Chapter - 5

The study of respiratory metabolism and starch metabolism in the spongy tissue.
1. Introduction

Since there is a close relationship between the ethylene production, fruit softening and climacteric respiration in mango fruit, it is important to study the changes which occur during the respiratory climacteric stage. Starch hydrolysis, a concomitant increase in total sugar and a decrease in titratable acid level are some of the changes that characterise ripening of mango. Any change in the starch metabolism is going to affect the fruit quality and also indicates the damages to ripening process. This is mainly indicated by the respiration of the fruit tissues. Therefore to understand the changes associated with the respiration the activities of two enzymes involved in the respiratory metabolism namely pyruvate decarboxylase and malate dehydrogenase has been estimated in this study. Malic enzyme may play an important role in the oxidation and mobilization of storage malate into the respiratory pathway and in mango, the increase in malic and pyruvate decarboxylase enzymes activity occur in close parallel with the increase in climactic respiration. Pyruvate decarboxylase is the enzyme that is involved in the conversion of acetaldehyde to pyruvate.

Starch metabolism in the fruit tissue is an important parameter of fruit quality. During the fruit ripening process the insoluble starch in the cells are catabolised into soluble sugars by the activities of two important enzymes namely amylase and invertase. It has been reported by the earlier workers that amylase was found to increase at least 4-fold in activity during mango ripening and also there was higher accumulation of starch granules in the affected spongy tissue due to the lower activities of starch degrading enzymes. Hence the total starch content, sugar content and the activities of starch degrading enzymes were studied in the different alphonso mango fruit tissues including healthy and affected tissues. Total starch content and total sugars were estimated by Anthrone method. Reducing sugars and amylase activity were estimated by DNS method. The activity of invertase enzyme was assayed by phosphomolybdic acid method.
2. **Materials and methods**

2.1 **Assaying the enzyme activities of respiratory metabolism**

2.1.1 Pyruvate decarboxylase

2.1.2 Malate dehydrogenase

2.1.1 **Estimation of Pyruvate decarboxylase activity:** Assay was done as per the methods Selvaraj *et al.* (1995).

**Chemicals required:**

1) 0.1M Sodium phosphate buffer pH-6.0:-
   a) 0.2M monobasic sodium phosphate solution – 15.6gm dissolved in 500ml of distilled water.
   b) 0.2M dibasic sodium phosphate solution – 17.7gm dissolved in 500ml of distilled water.
87.7ml of a and 12.3ml of b were mixed and pH was adjusted to 6.0.

2) 10mM Sodium pyruvate:– 55mg of sodium pyruvate in 50ml of distilled water.

3) 0.1mM Thiamine pyrophosphate(TPP):– 0.0076gm of TPP in 25ml of distilled water.

4) 1mM Magnesium sulphate:– 0.02464gm of Magnesium sulphate was dissolved in 100ml of distilled water.

5) 0.16mM NADH solution:– 0.0056gm of NADH was dissolved in 50ml of distilled water.

6) 300units/ml of Yeast alcohol dehydrogenase solution(ADH):– 1mg of ADH was dissolved in 1ml of distilled water.
Enzyme extraction and assay method: 0.5gm of acetone powder was suspended in cold using 10ml of 0.1M sodium phosphate buffer pH- 6.0 for overnight incubation. Further that was ground using a chilled mortar and pestle. The homogenate was filtered and centrifuged at 14,000rpm for 15 minutes at 4°C. The resulting supernatent solution was used as crude enzyme extract. The assay mixture contained 0.3ml of phosphate buffer pH-6.0, 0.3ml of sodium pyruvate solution, 0.3ml of Thiamine pyro phosphate solution, 0.3ml of Magnesium sulphate, 0.2ml of NADH solution, 0.1ml of alcohol dehydrogenase solution and 0.5ml of crude enzyme extract. The control blank was run without sodium pyruvate. The assay mixture was shaken well and optical density of the solution was read spectrophotometrically at 340nm against the blank solution.

2.1.2 Malate dehydrogenase activity:- The activity was assayed as per the method by Selvaraj, Y., et.al.,(1989).

Chemicals required:-

1) Extraction medium pH-7.5:- 1.36gm of Potassium dihydrogen phosphate (0.05M KH₂PO₄), 34.23gm of Sucrose (0.5M), 0.372gm of EDTA (0.005M), 0.1404gm of Cystein (0.004M), 0.0813gm of Magnesium chloride (0.002M), 0.1491gm of potassium chloride (0.01M) and 150mg of Bovine serum albumin were dissolved in 200ml of distilled water and pH was adjusted to 7.5.

