Table 3.6: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in distilled water and incubated for upto 120 minutes at 40°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract = 1 ml</th>
<th>Garlic extract = 2 ml</th>
<th>Garlic extract = 3 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$10^3$ gms/litre</td>
<td>$10^3$ gms/litre</td>
<td>$10^3$ gms/litre</td>
</tr>
<tr>
<td>0</td>
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<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.07</td>
<td>0.13</td>
<td>0.20</td>
</tr>
<tr>
<td>30</td>
<td>0.15</td>
<td>0.28</td>
<td>0.37</td>
</tr>
<tr>
<td>45</td>
<td>0.23</td>
<td>0.43</td>
<td>0.56</td>
</tr>
<tr>
<td>60</td>
<td>0.32</td>
<td>0.54</td>
<td>0.70</td>
</tr>
<tr>
<td>75</td>
<td>0.33</td>
<td>0.68</td>
<td>0.78</td>
</tr>
<tr>
<td>90</td>
<td>0.34</td>
<td>0.69</td>
<td>0.79</td>
</tr>
<tr>
<td>120</td>
<td>0.36</td>
<td>0.71</td>
<td>0.81</td>
</tr>
</tbody>
</table>

Notes: O.D. = Optical Density
Table 3.7: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in distilled water and incubated for upto 120 minutes at 50°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O.D.</td>
<td>O.D.</td>
<td>O.D.</td>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.06 1.841</td>
<td>0.10 3.024</td>
<td>0.14 4.207</td>
</tr>
<tr>
<td>30</td>
<td>0.12 3.615</td>
<td>0.20 5.981</td>
<td>0.27 8.052</td>
</tr>
<tr>
<td>45</td>
<td>0.17 5.094</td>
<td>0.29 8.643</td>
<td>0.42 12.488</td>
</tr>
<tr>
<td>60</td>
<td>0.23 6.869</td>
<td>0.40 11.897</td>
<td>0.56 16.629</td>
</tr>
<tr>
<td>75</td>
<td>0.26 7.756</td>
<td>0.42 12.488</td>
<td>0.59 17.516</td>
</tr>
<tr>
<td>90</td>
<td>0.27 8.052</td>
<td>0.45 13.376</td>
<td>0.61 18.108</td>
</tr>
<tr>
<td>120</td>
<td>0.29 8.643</td>
<td>0.47 13.967</td>
<td>0.63 18.699</td>
</tr>
</tbody>
</table>
Figure 3.2: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in distilled water and incubated at 30°C for up to 120 min.
Figure 3.3: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in distilled water and incubated at 35°C for upto 120 min.
Figure 3.4: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in distilled water and incubated at 40°C for up to 120 min.
Figure 3.5: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in distilled water and incubated at 50°C for upto 120 min.
Table 3.8: The values of rate constants ($K_{\text{obs}}$) at various incubation temperature in distilled water obtained from slopes of figures 3.2-3.5

<table>
<thead>
<tr>
<th>Experimental Garlic extract (ml.)</th>
<th>Incubation Temperature</th>
<th>$\langle -- (K_{\text{obs}}) 10^4 \text{ gm/lt. min.} --\rangle$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30°C</td>
<td>2.414</td>
</tr>
<tr>
<td>2</td>
<td>35°C</td>
<td>3.529</td>
</tr>
<tr>
<td>3</td>
<td>40°C</td>
<td>2.772</td>
</tr>
<tr>
<td>3</td>
<td>50°C</td>
<td>1.104</td>
</tr>
</tbody>
</table>
It is apparent from the results (Tables 3.4-3.7 and Figures 3.2-3.5) that the rate constants for formation of pyruvic acid or conversion of alliin to allicin were temperature dependent. The rate constants ($K_{\text{obs.}}$) were high for 35°C than those for 30°C, 40°C and 50°C suggesting optimum temperature for alliinase at about a temperature of 35°C. This rate constant shall serve as reference point for assessment of effects of 5-30% acetic acid, 5-30% rectified spirit/ethanol, and buffers of pH 4.0-6.0 used as macerating medium.

3.3 Effect of Acetic Acid

The amounts of pyruvic acid formed in garlic macerates, obtained by macerating de-skinned garlic cloves with solutions of acetic acid of 5-30% strengths, as a function of time, were determined by using 1 ml, 2 ml, and 3 ml filtered extracts are reported in Tables 3.9-3.12.

