CHAPTER - 1
CHAPTER - I

GENERAL INTRODUCTION

Fermentation is a natural process which occurs when environmental conditions permit interaction between microorganisms and susceptible organic substrates. Such interactions are fundamental to the decomposition of natural materials and to the ultimate return of chemical elements to the soil and air without which life could not be sustained. Fermentation have played a vital role in a man's development from earliest times to the present. Some of the fermentations that bring changes in the food materials which are recognised to be desirable for human consumption. In other words Fermentation is a natural process in which alcohol and other related and non-related compounds are formed from the carbohydrate in the absence of air with the activities of micro-organisms. It is a biological oxidation confined to micro-organism when the final hydrogen acceptor is an organic compound. In fermentation breakdowns of the substrates is never complete and hence there is always incomplete liberation of energy. The pyruvic acid is the key intermediate in the fermentative metabolism of carbohydrate. Fermentation involves varieties of micro-organisms ranging from
bacteria to fungi, and with the variation in the microbial involvements the end products of the process also differ. The important products of fermentation are alcohol, Lactic acid and organic acids.

The production of these substances are made by the different micro-organism from the key intermediate, the pyruvic acid. The reaction after Joklik and Willet 1963, is given below:
<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Pyruvic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalacetic acid + 4H</td>
<td>Acetolactic acid</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>Acetoin + 2H</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>2,3-butanoid</td>
</tr>
<tr>
<td>Acetyl CoA</td>
<td></td>
</tr>
<tr>
<td>Ethanol + 4H</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>Acetone + 2H</td>
<td>Butyryl CoA + 4H</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>Butanol</td>
</tr>
</tbody>
</table>
The term "Fermentation" has come to have somewhat different meanings as its underlying causes have become better understood. The derivation of the word fermentation signifies a gentle bubbling or boiling condition. The term was first applied to the production of wine more than a thousand years ago. The bubbling action was due to carbon-dioxide gas liberated during the conversion of sugar, although this reaction was yet to be defined. When it was following the studies of Gay-Lussac, came to mean breakdown of sugar into alcohol and carbon-dioxide. Pasteur later demonstrated the relationship of yeast to this reaction, and the word fermentation became associated with micro-organisms, and still later with enzymes. The early research on fermentation dealt mostly with carbohydrates and reactions that liberated carbon-dioxide gas. It was soon recognised, however, that micro-organisms or enzymes acting on sugar did not always evolve gas. Further, many of the micro-organisms and enzymes studied also had the ability to break down noncarbohydrate materials such as proteins and fats, which yielded carbon-dioxide, other gases, and a wide range of additional materials. Currently the term fermentation is used in various ways which require clarification. Many of the fermentations are to make new and desired products, and preservative effects are accidental. The fermentations
may be brought by yeast, bacteria, molds or combination of the organisms.

The word fermentation is used in a still broader or less precise manner. We say "fermented foods" to describe a special class of food products. These foods are characterized by various kinds of carbohydrate breakdown; but seldom is carbohydrate the only constituent acted upon. In fermented foods we are virtually always dealing with a complex mixture of carbohydrates, proteins, fats, etc., undergoing modification simultaneously, or in some sequence, under the action of a variety of micro-organism and enzyme types present. This creates the need for additional terms to distinguish between major types of changes. Those reactions involving carbohydrates and carbohydrate like materials (true fermentations) are referred to as "fermentative" changes, however, in proteinaceous materials it is designated as "Proteolytic" or "putrefactive" changes. Breakdowns of fatty substances are described as "bipolytic". When complex foods are "fermented" under natural conditions they invariably undergo different degrees of each of these types of change. Whether fermentative, proteolytic, or lipolytic end products dominate will depend upon the nature of the food, the types of micro-organisms present, and
environmental conditions affecting their growth and metabolic patterns. In specific food fermentations, of course, there is always endeavour to control the types of micro-organisms and environmental conditions to produce desired product characteristics.