2) 0.2M Tris – HCl buffer pH-7.5
   a) 0.2M tris aminomethane solution:- 24.2gm in 1000ml of distilled water.
   b) 0.2M HCl:- 17.8ml of concentrated HCl diluted to 1000ml with water.
   50ml of a and 39.9ml of b were diluted to 200ml and pH was adjusted to 7.5.

3) 1mM NADH solution:- 0.03547gm of NADH was dissolved in 50ml of water.
4) 15mM Oxaloacetate solution:- 0.1981gm of oxaloacetate was dissolved in 100ml of distilled water.

**Enzyme extraction and assay method:-** 0.5gm of acetone powder was suspended overnight in cold condition using 10ml of chilled extraction medium pH-7.5 consisting of 0.5M sucrose, 0.05M KH₂PO₄, 0.005M EDTA, 0.004M cystein, 0.002M MgCl₂, 0.01M KCl and 0.75mg bovine serum albumin per mL. That was homogenized using a chilled mortar and pestle. The homogenate was filtered through four layers of cheese cloth and the fluid was centrifuged at 2,000g for 10 minutes at 4°C to eliminate cell debris. The supernatent was then centrifuged at 1500g for 10 minutes at 4°C. The precipitate was washed with grinding medium and centrifuged again at 15,000g for 15 minutes. The final mitochondrial precipitate was suspended in 2.0ml of 0.17gm of sucrose(0.25M) in 0.05M tris buffer (pH-7.5) and was used as enzyme source. The assay mixture contained 0.45ml of 0.2M tris-HCl buffer(pH-7.5), 0.5ml of 1mM NADH, 0.5ml of 15mM oxaloacetate and 0.2ml of crude enzyme extract. The final volume was adjusted to 3.0ml with triple distilled water. The mixture was shaken well and optical density of the solution was read at 340nm against the blank solution at 30°C.

**2.2. Estimation of total starch and sugar content and assaying the Enzyme activities of starch metabolism.**

2.2.1 Starch estimation,
2.2.2 Sugar estimation,
2.2.3 Amylase activity and
2.2.4 Invertase activity

**2.2.1 Estimation of Starch:-** Estimation of starch was done as per the method by Sadasivam *et.al.*, (1996)
Chemicals Required:-

1) 0.2% Anthrone reagent (freshly prepared): - 50mg of Anthrone reagent was dissolved in 25ml of 95% concentrated Sulphuric acid.

2) 26% Perchloric acid: - 37ml of 70% perchloric acid was diluted to 100ml with water.

Starch extraction method: - About 100gm of fruit pulp was kept in 80% alcohol for few days. That was extracted continuously in a soxlet apparatus till the pulp turned colourless. The colourless alcohol insoluble fraction was dried and used for starch estimation. Where as the alcohol soluble fraction was dried completely in a water bath and was used for sugar estimation. The dried pulp was weighed and finely powdered. 100mg of the powder was used for starch estimation which was mixed with 6ml of 26% perchloric acid and allowed to stand for a minimum of 30 minutes at room temperature. It was filtered through glass wool and the solid mass was repeatedly washed with water. Volume of the extract and the washings were made up to 50ml and was taken for starch estimation.

Starch estimation method: - The reaction mixture contained 0.5ml of starch solution to be estimated, 0.75ml of distilled water and 2.5ml of freshly prepared ice cold anthrone reagent. The reaction mixture was mixed well carefully and placed in a boiling water bath for exactly 10 minutes. Then cooled to room temperature and optical density was read at 625nm against a blank solution.

2.2.2 Estimation of total, reducing and non reducing sugars.

2.2.2.a. Estimation of total sugar: - Estimation was done as per the method by Sadasivam. S. and Manickam. A(1996).
Chemicals required:

1) 0.2% Anthrone reagent:– 50mg of anthrone reagent was dissolved in 25ml of 95% concentrated sulphuric acid.

About 100gm of fruit pulp was kept in 80% alcohol for few days. That was extracted continuously in a soxlet apparatus till the pulp turned colourless. The colourless alcohol insoluble fraction was dried and kept separately where as the alcohol soluble fraction was dried completely in a water bath and was used for sugar estimation. Dried alcohol extract was redissolved in distilled water and the volume was made up to 100ml(stock solution) and 1ml of the stock solution was further made up to 50ml.

Estimation:– The reaction mixture contained 0.1ml of diluted sugar solution. 2.4ml of distilled water and 5ml of freshly prepared ice cold anthrone reagent. The assay mixture was gently shaken and placed in a boiling water bath for exactly 7.5 minutes. Then cooled immediately in an ice bath and optical density of the solution was read at 625nm against a blank solution.