The plots of [pyruvic acid] versus incubation time in presence of various initial acetic acid concentrations are represented graphically in Figures 3.6-3.9. These plots are linear and, therefore, the rate constants ($K_{\text{obs.}}$) for the formation of pyruvic acid, i.e., rate of decomposition of alliin to allicin, have been evaluated from the slopes of these straight lines. The values of rate constants ($K_{\text{obs.}}$) are summarised in Table 3.13.
Table 3.9: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 5% acetic acid and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract = 1 ml [Pyruvic acid] 10^3 gms/litre</th>
<th>Garlic extract = 2 ml [Pyruvic acid] 10^3 gms/litre</th>
<th>Garlic extract = 3 ml [Pyruvic acid] 10^3 gms/litre</th>
</tr>
</thead>
<tbody>
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<td>0.00 -</td>
</tr>
<tr>
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<td>0.14 4.207</td>
<td>0.19 5.686</td>
<td>0.14 4.207</td>
</tr>
<tr>
<td>30</td>
<td>0.17 5.094</td>
<td>0.30 8.939</td>
<td>0.30 8.939</td>
</tr>
<tr>
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<td>0.24 7.164</td>
<td>0.39 11.601</td>
<td>0.43 12.784</td>
</tr>
<tr>
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<td>0.32 9.531</td>
<td>0.48 14.263</td>
<td>0.56 16.629</td>
</tr>
<tr>
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<td>0.36 10.714</td>
<td>0.57 16.925</td>
<td>0.72 21.361</td>
</tr>
<tr>
<td>90</td>
<td>0.40 11.897</td>
<td>0.78 23.136</td>
<td>0.85 25.206</td>
</tr>
<tr>
<td>120</td>
<td>0.40 11.897</td>
<td>0.78 23.136</td>
<td>1.10 32.600</td>
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</tbody>
</table>
Table 3.10: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 10% acetic acid and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction/Incubation time (min)</th>
<th>Garlic extract = 1 ml [Pyruvic acid] (10^3) gms/litre</th>
<th>Garlic extract = 2 ml [Pyruvic acid] (10^3) gms/litre</th>
<th>Garlic extract = 3 ml [Pyruvic acid] (10^3) gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.05 1.545</td>
<td>0.08 2.432</td>
<td>0.13 3.911</td>
</tr>
<tr>
<td>30</td>
<td>0.10 3.024</td>
<td>0.17 5.094</td>
<td>0.25 7.460</td>
</tr>
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<td>0.15 4.503</td>
<td>0.27 8.052</td>
<td>0.40 11.897</td>
</tr>
<tr>
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<tr>
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<td>0.45 13.376</td>
<td>0.68 20.178</td>
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<tr>
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<td>0.30 8.939</td>
<td>0.54 16.037</td>
<td>0.82 24.319</td>
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<tr>
<td>120</td>
<td>0.41 12.192</td>
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<td>1.08 32.009</td>
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</tbody>
</table>
Table 3.11: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 20% acetic acid and incubated for upto 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml [Pyruvic acid] (10^3) gms/litre</th>
<th>Garlic extract =2 ml [Pyruvic acid] (10^3) gms/litre</th>
<th>Garlic extract =3 ml [Pyruvic acid] (10^3) gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00 -</td>
<td>0.00 -</td>
<td>0.00 -</td>
</tr>
<tr>
<td>15</td>
<td>0.04 1.249</td>
<td>0.06 1.841</td>
<td>0.11 3.320</td>
</tr>
<tr>
<td>30</td>
<td>0.08 2.432</td>
<td>0.13 3.911</td>
<td>0.22 6.573</td>
</tr>
<tr>
<td>45</td>
<td>0.12 3.615</td>
<td>0.20 5.981</td>
<td>0.34 10.122</td>
</tr>
<tr>
<td>60</td>
<td>0.16 4.798</td>
<td>0.26 7.756</td>
<td>0.43 12.784</td>
</tr>
<tr>
<td>75</td>
<td>0.20 5.981</td>
<td>0.32 9.531</td>
<td>0.56 16.629</td>
</tr>
<tr>
<td>90</td>
<td>0.23 6.869</td>
<td>0.39 11.601</td>
<td>0.67 19.882</td>
</tr>
<tr>
<td>120</td>
<td>0.32 9.531</td>
<td>0.52 15.446</td>
<td>0.88 26.093</td>
</tr>
</tbody>
</table>
Table 3.12: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 30% acetic acid and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction/Incubation time (min)</th>
<th>Garlic extract = 1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract = 2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract = 3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00 [ ]</td>
<td>0.00 [ ]</td>
<td>0.00 [ ]</td>
</tr>
<tr>
<td>15</td>
<td>0.03 0.953</td>
<td>0.06 1.841</td>
<td>0.08 2.432</td>
</tr>
<tr>
<td>30</td>
<td>0.06 1.841</td>
<td>0.11 3.320</td>
<td>0.16 4.798</td>
</tr>
<tr>
<td>45</td>
<td>0.09 2.728</td>
<td>0.16 4.798</td>
<td>0.23 6.869</td>
</tr>
<tr>
<td>60</td>
<td>0.12 3.615</td>
<td>0.22 6.573</td>
<td>0.32 9.531</td>
</tr>
<tr>
<td>75</td>
<td>0.15 4.503</td>
<td>0.27 8.052</td>
<td>0.39 11.601</td>
</tr>
<tr>
<td>90</td>
<td>0.18 5.390</td>
<td>0.34 10.122</td>
<td>0.47 13.967</td>
</tr>
<tr>
<td>120</td>
<td>0.23 6.869</td>
<td>0.43 12.784</td>
<td>0.62 18.403</td>
</tr>
</tbody>
</table>
Figure 3.6: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 5% acetic acid and incubated at 35°C for up to 120 min.
Figure 3.7: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 10% acetic acid and incubated at 35°C for up to 120 min.
Figure 3.8: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 20% acetic acid and incubated at 35°C for up to 120 min.
Figure 3.9: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 30% acetic acid and incubated at 35°C for up to 120 min.
Table 3.13: The values of rate constants ($K_{obs}$) at various initial concentration of acetic acid obtained from slopes of figures 3.6 - 3.9

