CHAPTER - 1

INTRODUCTION

1.1 Food -

Food is any substance or mixture of substances which when taken into body serves to nourish body or to supply energy to the body (Rahman, 2006). Food is any substance that can be eaten or drunk by human being. It does not include water and pharmaceutical (drug) products. It is usually composed of carbohydrates, fats, proteins, minerals, vitamins and growth factors. The ingredients used during preparation of food (spices, condiments and flavouring agents) are also included in food.

Food is essential requirement for existence of life. It meets the needs for energy, building the body, homoeostasis and protection of the body. Good health for healthy appearance, efficiency and emotional well being is result of right choice of food with respect to kind and amount.

Food of human being is used to ensure growth of children, maintain health and to meet special needs of pregnancy, lactation, recovery from illness and exhaustive work.

Being rich in nutrients food provides good nutritional environment for growth of microorganisms, leading to food spoilage. Depending upon the ease of microbial spoilage, various types of foods viz. vegetables, fruits, milk and dairy products, meat and poultry, eggs, seafood, sugar and sugar products, cereal and cereal products, are categorised in three major categories as perishable, semiperishable and non-perishable. Vegetables and fruits come under perishable and semi perishable category (Frazier, 1999; Jay et al., 2005).

1.2 Methods of food preservation -

Perishable and semiperishable foods can be preserved by various methods of preservation which include physical methods (refrigeration and dehydration), chemical methods (addition of vinegar, lactic acid, salt, sugar, chemical
preservatives like benzoic acid, parabens etc.) and microbial fermentation method (e.g. pickle preparation). Fruits and vegetables which come under perishable and semiperishable categories need to protect from microbial spoilage and hence can be preserved by pickling methods (Giridharilal, 1986). These are conventional methods of preservation which have limitations. Use of chemical preservatives in foods although increases shelf life, can cause health hazards and may change natural aroma and flavour. The recent trend in consumers is to buy the product without chemical preservatives, free from additives, just natural with assured safety and better shelf-life. Considering these needs naturally occurring biopreservatives which are produced by microorganisms, are practiced to preserve foods. Fermented milk products like curd and pickles used from ancient times, are good examples of biopreservation of food by using microorganisms.

1.3 **Fermentation and fermented foods –**

Fermentation is the process in which chemical changes are brought about in an organic substrate whether carbohydrate or protein or fat or some other type of organic material, through the action of the biochemical catalysts known as “enzymes” elaborated by specific types of living microorganism (Prescott and Dunn, 2002). In this metabolic process carbohydrates and related compounds are oxidised with release of energy in the absence of any external electron acceptors. The final electron acceptors are organic compounds produced directly from the breakdown of the carbohydrates (Tortora *et al.*, 2004; Thieman and Michael, 2009). Thus fermented foods are those which have been subjected to the actions of microorganisms which partially oxidise carbohydrates and other nutrients to the products like alcohols, acids, amino acids and other aroma and flavouring compounds.

The microbial food fermentation is carried out for (Sivasankar, 2002):

1. Modification of food or production of new form of food e.g. bread, idli.
2. Production of aroma and flavouring compounds in food e.g. buttermilk
3. Preservation of food to increase shelf-life e.g. sauerkraut and pickles

4. Production of food chemicals and additives e.g. vinegar, citric acid, etc.

Alcoholic beverages, vinegars, fermented milk products, fermented pickles, fermented fish and meat products, fermented other plant foods are six types of traditional fermented foods (Joshi and Pandey, 1999).

Vegetables and fruits are preserved by pickle formation. It is combination of salting and fermentation. Salting process selectively controls microorganisms while in fermentation different acids and other compounds are produced by the action of microorganisms to cause stabilisation of fruits and vegetables.

1.4 Pickles -

Pickle is the preparation made from sound, clean, raw or sufficiently matured fruit or vegetable or combination of both, free from insect damage or fungus attack, preserved in salt, acid, sugar or any combination of the three. They belong to the products stabilised with salt and lactic acid which is accumulated by lactic acid bacterial fermentation (Aktan et al., 1999). However, the products prepared by using vinegar are also included under pickles (MCCIA, 1955).