2.2.2.b. Estimation of reducing sugar:–
Reducing sugar was estimated as per the method by Miller,G.L(1972).

Chemicals required:

1) DNS reagent:– 1gm of DNS reagent, 2gm of phenol reagent, 0.5gm of sodium sulphite, 200gm of Rochelle salt was dissolved in 500ml of 2% sodium hydroxide solution and then diluted to 1litre with distilled water.

Estimation:– The reaction mixture contained 0.75ml of diluted sugar solution, 0.75ml distilled water and 3.0ml of DNS reagent. The assay mixture was mixed well and kept in boiling water bath for 5 minutes, cooled under running tap water and then diluted with
20ml of distilled water. Absorbance of the solution was read at 540nm against the blank solution.

2.2.2.c. Estimation of Non reducing sugar:-

Non reducing sugar content = total sugar content – reducing sugar content.

2.3 Assaying the activity of α-amylase enzyme:- Estimation was done as per the methods by Peter Bernfeld.

Chemicals required:-

1) 0.016M Acetate buffer pH-4.8:-
   a) 0.2M solution of acetic acid – 11.55ml diluted 100ml.
   b) 0.2M solution of sodium acetate – 16.4gm of sodium acetate dissolved in 100ml of distilled water.

20ml of a and 30ml of b, mixed, diluted to 200ml to get a stock solution of 0.05M. 80ml of 0.05M stock solution was further diluted to 250ml to get 0.016M buffer and pH was adjusted to 4.8.

2) 1% amylopectin solution :- 1gm of amylopectin was dissolved in 100ml of 0.016M acetate buffer pH-4.8.

3) DNS reagent:- 1gm of DNS reagent, 2gm of phenol reagent, 0.5gm of sodium sulphite, 200gm of Rochelle salt was dissolved in 500ml of 2% sodium hydroxide solution and then diluted to 1litre with distilled water.

Enzyme extraction:- 0.5gm of acetone powder was kept in the refrigerator for overnight incubation in 10ml of 0.016M acetate buffer pH - 4.8. That was homogenized in a mortar and pestle, filtered and centrifuged at 10,000rpm for 15 minutes. The supernatent was used as crude enzyme extract.
Enzyme assay: - The assay mixture contained 1.0ml of enzyme extract and 1.0ml of 1% soluble amylopectin solution. The assay mixture was shaken well and incubated for 1 hour at 37°C. Then the reaction was stopped by adding 2ml of DNS reagent. All the tubes were kept in boiling water bath for 5 minutes, cooled in running tap water and 20ml of distilled water was added for dilution. Absorbance of the solution was read at 540nm against the blank. One unit of enzyme activity was expressed as mg reducing sugar formed /hr.

2.4 Assaying the activity of invertase enzyme: - Estimation was done as per the methods by Selvaraj and Kumar (1995).

Chemicals required: -

1. Phosphomolybdic acid solution: - Add 200mL 10% NaOH solution and 200mL water to 35 g of molybdic acid and 5g of sodium tungstate in 1 L beaker. Boil vigorously for 20- 40 minutes, cool, dilute to 350mL and add 125mL phosphoric acid (85%) and dilute to 500mL and mix well.

2. Alkaline copper solution: - This was prepared by dissolving 40 g of sodium carbonate in 400 mL of water. Dissolve 7.5 g of tartaric acid in this solution and add 4.5 g of copper sulphate and mix well and make up the volume to 1 litre.

3. 0.05M citrate phosphate buffer (pH 4.5): -
   a) 0.1M citric acid – 21.01gm dissolved in distilled water and made up to 1000ml (stock solution).
   b) 0.2M Na₂HPO₄·7H₂O – 53.65gm dissolved in distilled water and made up to 1000ml (stock solution).

25.4ml of a and 24.6ml of b were mixed, diluted to 200ml and pH was adjusted to 4.5.
4. 62.5mM sucrose solution in 0.1mM Na-acetate buffer (pH 5.0):- 4.278gm of sucrose was dissolved in 200 ml of 0.1mM of Na-acetate buffer and pH was adjusted to 5.0.

**Enzyme extraction:-** 0.5 gram acetone powder was incubated in 10ml of 0.05M citrate phosphate buffer (pH 4.5)) overnight. The mixture was ground thoroughly and squeezed through 4 layers of muslin cloth and centrifuged at 1500 rpm for 30 minutes. The supernatant was used as the crude enzyme extract.