<table>
<thead>
<tr>
<th>Experimental Garlic extract (ml.)</th>
<th>Acetic acid = 5%</th>
<th>$K_{obs} \times 10^4$ gm/l min. --&gt;</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.069</td>
<td>1.005</td>
<td>0.782</td>
<td>0.577</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.902</td>
<td>1.787</td>
<td>1.286</td>
<td>1.073</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.746</td>
<td>2.690</td>
<td>2.182</td>
<td></td>
<td>1.533</td>
</tr>
</tbody>
</table>
It is apparent from the results (Tables 3.9-3.12 and Figures 3.6-3.9) that increasing strengths of acetic acid in macerating medium decreased rate of formation of pyruvic acid (indicative of the rates of conversion of alliin to allicin) and thereby effected and decreased the rate constants ($K_{\text{obs}}$) for decomposition of alliin (Table 3.13).

3.4 Effect of Rectified Spirit

The amounts of pyruvic acid formed in garlic macerates, obtained by macerating de-skinned garlic cloves in 5-30% aqueous solutions of rectified spirit/ethanol, as a function of time, were determined by using 1 ml, 2 ml, and 3 ml filtered extracts are reported in Tables 3.14-3.17. The plots of [pyruvic acid] versus incubation time in presence of various initial rectified spirit/ethanol concentrations are represented graphically in Figures 3.10-3.13. These plots are linear for all aliquots of garlic extract in 5-30% rectified spirit for incubation periods of about 60 min beyond which these tend to flatten out, especially for 2 and 3 ml samples of garlic extract in 10-30% rectified spirit. Therefore, the rate constant ($K_{\text{obs}}$) for the formation of pyruvic acid, i.e., rate of decomposition of alliin to allicin have been evaluated from the slopes of the straight line portions of these plots. The values of rate constants ($K_{\text{obs}}$) are summarised in Table 3.18.
Table 3.14: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 5% rectified spirit and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml O.D.</th>
<th>[Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml O.D.</th>
<th>[Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml O.D.</th>
<th>[Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>0.10</td>
<td>3.024</td>
<td>0.15</td>
<td>4.503</td>
<td>0.18</td>
<td>5.390</td>
</tr>
<tr>
<td>30</td>
<td>0.26</td>
<td>7.756</td>
<td>0.30</td>
<td>8.939</td>
<td>0.37</td>
<td>11.009</td>
</tr>
<tr>
<td>45</td>
<td>0.32</td>
<td>9.531</td>
<td>0.45</td>
<td>13.376</td>
<td>0.56</td>
<td>16.629</td>
</tr>
<tr>
<td>60</td>
<td>0.39</td>
<td>11.601</td>
<td>0.61</td>
<td>18.108</td>
<td>0.74</td>
<td>21.953</td>
</tr>
<tr>
<td>75</td>
<td>0.46</td>
<td>13.671</td>
<td>0.66</td>
<td>19.587</td>
<td>0.81</td>
<td>24.023</td>
</tr>
<tr>
<td>90</td>
<td>0.47</td>
<td>13.967</td>
<td>0.68</td>
<td>20.178</td>
<td>0.83</td>
<td>24.615</td>
</tr>
<tr>
<td>120</td>
<td>0.49</td>
<td>14.559</td>
<td>0.70</td>
<td>20.770</td>
<td>0.85</td>
<td>25.206</td>
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</table>
Table 3.15: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 10% rectified spirit and incubated for upto 120 minutes at 35 °C.

