CHAPTER - 6

STUDIES ON THE CHANGES IN THE LEVELS OF CARBOHYDRATES DURING THE FERMENTATION OF HAMEI

INTRODUCTION

During the pathogenesis by certain fungi and bacteria in the tissues significant rise or fall in carbohydrates was recorded (Mc Combs and Winstead, 1961, 1964; Ghosh et al 1964, 1969; Bilgrami, 1970 and Prasad 1977. The accumulation of sugars in the infected sites might be due to the transport of sugars by the pathogen for the growth (Irman 1965). Yarwood and Jacobson 1955 explained that sugars might be transformed to the infected sites from non-infected sites due to the metabolic sink which may result from increased metabolic activity at the infection sites. Mandokhot et al (1976) and Bilgrami et al(1980) gave their views that the accumulation of the carbohydrates appears probably due to the hydrolysis of the starch. Garg and Mandhar (1975) also reported that sugar content in the infected leaves was 31.86% higher than that of the control on the other hand starch content was highly reduced in the infected as compared to the control. This may be due to the probable higher rate of conversion of starch to sugar in them. The increased in
Sugar contents due to the fungal infestation has earlier been recorded by Grewitz and Durbin (1960), Swamy (1964), Prasad and Poddar (1976). Virus infection influences the carbohydrate level of the leaves by influencing the rates of synthesis or the rates of translocation and it disturbs the carbohydrate nitrogen ratio especially on mosaic affected leaves (Danlop 1930). Owing to the activation of the carbohydrate hydrolysis in the infected tissue the carbohydrate level is depleted (Schipper and Mirocha, 1960). Thite et al. (1980) also reported that in the powdery mildew fungus infected leaves a significant reduction of carbohydrates was recorded.

Reduction in starch contents in infected tissues has been earlier reported and attributed to the starch hydrolysing enzyme Bmymlase which converts starch into simpler sugars (Schipper and Mirocha 1968, and Kandyawamy 1972). Degradation of starch has been suggested to be mainly due to involvement of L-amysase enzyme (Manner 1974).

Reducing sugars increase while starch, total soluble sugars and non-reducing sugars decreased due to the disease development in *Tephrina maculans* infected leaves of *Curcuma longa* (Agrawal et al. 1982). Ghosh et al.
(1969) reported the reduction of reducing sugars in leaves of *Lagenaria Vulgaris* within 10 days after inoculation with bottle gourd mosaic virus. In the low sugar diseases like helminthosporiola, the sugar content decreased due to infection (Dayal and Joshi, 1966). The decrease of carbohydrate was also reported by Thind et al. (1981) in *Xanthomonas Vesicatoria* infected leaves of *Capsicum annum*. They attributed this to partial utilization by the Pathogen or partial hydrolysis resulting into simple sugars.

In this chapter the changes in the levels of carbohydrates during the fermentation of Hamei were investigated.

**MATERIALS AND METHODS**

Powdered materials were extracted twice or thrice with hot 80% ethanol solution on a hot water bath. The alcohol extract was evaporated until it was free from alcohol. Then it was again evaporated with a few ml. of water (2-3) ml. and the aqueous fraction was used for the analysis of total soluble sugars, reducing and non-reducing sugars. A reagent blank without the sample was determined in triplicate.
Estimation of total soluble sugars

The amount of total soluble sugars was estimated by Anthrone method (Dubois et al., 1951). In this method anthrone reagent was used as a colour development resulting in blue-green solution. After boiling for 10 min. the absorbance was measured at 625 nm. The amount of sugar content was calculated from the standard curve using glucose.

Estimation of reducing and non-reducing sugars

The evaporated aqueous fraction was used for the detection of reducing sugars. In case of non-reducing sugars the extract was acid hydrolysed at 49° for 30 min. in a water bath for the conversion of non-reducing sugars to monosaccharide components. Nelson's modification of Somogyi's method (Nelson, 1944) was followed for the estimation of these sugars. From each extract 1 ml of the aliquot was mixed with 1 ml of copper reagent (25 ml of reagent A + 1 ml of reagent B) and boiled for 20 minutes. Arsenomolybdate reagent was then added and after 15 min. the colour intensity was measured at 500 nm. The amount of reducing sugars was determined from glucose's standard curve. The difference between the total soluble sugars and the reducing sugars estimated without hydrolysis corresponds to the non-reducing sugars.
QUALITATIVE ANALYSIS OF INDIVIDUAL SOLUBLE SUGAR

10 g. of powdered sample were taken and 100 ml. of 80% ethanol was added and boiled for 5 minutes. The whole mass was then thoroughly ground in a mortar and the homogenate was centrifuged for 20 min. The residue was again extracted with ethanol for complete removal of the sugars. After filtration the residue was washed down several times with boiling ethanol and the aqueous extract thus obtained was evaporated on a water bath maintained at 40°C. The alcohol free extract was redissolved in water and the clear filtrate was then treated with Dowex (20-50 mesh, Cl\(^-\)) and kept for at least one hour with occasional shaking. Then it was filtered through a Whatmann No. 1 filter paper and the filtrate was concentrated in a water bath before spotting. For the detection of sugars TLC method of Stahl(1969) was employed using the following solvent and spraying reagent.

Solvent system - Ethyl acetate : Acetic acid :
Methanol : water : (60:15:15:10)
v/v.