**Enzyme assay:-** Enzyme activity was estimated by mixing 0.2 mL of enzyme extract, 0.8 mL of sucrose solution and 1 mL of buffer (citrate phosphate buffer). The mixture was incubated for 1 hr at 37°C and the reaction was stopped by adding 2 mL of alkaline copper reagent. Reducing sugar in the reaction mixture was estimated using phosphomolybdic acid method by taking optical density at 420 nm against a blank solution. Enzyme activity was expressed in terms of amount of reducing sugar produced per unit of enzyme per unit time using a standard curve.

**Statistical analysis:-** Analysis of variations (ANOVA) of data was done using CRD (completely randomized design) method. Critical difference (CD) values were calculated at P = 0.05 wherever ANOVA was found significant.
3. **Results**

3.1 **Enzyme activities of respiratory metabolism.**

3.1.1 **Pyruvate decarboxylase activity:** According to the analysed data pyruvate decarboxylase activity was significantly different at 5% significant level \((P = 0.05)\) between the different tissues. However completely ripen healthy tissue and healthy tissue surrounding spongy tissue and also spongy tissue and induced spongy tissues were on par with each other as their differences were less than the CD value. Data indicated that pyruvate decarboxylase activity was 28 times more in the completely ripen healthy tissue and healthy tissue surrounding spongy tissue when compared to the activity of spongy tissue. The activities of completely ripen healthy tissue; healthy tissue surrounding spongy tissue and spongy tissue were 6.5215 U, 6.4938 U and 0.2325 U respectively. Induced spongy tissue showed lowest enzyme activity of 0.1811 U. Enzyme showed highest activity in 3/4th ripen tissue (28.8200 U) and the activity in mature unripen tissue was 1.2811 U which was in between 3/4th ripe and fully ripen tissues.

The specific activity of the pyruvate decarboxylase enzyme was maximum in the 3/4th ripen tissue (3.6323 U/mg protein) indicating the higher enzyme activity in this tissue. Completely ripen healthy tissue and healthy tissue surrounding spongy tissue showed almost same specific activity of 2.7175 U/mg protein and 2.6224 U/mg protein respectively. Among spongy tissues the specific activity was more in the natural spongy tissue (0.4110 U/mg protein) when compared to the induced spongy tissue (0.3037 U/mg protein). The specific activity in the mature unripen tissue was (1.7106 U/mg protein) half of the activity of 3/4th ripen tissue (3.6323 U/mg protein).

3.1.2 **Malate dehydrogenase activity:** As per the analysed data malate dehydrogenase activity was significantly different between the different tissues. Healthy tissue surrounding spongy tissue and completely ripen healthy tissue and also spongy tissue as well as induced spongy tissue were on par with each other. However the activity was maximum in the 3/4th ripen tissue (12.0941 U) followed by healthy tissue
surrounding spongy tissue (8.7987 U), completely ripen healthy tissue (8.6122 U),

spongy tissue (6.1436 U) and induced spongy tissue (6.1085 U). Spongy tissue showed

slightly lesser enzyme activity (6.1436 U) than the completely ripen healthy tissue (8.6122

U). Enzyme activity was minimum in the mature unripe tissue (4.2311 U).

Specific activity of the enzyme was maximum in the 3/4\textsuperscript{th} ripen tissue (7.1402

U/mg protein) followed by mature unripe tissue (5.4158 U/mg protein), healthy ripen

tissue (4.5348 U/mg protein), healthy surrounding spongy tissue (4.2332 U/mg protein

and spongy tissue (3.2015 U/mg protein). Induced Spongy tissue showed slightly more

specific activity of 4.4160 U/mg protein than the natural Spongy tissue (3.2015 U/mg

protein).

The activities of Pyruvate decarboxylase and Malate dehydrogenase enzymes are

presented in Table - 3 and figure - 5.
Table – 3. The activities of Pyruvate decarboxylase and Malate dehydrogenase enzymes.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Pyruvate decarboxylase</th>
<th>Malate dehydrogenase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/100gmfw</td>
<td>U/mg protein</td>
</tr>
<tr>
<td>MU</td>
<td>1.2811</td>
<td>1.7106</td>
</tr>
<tr>
<td>CR</td>
<td>6.5215</td>
<td>2.7175</td>
</tr>
<tr>
<td>HSS</td>
<td>6.4938</td>
<td>2.6224</td>
</tr>
<tr>
<td>Sp</td>
<td>0.2325</td>
<td>0.4110</td>
</tr>
<tr>
<td>ISp</td>
<td>0.1811</td>
<td>0.3037</td>
</tr>
<tr>
<td>SEM</td>
<td>0.0533</td>
<td>0.0333</td>
</tr>
<tr>
<td>CD(P=0.05)</td>
<td>0.1601</td>
<td>0.1000</td>
</tr>
</tbody>
</table>


The activities of pyruvate decarboxylase and malate dehydrogenase enzymes were significantly different at 5% significant level (P = 0.05) between the different tissues.
Figure 5. The activities of Pyruvate decarboxylase and Malate dehydrogenase enzymes.