<table>
<thead>
<tr>
<th>Enzymic reaction/Incubation time (min)</th>
<th>Garlic extract =1 ml O.D. [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml O.D. [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml O.D. [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.09 2.728</td>
<td>0.12 3.615</td>
<td>0.16 4.798</td>
</tr>
<tr>
<td>30</td>
<td>0.22 6.573</td>
<td>0.25 7.460</td>
<td>0.34 10.122</td>
</tr>
<tr>
<td>45</td>
<td>0.28 8.348</td>
<td>0.37 11.009</td>
<td>0.51 15.150</td>
</tr>
<tr>
<td>60</td>
<td>0.37 11.009</td>
<td>0.51 15.150</td>
<td>0.69 20.474</td>
</tr>
<tr>
<td>75</td>
<td>0.36 10.714</td>
<td>0.53 15.742</td>
<td>0.77 22.840</td>
</tr>
<tr>
<td>90</td>
<td>0.38 11.305</td>
<td>0.55 16.333</td>
<td>0.79 23.431</td>
</tr>
<tr>
<td>120</td>
<td>0.42 12.488</td>
<td>0.57 16.925</td>
<td>0.81 24.023</td>
</tr>
</tbody>
</table>
Table 3.16: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 20% rectified spirit and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml</th>
<th>Garlic extract =2 ml</th>
<th>Garlic extract =3 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.08</td>
<td>2.432</td>
<td>0.13</td>
</tr>
<tr>
<td>30</td>
<td>0.14</td>
<td>4.207</td>
<td>0.26</td>
</tr>
<tr>
<td>45</td>
<td>0.22</td>
<td>6.573</td>
<td>0.38</td>
</tr>
<tr>
<td>60</td>
<td>0.29</td>
<td>8.643</td>
<td>0.51</td>
</tr>
<tr>
<td>75</td>
<td>0.31</td>
<td>9.235</td>
<td>0.55</td>
</tr>
<tr>
<td>90</td>
<td>0.32</td>
<td>9.531</td>
<td>0.57</td>
</tr>
<tr>
<td>120</td>
<td>0.34</td>
<td>10.122</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Table 3.17: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in 30% rectified spirit and incubated for upto 120 minutes at 35 °C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00 -</td>
<td>0.00 -</td>
<td>0.00 -</td>
</tr>
<tr>
<td>15</td>
<td>0.02 0.658</td>
<td>0.06 1.841</td>
<td>0.12 3.615</td>
</tr>
<tr>
<td>30</td>
<td>0.07 2.136</td>
<td>0.15 4.503</td>
<td>0.25 7.460</td>
</tr>
<tr>
<td>45</td>
<td>0.13 3.911</td>
<td>0.24 7.164</td>
<td>0.39 11.601</td>
</tr>
<tr>
<td>60</td>
<td>0.18 5.390</td>
<td>0.34 10.122</td>
<td>0.52 15.446</td>
</tr>
<tr>
<td>75</td>
<td>0.21 6.277</td>
<td>0.37 11.009</td>
<td>0.55 16.333</td>
</tr>
<tr>
<td>90</td>
<td>0.23 6.869</td>
<td>0.39 11.601</td>
<td>0.56 16.629</td>
</tr>
<tr>
<td>120</td>
<td>0.26 7.756</td>
<td>0.41 12.192</td>
<td>0.58 17.220</td>
</tr>
</tbody>
</table>
Figure 3.10: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 5% rectified spirit and incubated at 35°C for upto 120 min.
Figure 3.11: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 10% rectified spirit and incubated at 35°C for upto 120 min.
Figure 3.12: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 20% rectified spirit and incubated at 35°C for upto 120 min.
Figure 3.13: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in 30% rectified spirit and incubated at 35°C for upto 120 min.
Table 3.18: The value of rate constants ($K_{obs}$) at various initial concentration of rectified spirit obtained from slopes of figures 3.10-3.13