Spraying reagent-Aniline-Diphenylamine-Phosphoric acid.

The standard sugars used as reference were glucose, galactose, ribose, xylose and maltose. A number of runs
were made in ascending and one-dimensional ways and the spots were detected after spraying. The identification of spots was done by running with 1 ml of each standard solution along with the unknown aliquots and then by comparing their colour and Rf values. The spots were individually scratched out and eluted in 1 ml of water. Then the individual sugars were quantitatively estimated following Nelson's method (1944).

**TABLE - 7**

Table: Changes in carbohydrate content (mg/g material wt. in the fresh and fermented Hamei).

<table>
<thead>
<tr>
<th>Material</th>
<th>Reducing sugar (mg/100 g)</th>
<th>Non-reducing sugar (mg/100 g)</th>
<th>Total soluble sugar (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>1062.37</td>
<td>426.30</td>
<td>1528.67</td>
</tr>
<tr>
<td>Fermented</td>
<td>101.62</td>
<td>415.6</td>
<td>141.10</td>
</tr>
<tr>
<td>Hamei</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean of the triplicate determination is presented as a mean value for every readings.
6.3 Comparison of starch contents of freshly prepared Hamei and fermented Hamei.

MATERIALS AND METHOD

Determination of starch was carried out according to Mahadevan & Sridhar. 100mg of the dried sample were extracted with ethanol and 5 ml of water were added to the residue followed by 6.5 ml of 52 percent perchloric acid. Then 20 ml of water were added and centrifuged after constant stirring. The extraction were repeated for the 30 minutes. Then, the contents were transferred into the volumetric flask and the volumes were made up to 100 ml with water and filtered through whatman No. 42 filter paper. The first few ml of the filtrate were discarded. An aliquot of the filtrate is diluted to a known volume and the sugar were analysed with Anthrone reagent. The sugar content in terms of Glucose was calculated by using a conversion factor 0.9 to convert the values of glucose to starch.
TABLE - 8

Table: Changes in starch contents during the 5th day fermentation in Hamei. The results are given in mg/100 g.

<table>
<thead>
<tr>
<th>DAYS</th>
<th>Reduction in the content of starch during 5th day of Hamei starch contents 1179.00mg</th>
<th>1142.25mg</th>
<th>26.75 mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Results

The changes of total soluble sugars, reducing sugars, non-reducing sugar are shown in Table - and all the triplicate mean values are given for every experiment. The result shows the gradual decrease of total carbohydrates from fresh to fermented Hamei. In the investigation made for the individual carbohydrate Fructose, Glucose & Sucrose, the same relationship also was observed. During the analysis of materials having different stages of fermentation it was found that glucose was utilized completely by the micro-organism. The other
sugars also had appreciable amounts though their concentration were not as high as glucose. The identified individual sugars are sucrose, Glucose, Maltose and Galactose. The individual sugar contents in the fresh and fermented Hamei were observed unchanged. However, the amount of individual sugar was found increased in the fermented Hamei. Table 4 shows the changes in the starch contents during the 5th day of Hamei fermentation. The amount of starch content was also found decreased in the fermented Hamei to that of the unfermented (79.00 mg to 52.25 mg).

Discussion

As a result of fermentation various metabolites of host tissues were affected, thereby reducing nutritional value. The result showed the decrease of total carbohydrates from the fresh to fermented Hamei; similar conclusions were also made by Schipper and Mirocha, 1988, Dayal & Joshi 1968.

In the investigation made for the individual carbohydrates, like,Glucose and Sucrose, the same relationship was also observed (i.e. decrease in concentration of the fermentation investigation agreee with the findings of Chan and Thower (1980).
There was not much change in the Galactose content but an increased in the Maltose content was noticed. The high concentration of Maltose in the fermented Hamei might be, perhaps, due to the production of maltose during the microbial metabolism. The above results are supported from the findings of Achineuhu J (1987) which says "Fermentation decreased the total disaccharide to about 3.4 p.c. and increased the total monosachcharide to 66 p.c. of the total carbohydrate".
TABLE - 9

CHROMATOGRAPHIC ANALYSIS OF INDIVIDUAL SOLUBLE SUGARS IN UNFERMENTED AND FERMENTED HAMEI

<table>
<thead>
<tr>
<th>Samples</th>
<th>Compounds</th>
<th>Amount of individual Sugars present mg/100 g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unfermented Hamei</td>
<td>Glucose</td>
<td>.511</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>.520</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>.321</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>.387</td>
</tr>
<tr>
<td>Fermented Hamei</td>
<td>Glucose</td>
<td>.328</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>.410</td>
</tr>
<tr>
<td></td>
<td>Maltose</td>
<td>.520</td>
</tr>
<tr>
<td></td>
<td>Galactose</td>
<td>.379</td>
</tr>
</tbody>
</table>

Reference standards : Glucose, Sucrose, Galactose, Ribose, Maltose & Xylose.
Single dimensional separation of free amino acids of Hamei using standard amino acids ($S_1$-Lysine, $S_2$-glutamic acid, Tryptophan, $S_3$-Methionine, Leucine, Glycine, Cysteine, H-Hamei (fermented) $S_4$-Proline, Arginine) in solvent system n-butanol:acetic acid:water (4:1:5) V/V.