Each bar in the graphs represent enzyme activity/gm FW.

Mu – Mature unripen tissue, 3/4thR – 3/4th ripen tissue, CR – completely ripen tissue,
HSS – healthy tissue surrounding spongy tissue, Sp – spongy tissue, ISp – induced spongy tissue

PDC – Pyruvate decarboxylase  MDH – Malate dehydrogenase
3.2 Starch, sugar content and enzyme activities of starch metabolism.

3.2.1 Starch estimation:- According to the statistical analysis (CRD method) total starch content was significantly different at 5% significant level between the different tissues except for the completely ripen healthy tissue and healthy tissue surrounding spongy tissue which were on par with each other as the difference between the two were less than the CD value. Starch content in the spongy tissue was almost 6 times more (4.394 %) than the completely ripen healthy tissue (0.696 %). Mature but unripe tissue showed maximum amount of starch than other tissues (11.712 %). The starch content was slightly less in the induced spongy tissue (3.530 %) when compared to the natural spongy tissue. Among the ripening tissues 3/4* ripe tissue had higher starch (1.458 %) when compared to the completely ripen healthy tissue (0.696 %) and healthy tissue surrounding spongy tissue (0.702 %). Data clearly indicated that the starch content decreases with the increase in stage of ripening. Retention of more starch content in the spongy tissue indicates that starch is not getting converted to the sugars may be due to the lower activities of the starch degrading enzymes.

3.2.2 Sugar estimation :- Total, reducing and non-reducing sugars were estimated. According to the statistical analysis (CRD method) total sugar content was significantly different at 5% significant level (P = 0.05) between the different tissues (Table - 4 and figure - 6). The total sugar content was almost 4 times more in the completely ripen healthy tissue (8.028 %) when compared to the spongy tissue (2.214 %). Total sugar content was slightly less in the healthy tissue surrounding spongy tissue (7.732 %) than the completely ripen healthy tissue. Induced spongy tissue showed more of sugar content (2.532 %) in comparison with the natural spongy tissue. Mature unripe tissue and 3/4* ripe tissue showed total sugar content of 1.182 % and 3.242 % respectively.
As per the analysis (CRD method) reducing sugar content was significantly different at 5% significant level between the different tissues. However the 3/4<sup>th</sup> ripen tissue and spongy tissues were on par with each other. Reducing sugar was 2 times more in the completely ripen healthy tissue (1.634 %) as compared to the spongy tissue (0.642 %). The reducing sugar content was less in the natural spongy tissue than the induced spongy tissue (0.784 %). The reducing sugar content in the healthy tissue surrounding spongy tissue (1.208 %) was less than the completely ripen healthy tissue (1.634 %). The sugar content increased 4 times from mature unripen tissue (0.152 %) to 3/4<sup>th</sup> ripen tissue (0.684 %).

According to the analysis of variations (ANOVA, CRD method) nonreducing sugar content was significantly different at 5% significant level (P = 0.05) between the different tissues. The non-reducing sugar also followed the same trend as that of total sugar and reducing sugar content with completely ripen healthy tissue showing maximum non reducing sugars of 6.510 % followed by healthy tissue surrounding spongy tissue (6.396 %), 3/4<sup>th</sup> ripen tissue (2.558%), induced spongy tissue (1.748 %), natural spongy tissue (1.572 %) and mature unripen tissue (1.030 %).

Total starch content, total sugar content, reducing and non reducing sugar contents in the different fruit tissues are presented in Table - 4 and figures – 6 and 7.
The total starch content, total sugar content, reducing and non reducing sugar contents were significantly different at 5% significant level (P = 0.05) between the six different fruit tissues.