1.4.1 Consumption of pickles -

Pickles represent an essential part of people’s diet worldwide. They represent a traditional dietary element. Pickle formation is done on domestic scale and is inexpensive. The process usually enhances the organoleptic and nutritional quality of final fermented pickle which is highly appreciated by consumer. Pickles are usually used as condiments accompanying the main diet constituent. They are an essential part of Indian foods where in addition to regular meals they are also eaten along with breakfast dishes.

The pickles obtained by fermentation have been accepted due to protective effect on human health especially observed in the case of gut where
the microorganisms involved in fermentation play role by synthesising vitamins, essential amino acids and proteins (Giraffa, 2004). Due to their organoleptic properties like rich taste, palatability, stimulation of flow of gastric juice and appetizing effect, the pickles are advised to the patients with fever who have lost the taste and developed nausea for food (Khader, 2001).

1.4.2 Main raw materials used for pickles -

The microbially fermented pickles are prepared using different types of materials like brassicaceous vegetables (cabbage, cauliflower, mustard vegetable), root vegetables (carrot, turnip, beet-root, radish), vegetative fruits (cucumber, olive, tomato, pepper, green bean, chilli, drumstick), bulbs (garlic and onion) and fruits (apple, pear, lemon, mango) (Montet et al., 1999).

In addition to different fruits and vegetables, though rare, some fishes and mutton is also used for non vegetarian pickle production in India using common fishes like surmai (Cybium commersoni), kolambi (prauns: Penaceus sp.), bombil (Bombay duck: Harpadon nehereus) and mutton of sheep (Capra hyrchush). Some non vegetarian pickles found in countries other than India include egg pickles (British), pickled pig’s feet (South America) and pickled sausages of poultry (Poland and Czech Republic) (http://www.bbc.co.uk.2007).

1.4.3 Other ingredients of pickle:

In addition to main raw materials, ingredients added during pickle production include chilli, turmeric, compounded asafoetida, fenugreek seed powder, common salt, garlic, mustard seeds, fennel seeds, clove, black pepper, jaggery, edible oil, lemon juice, vinegar and permitted class II chemical preservatives like benzoic acid and parabens (Jay et al., 2005).

1.4.4 Classification of pickles -

Pickles are classified on the basis of method of preparation and ingredients used for pickle preparation. There are main two types of pickles.
1. **Quick pickles** - These are unfermented pickles, made by adding acids like acetic acid (vinegar), citric acid etc. or by addition of common salt.

Quick pickles made by addition of only common salt are called ‘Dry salted pickles’; while those prepared by combination of acid and salt are called ‘Brined pickles’.

2. **Fermented pickles** - These pickles are prepared using microbial fermentation especially through action of lactic acid bacteria. The naturally occurring lactic acid bacteria are stimulated by the addition of salt and other ingredients. As lactic acid bacteria grow; they ferment sugar of raw materials and produce lactic acid. The fermentation based pickles require ripening period of several weeks.

Fabian and Switzer (1941) classified pickles depending upon the methods of preparation: as dill pickles, sour pickles and sweet pickles (Prescott and Dunn, 2002). However, the pickles prepared in India are mostly fermented pickles where natural microflora especially the lactic acid bacteria act as the principal fermenting organisms.

1.4.5 **Role of microorganisms in pickle fermentation** -

Vegetables and fruits harbour variety of microorganisms including useful as well as spoilage causing bacteria, yeasts and molds. Spoilage causing organisms are more in number than number of lactic acid and other useful bacteria. The natural composition of fruits and vegetables is not favourable for spontaneous growth of lactic acid bacteria. During pickle formation, favourable environment for lactic acid bacteria is created by adding high concentration of salt and other ingredients and by overlaying edible oil on it. Promotion of lactic acid bacteria forms principally lactic acid from available sugar, dropping the pH of fermenting contents to about 4.0. This acidic pH inhibits growth of spoilage causing microflora and pathogenic bacteria (Montet et al., 1999). The pickle fermentation is the oldest method of food preservation which is accomplished by the natural (spontaneous/traditional/conventional) fermentation process or by
controlled fermentation i.e. inoculation with desired and selected starter organisms.

a) Natural/Spontaneous/Traditional/Conventional pickle fermentation

Raw material used for pickle production includes vegetables and fruits which harbour gram negative and positive bacteria including lactic acid and some spore forming bacteria. The number of lactic acid bacteria generally found is about 0.1% of total microbial population, indicating predominance of undesirable and spoilage causing organisms (Daeschel and Flemming, 1987; Frazier, 1999). During traditional pickle fermentation 20-80 g/L or more sodium chloride (common salt) is added (Montet et al., 1999). This high salt concentration causes plasmolysis of unwanted bacteria (especially gram negative bacteria) and plant cells creating anaerobic conditions (Montet et al., 1999). Release of nutrients from plant cells promotes growth of salt tolerant lactic acid bacteria.