<table>
<thead>
<tr>
<th>Experimental Garlic extract (ml.)</th>
<th>Rectified Spirit = 5%</th>
<th>$10^4$ gm/lt min. 10%</th>
<th>$10^4$ gm/lt min. 20%</th>
<th>$10^4$ gm/lt min. 30%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.980</td>
<td>1.866</td>
<td>1.399</td>
<td>1.064</td>
</tr>
<tr>
<td>2</td>
<td>3.005</td>
<td>2.666</td>
<td>2.484</td>
<td>1.833</td>
</tr>
<tr>
<td>3</td>
<td>3.601</td>
<td>3.200</td>
<td>3.115</td>
<td>2.642</td>
</tr>
</tbody>
</table>
It is apparent from the results that (Tables 3.14 - 3.17 and Figures 3.10 - 3.13) that increasing strengths of rectified spirit/ethanol in macerating medium, decreased rate of formation of pyruvic acid (indicative of the rate of conversion of alliin to allicin) and thereby effected and decreased the rate constants ($K_{obs}$) for decomposition of alliin (Table 3.18). However, ($K_{obs}$) for conversion of alliin to allicin in presence of experimental levels of rectified spirit/ethanol in macerating medium were higher/greater than those observed in experimental levels of acetic acid in macerating medium. This could perhaps explain the observed tendency of the plots in Figures 3.10 - 3.13 of flattening out with incubation time exceeding 60 min.

3.5 Effect of pH:

The amounts of pyruvic acid formed in garlic macerates, obtained by macerating de-skinned garlic cloves with buffers of pH 4.0 - 6.0, as a function of time, were determined by using 1 ml, 2 ml and 3 ml filtered extracts are reported in Tables 3.19 - 3.23.