For centuries fermented vegetables and crops in varied forms are widely used by different races of the world, however, different races have their own models of fermentations; and most of these processes have been based on the acts mystic arts and science "Vandomme 1982". Fermentation has wide impact on nutritional possibilities in future (Wesley, 1981, Kretschnur, 1982) and now-a-days in Japanese communities the knowledge of advance fermentation technology is understandable even to the common people. The traditional models of fermentation are becoming the basis for orienting modern techniques in food industry (Steinkraws, 1983) and considerable advances have been evidenced as noted in cucumber preservation (Rodrigio et al 1984).

CHAPTER 1.2

REVIEW OF LITERATURE

The literature relevant to the relationship of Physiology and Biochemistry of Hamei is reviewed under the
following heads: (a) Preparation of fermented foods of the world, (b) Biochemical changes involved in the fermented foods, (c) Possibilities of Alcohol production from fermented food stuff.

From the Text of several authors it can be known that the fermentation of vegetable is mostly involved with the activities of various kinds of bacteria and yeast. The typical fermentation of Sauerkraut and Pickles are carried out with the treatment of salt and in brine respectively. Fermentation has a wide impact on the nutritional possibilities in future (Wesky, 1981, Kresteechra 1982 and now-a-days in Japanese community the knowledge at advance fermentation technology is understandable even to the common people. Food products like bread, beer, wine and different liquors are brought about by the yeast; yeast and bacteria are involved in the manufacture of vinegar from sugar bearing materials and bacteria chiefly in the production of fermented milks. Molds are important in the preparation of some cheeses and oriental food (Frezier & Westhoff, 1979). The commercial manufacture and sometimes the "domestic or home" production of many of these fermented foods are began by appropriate microbial starter cultures.
The common fermented foods used by the different races of the world are given:

**TABLE - I**

<table>
<thead>
<tr>
<th>Food or product</th>
<th>Raw starting material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy sauce (Shoyu)</td>
<td>Soybeans, wheat &amp; rice</td>
</tr>
<tr>
<td>Tamari sauce</td>
<td>Soybean</td>
</tr>
<tr>
<td>Miso</td>
<td>Steamed soybeans</td>
</tr>
<tr>
<td>Tempeh</td>
<td>Soaked soybeans</td>
</tr>
<tr>
<td>Angkhak</td>
<td>Autoclaved rice</td>
</tr>
<tr>
<td>Natto</td>
<td>Boiled soybeans</td>
</tr>
<tr>
<td>Ton-fu-ru</td>
<td>Soaking soybeans</td>
</tr>
<tr>
<td>Minchin</td>
<td>Wheat gluten</td>
</tr>
<tr>
<td>Sauerkraut</td>
<td>Cabbage</td>
</tr>
<tr>
<td>Idli (India)</td>
<td>Rice &amp; black gram</td>
</tr>
<tr>
<td>Fermented fish (Japan)</td>
<td>Fish strips</td>
</tr>
<tr>
<td>Pidan (Chinese)</td>
<td>Preserved eggs</td>
</tr>
<tr>
<td>Poi</td>
<td>Taro-roots</td>
</tr>
<tr>
<td>(Hawaijjan)</td>
<td></td>
</tr>
<tr>
<td>Cakes rolls etc.</td>
<td>Wheat flours</td>
</tr>
<tr>
<td>San Francisco</td>
<td>Wheat flours</td>
</tr>
<tr>
<td>Sourdough bread</td>
<td></td>
</tr>
</tbody>
</table>
Acidophilus milk
Cheese (Ripened)
Cultured buttermilk
Yogurt
Country cured hams
Dry sausages
(Salami etc.)
Olives
Pickles
Dosa
Dhokia
Ambali
Kanji (Ganji)
Kali
Soibum
(Manipur name)
Ngari (Manipur name)
Hawaijar
(Manipur name)
Hentak (Manipur name)
Hamei (Manipur
... Milk
... Milk Curd
... Milk
... Milk and milk solids
... Pork hams
... Pork, beef,
... Green olives
... Cucumbers
... Rice & Pulse
... Rice & Bengal gram
... Finger milt flour and
... Cooked rice
... Cooked rice
... Soft bamboo shoot
... Phabou (Small fishes)
... Soybean
... Small dry fishes + Stem
of Arum
... Rice + bark of Albizia
   myriophylla.
The chemical changes which take place during food fermentation have been fully investigated in Nigeria where fermented foods are in common use. The understanding of the chemical changes during fermentation may help the fermenter direct process to the desired product. The changes in the levels of nitrogenous substances during fermentation had been reported by Kim & Masalygina, 1970. The nutritional values of vegetable was said to be increased during fermentation with the liberation of more amounts of free amino acids (Tashupher et al 1981, Tongual et al 1981). The fermentation was noted from the works of Noda et al 1982, Sakata et al 1982), Shiniza and Watanabse 1982.