Mu – Mature unripen stage, 3/4⁰R – 3/4⁰ ripen stage, CR – completely ripen stage,
HSS – healthy tissue surrounding spongy tissue, Sp – spongy tissue, ISp – induced spongy tissue.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Starch (%)</th>
<th>Total sugar (%)</th>
<th>Reducing sugar (%)</th>
<th>Non-reducing sugar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MU</td>
<td>11.712</td>
<td>1.182</td>
<td>0.152</td>
<td>1.030</td>
</tr>
<tr>
<td>3/4⁰R</td>
<td>1.458</td>
<td>3.242</td>
<td>0.684</td>
<td>2.558</td>
</tr>
<tr>
<td>CR</td>
<td>0.696</td>
<td>8.028</td>
<td>1.634</td>
<td>6.510</td>
</tr>
<tr>
<td>HSS</td>
<td>0.702</td>
<td>7.732</td>
<td>1.208</td>
<td>6.396</td>
</tr>
<tr>
<td>Sp</td>
<td>4.394</td>
<td>2.214</td>
<td>0.642</td>
<td>1.572</td>
</tr>
<tr>
<td>ISp</td>
<td>3.530</td>
<td>2.532</td>
<td>0.784</td>
<td>1.748</td>
</tr>
<tr>
<td>SEM</td>
<td>0.01305</td>
<td>0.00708</td>
<td>0.01276</td>
<td>0.02</td>
</tr>
<tr>
<td>CD(P=0.05)</td>
<td>0.08542</td>
<td>0.04635</td>
<td>0.08351</td>
<td>0.07</td>
</tr>
</tbody>
</table>
**Figure – 6.** Total starch and total sugar contents in the six different tissues.

Figure 7. Reducing and non reducing sugar contents.

RS

NRS

3.2.3 α-Amylase activity:- Amylase is one of the starch degrading enzyme which was estimated in all the 6 fruit tissues. And according to the ANOVA, CRD method α-Amylase activity was significantly different at 5% significant level (P = 0.05) between all the six different fruit tissues. As per the results in Table - 5 amylase activity was almost double in the completely ripen healthy tissue (82.8874 U) when compared to the spongy tissue (34.0210 U). Healthy tissue surrounding spongy tissue showed slightly less activity (73.5465 U) when compared to the completely ripe healthy tissue. The activity was more in the induced spongy tissue (41.9225 U) when compared to the natural spongy tissue (34.0210 U). Mature unripe tissue showed very less enzyme activity (23.6445 U) than the rest of the tissues. 3/4th ripen tissue showed an activity of 65.2614 Units. Due to the lesser activity of amylase enzyme accumulation of starch was more in the affected spongy tissue.

Specific activity of amylase enzyme was also more in the completely ripen healthy tissue (4.5063 U/mg protein) when compared to the spongy tissue (3.9187 U/mg protein). Induced spongy tissue showed slightly more activity (4.5089 U/mg protein) than the natural spongy tissue. 3/4th ripen tissue and healthy tissue surrounding spongy tissue showed the specific activities of 3.9105 U/mg protein and 4.8284 U/mg protein respectively. Mature unripe tissue showed least activity of 2.4428 U/mg protein.

3.2.4 Invertase activity:- As per the statistical analysis of variation, CRD method invertase activity was significantly different at 5% significant level (P = 0.05) between the six different fruit tissues. And also the Invertase activity increased with the continuation of ripening process from mature unripe stage (15.9148 U), 3/4th ripen stage (26.8057 U) to the completely ripen stage (32.0159 U). The enzyme activity was nearly 1.5 times more in the completely ripen healthy tissue (32.0159 U) in comparison with the spongy tissue (17.4358 U). Healthy tissue surrounding spongy tissue showed an activity of 31.2236 U. The activity was slightly more in the induced spongy tissue (18.9294 U) when compared to the natural spongy tissue (17.4358 U). Mature unripe tissue showed minimum enzyme activity (15.9148 U) than the rest of the tissues.
Specific activity of invertase enzyme was also more in the completely ripen healthy tissue (5.0307 U/mg protein) and healthy tissue surrounding spongy tissue (4.9773 U/mg protein) when compared to the spongy tissue (2.4486 U/mg protein). Induced spongy tissue showed slightly more activity (2.9839 U/mg protein) than the natural spongy tissue. 3/4th ripen tissue and mature unripe tissue showed the specific activities of 4.1545 U/mg protein and 1.7222 U/mg protein respectively.

The activities of α – amylase and invertase enzymes are presented in Table - 5 and Figure - 8.
Table 5. The activities of α - amylase and invertase enzymes.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Amylase</th>
<th>Invertase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µgm maltose/min/gm FW</td>
<td>µgm maltose/mg protein</td>
</tr>
<tr>
<td>MU</td>
<td>23.6445</td>
<td>2.4428</td>
</tr>
<tr>
<td>CR</td>
<td>82.8874</td>
<td>4.5063</td>
</tr>
<tr>
<td>HSS</td>
<td>73.5465</td>
<td>4.8284</td>
</tr>
<tr>
<td>Sp</td>
<td>34.0210</td>
<td>3.9187</td>
</tr>
<tr>
<td>ISp</td>
<td>41.9225</td>
<td>4.5089</td>
</tr>
<tr>
<td>SEM</td>
<td>0.3772</td>
<td>0.0160</td>
</tr>
<tr>
<td>CD(P=0.05)</td>
<td>1.1038</td>
<td>0.1049</td>
</tr>
</tbody>
</table>

The activities of α - amylase and invertase enzymes were significantly different at 5% significant level (P = 0.05) between all the six different fruit tissues.