The plots of [pyruvic acid] versus incubation time for different buffers corresponding to pH 4.0 - 6.0 are represented graphically in Figures 3.14 - 3.18. These plots exhibited a trend similar to that depicted by plots of effect of rectified spirit and, therefore, the rate constants ($K_{obs}$) for the formation of pyruvic acid i.e. rate of decomposition of alliin to allicin have been evaluated from the slopes of the straight line portions of the plots of [pyruvic acid] versus incubation time. The values of rate constants for the formation of pyruvic acid ($K_{obs}$) are summarised in Table 3.24.
Table 3.19: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in buffer of pH 4.0 and incubated for upto 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O.D.</td>
<td>O.D.</td>
<td>O.D.</td>
</tr>
<tr>
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<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
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<td>0.04</td>
<td>0.07</td>
<td>0.10</td>
</tr>
<tr>
<td>30</td>
<td>0.09</td>
<td>0.12</td>
<td>0.20</td>
</tr>
<tr>
<td>45</td>
<td>0.13</td>
<td>0.23</td>
<td>0.31</td>
</tr>
<tr>
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<td>0.16</td>
<td>0.29</td>
<td>0.42</td>
</tr>
<tr>
<td>75</td>
<td>0.20</td>
<td>0.31</td>
<td>0.44</td>
</tr>
<tr>
<td>90</td>
<td>0.22</td>
<td>0.33</td>
<td>0.46</td>
</tr>
<tr>
<td>120</td>
<td>0.25</td>
<td>0.32</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 3.20: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in buffer of pH 4.5 and incubated for upto 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.08</td>
<td>0.10</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>2.432</td>
<td>3.024</td>
<td>3.911</td>
</tr>
<tr>
<td>30</td>
<td>0.13</td>
<td>0.22</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>3.911</td>
<td>6.573</td>
<td>7.756</td>
</tr>
<tr>
<td>45</td>
<td>0.20</td>
<td>0.32</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>5.981</td>
<td>9.531</td>
<td>11.601</td>
</tr>
<tr>
<td>60</td>
<td>0.25</td>
<td>0.44</td>
<td>0.53</td>
</tr>
<tr>
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<td>7.460</td>
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<td>75</td>
<td>0.26</td>
<td>0.46</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>7.756</td>
<td>13.671</td>
<td>16.629</td>
</tr>
<tr>
<td>90</td>
<td>0.28</td>
<td>0.47</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>8.348</td>
<td>13.967</td>
<td>16.925</td>
</tr>
<tr>
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<td>0.29</td>
<td>0.48</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>8.643</td>
<td>14.263</td>
<td>17.516</td>
</tr>
</tbody>
</table>
Table 3.21: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in buffer of pH 5.0 and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract =1 ml O.D.</th>
<th>[Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =2 ml O.D.</th>
<th>[Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract =3 ml O.D.</th>
<th>[Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
<td>-</td>
<td>0.00</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>0.07</td>
<td>2.136</td>
<td>0.13</td>
<td>3.911</td>
<td>0.15</td>
<td>4.503</td>
</tr>
<tr>
<td>30</td>
<td>0.14</td>
<td>4.325</td>
<td>0.24</td>
<td>7.164</td>
<td>0.32</td>
<td>9.531</td>
</tr>
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<tr>
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<td>0.44</td>
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<td>16.333</td>
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<td>0.26</td>
<td>7.756</td>
<td>0.51</td>
<td>15.150</td>
<td>0.63</td>
<td>18.699</td>
</tr>
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<td>90</td>
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<td>8.052</td>
<td>0.53</td>
<td>15.742</td>
<td>0.65</td>
<td>19.291</td>
</tr>
<tr>
<td>120</td>
<td>0.28</td>
<td>8.348</td>
<td>0.55</td>
<td>16.333</td>
<td>0.68</td>
<td>20.178</td>
</tr>
</tbody>
</table>
Table 3.22: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in buffer of pH 5.5 and incubated for upto 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract = 1 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract = 2 ml [Pyruvic acid] $10^3$ gms/litre</th>
<th>Garlic extract = 3 ml [Pyruvic acid] $10^3$ gms/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00 -</td>
<td>0.00 -</td>
<td>0.00 -</td>
</tr>
<tr>
<td>15</td>
<td>0.09 2.728</td>
<td>0.13 3.911</td>
<td>0.21 6.277</td>
</tr>
<tr>
<td>30</td>
<td>0.17 5.094</td>
<td>0.28 8.348</td>
<td>0.55 16.333</td>
</tr>
<tr>
<td>45</td>
<td>0.26 7.756</td>
<td>0.40 11.897</td>
<td>0.61 18.108</td>
</tr>
<tr>
<td>60</td>
<td>0.35 10.418</td>
<td>0.53 15.742</td>
<td>0.67 19.882</td>
</tr>
<tr>
<td>75</td>
<td>0.39 11.601</td>
<td>0.59 17.516</td>
<td>0.69 20.474</td>
</tr>
<tr>
<td>90</td>
<td>0.41 12.192</td>
<td>0.61 18.108</td>
<td>0.72 21.361</td>
</tr>
<tr>
<td>120</td>
<td>0.42 12.488</td>
<td>0.62 18.403</td>
<td>0.72 21.361</td>
</tr>
</tbody>
</table>
Table 3.23: Determination of Pyruvic acid in different aliquots of filtered garlic extract prepared by macerating garlic cloves in buffer of pH 6.0 and incubated for up to 120 minutes at 35°C.

<table>
<thead>
<tr>
<th>Enzymic reaction / Incubation time (min)</th>
<th>Garlic extract = 1 ml</th>
<th>Garage extract = 2 ml</th>
<th>Garlic extract = 3 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>15</td>
<td>0.10</td>
<td>3.024</td>
<td>0.14</td>
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<tr>
<td>30</td>
<td>0.19</td>
<td>5.686</td>
<td>0.30</td>
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<tr>
<td>45</td>
<td>0.30</td>
<td>8.939</td>
<td>0.47</td>
</tr>
<tr>
<td>60</td>
<td>0.41</td>
<td>12.192</td>
<td>0.63</td>
</tr>
<tr>
<td>75</td>
<td>0.45</td>
<td>13.376</td>
<td>0.68</td>
</tr>
<tr>
<td>90</td>
<td>0.48</td>
<td>14.263</td>
<td>0.69</td>
</tr>
<tr>
<td>120</td>
<td>0.49</td>
<td>14.559</td>
<td>0.71</td>
</tr>
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</table>
Figure 3.14: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in pH of buffer 4.0 and incubated at 35°C for up to 120 min.
Figure 3.15: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in pH of buffer 4.5 and incubated at 35°C for upto 120 min.
In 1 ml GARLIC EXTRACT
In 2 ml GARLIC EXTRACT
In 3 ml GARLIC EXTRACT