Fermentation decreased the total disaccharide and increased the total mono-saccharide composition where as it did not have much effect on the fatty acid composition except for a slight increase in the total unsaturated and decrease in total saturated fatty acid (Singh, R.P., 1987). During the 5th day fermentation, the soluble nitrogen increased was reported by S.C. Achiniautiu (1986). Increase in protease activity, Amylase activity and total reducing sugar during fermentation of soy sauce moromi had also been demonstrated by (S.C. Achiniautiu
The fermented Hamei has been used by the natives of Maniour belonging to Schedule castes and Tribes to feed the cattle as an ingredient of cattle feeds.

It is specially also used as a catalyst or accelerator in the preparation of local liquor after mixing with the cooked rice.

This liquor is not only consumed by some section of Manipuri, but also it has been reported to be used in the preparation of medicine. Hence, the Hamei may enrich the economy of Maniour.

Till this day no scientific studies on the Biochemistry and physiology of Hamei have been reported so the research work on this important fermented stuff has been taken up.
DEFINITION OF HAMEI

'Hamei' which is an indigenous feed of the cattle and also an indespensable catalyst in the preparation of locally made liquor, is prepared by mixing pre-soaked uncooked rice with the juice or small fragments of a special plant *Albizia myriophylla* locally known as Yangli. The preparation of Hamei is a popular domestic business for the people of Schedule Castes and Tribes in Manipur.

Hamei is prepared and used in the preparation of local liquor not only in Manipur but also in some states of North Eastern India, like, Tripura, Arunachal Pradesh and Mizoram etc. For Example, in Tripura, it is locally known as Chowan and is prepared with soaked uncooked rice by mixing with the following ingredients: (a) Tender portion of pineapple leaf (b) Jackfruit Leaves (after removing the mid-rib portion) (c) Dried chillies (d) Bark portion of a specified kind of tree which is locally known chowan tree (in Tripuri Language). The mixture so prepared is made fermented and
used for preparation of Liquor.

In Arunachal Pradesh it is locally known as Appu and it is prepared by crushing rice or millet with some husk and made fermented by covering with colocasia leaves for 5 days. After 5 days, the fermented Appu is ready for preparation of local liquor. The Hamei so prepared seems to contain important substances like Protein, Carbohydrate, Alkaloid, Vitamins, Minerals etc. It is used in one form or other by some section of people of Manipur for commercial purposes. However, till to date there is no record of scientific studies of this fermented food "Hamei". Keeping in view of the importance of this fermented mash the problem on the physiology and biochemistry of Hamei has been chosen for the present research work.

The thesis may be divided into the following chapters:

1. Preparation of Hamei
2. Improved method of Hamei preparation
3. Studies on the role of Hamei in the production of Alcohol in Manipur and comparative study of Alcohol Production at different doses of Hamei.

5. Studies on enzyme activities in different stages of Hamei fermentation.

6. Studies on by-products formed during the fermentation of Hamei.

7. Studies on alkaloid content of Hamei.