Figure 8. The activities of α-amylase and invertase enzymes.

Amylase

Invertase

Each bar in the graphs represent enzyme activity/gm FW
Mu – Mature unripen tissue, 3/4thR - 3/4th ripen tissue, CR – completely ripen tissue,
HSS – healthy tissue surrounding spongy tissue, Sp – spongy tissue and
ISp – induced spongy tissue.
4. **DISCUSSION**

4.1 **Respiratory metabolism**

Mango fruit ripening is associated with cell wall metabolism and also with respiratory metabolism. Respiratory metabolism involving the activities of malic enzyme, pyruvate decarboxylase enzyme. During ripening there is a rapid influx of respiratory substrates via malate oxidation and also via glycolytic and tricarboxylic acid pathways. And malic enzyme may play an important role in the oxidation and mobilization of storage malate into the respiratory (glycolytic) pathway. In the present study the malate dehydrogenase activity increased almost 3 times more from mature unripen stage to the 3/4th ripen stage. At the 3/4th ripen stage the activity reached the maximum peak and then decreased as the fruit further ripens. The completely ripen healthy tissue and healthy tissue surrounding spongy tissue showed about 1.5 times lesser activities than the 3/4th ripen stage.

Similar facts has been reported by Ian.A.Dubery et al. (1984). They studied the malic enzyme activity and related biochemical aspects during ripening of mango fruits cv.Haden. According to which malate dehydrogenase activity developed gradually during the climacteric rise, reached a maximum slightly ahead of the peak in respiration and then diminished again. Even Hamid Lazan et al. (1990) also reported that the malic enzyme activity increased to a peak level, and then decreased during ripening of Harumanis mango.

In the present study the Malate dehydrogenase activity in the spongy tissue was in between the mature unripen and 3/4th ripen stage i.e. its activity was more than the mature unripen stage but slightly less than the 3/4th ripen stage. This indicates that the spongy tissue is less ripe than the completely ripen healthy tissue. Shantha Krishnamurthy (1981) also reported the higher activity of malic enzyme in the affected pulp than in healthy pulp. Induced spongy tissue showed very less MDH activity. This is because of heat
treatment which suppressed the enzyme activity. Pyruvate decarboxylase (PDC) enzyme may also play a significant role in respiratory metabolism possibly via its involvement in the anaplerotic reaction necessary to sustain the tricarboxylic acid (TCA) cycle.

As per the present experimental results the PDC activity also increases rapidly with the onset of ripening process and diminishes upon ripening. Here also the activity showed a maximum peak at the 3/4th ripens stage. Spongy tissue and induced spongy tissue showed very least PDC activity which was about 28 times lesser the activity of ripen healthy tissue. Due to this also the breakdown tissue remains unripe and also there was accumulation of acetaldehyde content which may bring about the physiological deterioration of fruits. The specific activities of both MDH and PDC enzymes were maximum in the 3/4th ripens stage as compared to the completely ripen stage. Even though the protein concentration was more in the completely ripen tissue due to the lesser enzyme activity it showed lowered specific activity.

4.2 Starch metabolism

Mango fruit at the mature green stage contains some starch accumulated and during fruit ripening the starch is hydrolyzed and there is a concomitant increase in total sugar level. Here in the present study starch and the starch degrading enzyme activities namely α-amylase and invertase were estimated. According to the report the starch content decreases remarkably from mature unripen stage to 3/4th ripen stage and then to completely ripen stage i.e the accumulated starch in the mature unripen stage was hydrolyzed gradually with the onset of ripening process.

Similarly Katrodia et.al.,(1988) reported that the starch content of unripe fruit was as high as 12.87% while the healthy pulp around spongy as well as around sun-desiccated tissue behaved like ripe pulp leaving behind little quantities of unhydrolyzed starch. similar findings were reported by Leley et.al.,(1943) in respect of starch contents in ripe and unripe alphonso mango fruits. α-Amylase and invertase enzymes play a significant
role in the hydrolysis of starch. There was a steep increase of amylase activity and invertase activity from mature unripen stage to the 3/4th ripen stage. The amylase activity increased about 25 times more and the invertase activity increased 2 times more from the unripen stage to the 3/4th ripen stage.