Figure 3.16: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in pH of buffer 5.0 and incubated at 35°C for upto 120 min.
Figure 3.17: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in pH of buffer 5.5 and incubated at 35°C for upto 120 min.
Figure 3.18: Plot of [Pyruvic Acid] versus incubation time for garlic extract prepared in pH of buffer 6.0 and incubated at 35°C for upto 120 min.
Table 3.24: The values of rate constants ($K_{obs}$) at various buffers of different pH obtained from slopes of figures 3.14 - 3.18.

<table>
<thead>
<tr>
<th>Experimental Garlic extract (ml.)</th>
<th>pH = 4.0</th>
<th>$&lt;-- K_{obs} 10^4$ gm/lt min. --&gt;</th>
<th>4.5</th>
<th>5.0</th>
<th>5.5</th>
<th>6.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.79</td>
<td>1.253</td>
<td>1.267</td>
<td>1.724</td>
<td>2.019</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.46</td>
<td>2.160</td>
<td>2.209</td>
<td>2.631</td>
<td>3.143</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.076</td>
<td>2.608</td>
<td>2.710</td>
<td>3.084</td>
<td>3.735</td>
<td></td>
</tr>
</tbody>
</table>
It is apparent from the results (Tables 3.19 - 3.23 and Figures 3.14 - 3.18) that increasing pH of macerating medium in limited range 4.0 - 6.0 studied in this research increased rate of formation of pyruvic acid (indicative of the rate of conversion from alliin to allicin) and thereby effected and increased the rate constants (K_{obs.}) for decomposition of alliin (Table 3.24). These results indicate optimum pH for native enzyme for conversion of alliin to allicin to lie towards neutrality of the medium.

The results of the effect of incubation temperature, acetic acid, rectified spirit/ethanol, and pH on the rate of conversion of alliin to allicin/decomposition of alliin could be summarised as follows:

1. The plots of [pyruvic acid] versus incubation time in presence of different initial concentrations of rectified spirit/ethanol or at different pH or at different incubation temperatures were linear for incubation periods of 60 minutes, beyond which these tended to flatten out. Therefore, it was apparent that most of alliin converted to allicin in about 60-75 minutes under the aforesaid conditions. However, the plots of [pyruvic acid] versus incubation time in presence of various initial acetic acid concentrations were linear even up to 2hrs, suggesting relatively slow rates of conversion of alliin to allicin in presence of acetic acid. The slow rate of conversion of alliin to allicin, were also confirmed by low values of observed rate constants in presence of acetic acid.
(2) The rate of conversion of alliin to allicin decreased with increasing strengths of acetic acid or rectified spirit in macerating medium.

(3) It was observed that on increasing the pH of macerating medium in limited range (pH 4.0-6.0), the rate of conversion of alliin to allicin increased.

(4) The rate constants for conversion of alliin to allicin were observed to be temperature dependent. The plots of \( \frac{1}{K_{\text{obs.}}} \) versus incubation temperature for different aliquots of garlic extract (Fig. 3.15) suggested the optimum temperature of \( \sim 35^\circ \text{C} \) for the conversion of alliin to allicin, which was in good agreement with the reported optimum temperature of 37\(^\circ\text{C} \) for this enzymatic conversion.

(5) It was also observed that plots of \( \frac{1}{K_{\text{obs.}}} \) versus amount of garlic extract under specified experimental conditions were linear suggesting that the rate of conversion of alliin to allicin/decomposition of alliin increased with increasing substrate concentrations.

Thus, on the basis of experimental results the favourable conditions for conversion of alliin to allicin are:

(a) Enzymatic action temperature of about 35\(^\circ\text{C} \).
(b) pH of the reaction should be maintained towards neutrality of medium, as, the acidic conditions of the reaction-mix decreases the rate of decomposition.
Figure 3.19: Plot of $K_{obs}$ versus incubation temperature for different aliquots of garlic extract.
REFERENCES


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