Mattoo et.al.,(1975) and Majmudar et.al.,(1981) also reported that the α-amylase has been found to increase at least 4 fold in activity during mango ripening. The considerable decrease of starch content during fruit ripening can be correlated with the increased activity of α-amylase enzyme. The spongy tissue showed nearly 6 times more of starch content as compared to the completely ripen healthy tissue. And also the spongy tissue showed lowered activities of amylase and invertase enzymes which were about half the activities of completely ripen healthy tissue. Therefore it may be concluded that the accumulation of starch granules in the affected spongy tissue was due to the reduced activities of amylase and invertase enzymes. In the induced spongy tissue the starch content was lesser than the natural spongy tissue which could be because of slightly higher starch degrading enzyme activities in the induced spongy tissue.

Earlier workers have recorded Similar observations with respect to the accumulation of starch granules in the affected tissue. For instance Rangawala (1975) observed 1.47% starch in the spongy tissue against no starch in the healthy ripe pulp whereas Katrodia (1979) observed 7.98% starch in the spongy tissue, 0.25% in the healthy tissue and 12.87% in the mature unripe tissue. Nuevo et.al.,(1984a) reported that the parenchyma cells of the spongy tissue contained an average of 18 starch granules per cell in contrast to only 2 granules in the cells of healthy tissues. Gupta et.al.,(1985) reported that the spongy tissue exhibited about 16 times lesser amylase activity and 2.5 times higher invertase activity as compared to the healthy pulp.

According to Patkar (1984) starch was completely absent in the healthy ripe fruits, negligible in the unaffected part of the affected fruit, and 8.25% of starch in the spongy tissue. He reported that the reduced hydrolysis of starch in the spongy tissue was due to the decreased amylase activity. Chhatpar et.al.,(1968) also reported the decreased
amylase activity in the spongy tissue. Further Katrodia (1988) reported that amylase activity in the healthy ripen pulp was as high as 3.21U while the spongy tissue showed lower activity of 0.39U and sundesiccated tissue showed an activity of 0.50U. He stated that the decreased amylase activity in both the types of affected tissues seems to be the result of high temperature effect in the pulp during fruit ripening. Lima et al., (2001) also reported higher starch content and lower amylase activity in the spongy tissue of Tommy Atkins mango. Similar trend was also reported by Conn and Stumpf (1971). As the protein concentration was more in the completely ripen healthy tissue the specific activities of amylase and invertase enzymes were more than the spongy tissue.

In the present research study total sugars, reducing sugars and non reducing sugars were estimated in all the six different fruit tissues During normal ripening of the mango fruit, the starch is being broken down by higher activities of amylase and invertase and it is converted into glucose, fructose and sucrose in the fruits pulp. This phenomenon is well exhibited by the healthy ripen tissue. The total Sugar, reducing and non reducing sugars increases gradually from the mature unripen stage to the 3/4\textsuperscript{th} ripen stage and then to the completely ripen stage which may due to the normal enzymatic activities of amylase and invertase enzymes. The total Sugar content was almost 4 times more, the reducing sugars were nearly 2 times more and the non reducing sugars were 5 times more in the completely ripen healthy tissue in comparison with the Spongy tissue. Several reports are available in favor of the above facts.

For instance, Amin (1967) reported appreciable reduction of non-reducing sugars in the spongy tissue affected ripe fruit as compared to healthy one. Lima et al., (2001) also reported lesser reducing and non-reducing sugars in the spongy tissue of Tommy Atkins mango fruits. Similar findings were observed by Chhatpar et al., (1968-69), Subramanyam et al., (1971), Rangawala (1975) and Katrodia (1988). Contrary to this Patkar et al., (1984) reported slightly higher reducing sugars in the spongy tissue when compared to the healthy tissue. However the non-reducing sugars were considerably lower in the Spongy tissue. The lesser quantities of sugars in the natural Spongy tissue and heat induced spongy tissue may be attributed to the retarded activities of amylase and
invertase enzymes and as a result the fine regulatory phenomenon of starch catabolism was affected which leads to the accumulation of higher content of starch in the affected pulp.
5. References


Rangawala, A. D., 1975. Changes in chemical composition of Alphonso fruits during ripening with particular reference to spongy tissue. MSc.(Agri) thesis (unpublishshed) submitted to konkan krisihi vidhyapeeth, Dapoli (Ratnagiri), India.