As the fermentation proceeds acidity of the pickle gradually increases and the acid tolerant *Lactobacillus* species persist in the succession growth of *Pediococcus*, *Leucunostoc* and *Lactobacillus*. Total acidity as lactic acid is 0.6 to 0.8% after completion of fermentation. Traditional pickles require 6-9 weeks to complete fermentation depending upon salting and temperature applied (Frazier, 1999). The residual sugars allow growth of contaminant acidophilic yeasts which destroy lactic acid by oxidation further allowing spoilage organisms to grow (Frazier, 1999).

b) Controlled fermentation -

Natural fermentations cannot maintain constant quality of product due to microbial diversity in it. The variation in quality is tolerated, though on small scale production (home made pickles), it is not acceptable on industrial scale where the controlled fermentations are used to maintain the constant quality. In the controlled fermentation known microbial starter cultures are used for fermentation where there occurs promotion of growth and development of
commensal lactic acid bacteria concomitantly eliminating natural spoilage causing flora of raw material. The controlled fermentation is designed to minimise drawbacks of traditional or natural fermentation.

In controlled fermentation pretreatment is recommended for raw materials to eliminate spoilage causing microorganism, which includes:

1) Mild radiation treatment
2) Washing with chlorine solution
3) Acidification with glacial acetic acid.

Chlorination (80 ppm) and acidification (6 mL/gallon) effectively suppresses the undesired microflora. Mild radiation treatment (U.V.) allows radioresistant organisms i.e. lactic acid bacteria to survive. Such pretreated raw materials are then inoculated with special selective starter cultures of lactic acid bacteria (Frazier, 1999), the selection of which depends upon type of fruit or vegetable (Pederson, 2000).

1.4.6 Diatery importance of microbially fermented pickles -

1. Pickles are rich in fiber content, minerals, salts and vitamins.

2. Microorganisms involved in pickle formation have therapeutic value. Lactic acid bacteria are considered to decrease serum cholesterol. Lactic acid bacteria colonise in intestinal tract and prevent intestinal pathogens to establish (Khader, 2001). Hence the lactic acid bacteria are used as ‘Probiotic organisms’.

3. Biogenic amines like histamine produced by lactic acid bacteria cause headache and fever while the D (-) lactic acid produced by these organisms leads to loss of Ca$$^{++}$$ and Mg$$^{++}$$ from the body. Hence the lactic acid bacteria found in natural pickles and producing biogenic amines and D (-) lactic acid are to be eliminated from pickles by replacing with desired microbial starter cultures.
From ancient times pickles of various kinds are prepared in Indian homes; which are of traditional fermentation type and there is no major problem of uniform quality and taste, as they are prepared on very small scale. Salt and oil added are inhibiting spoilage causing microflora. Still many a time’s homemade pickles get spoiled by mold or yeast growth and rarely by formation of gas in the containers by undesirable bacterial action.

On the other hand at industrial scale also pickle production encounters many problems, viz. no uniform quality, microbial spoilages like gas formation, undesirable aroma, flavour, body and texture. Storage and distribution on long distance requires more shelf life which can be achieved by preventing growth of spoilage causing organisms.

Chemical preservatives, generally toxic but still invariably are used commercially to inhibit spoilage causing microorganisms. But these days’ alert customers demand for products without chemical preservatives and products with natural preservatives.

To achieve goal of discouraging indiscriminate use of toxic chemical preservatives and avoiding pickle spoilages caused by failure of natural starter organisms, the controlled fermentation using promising starter microorganisms and pretreated raw materials will be the right approach. The antimicrobial compounds produced by starter microorganisms can replace chemical preservatives and elimination of undesired and spoilage causing organisms by pretreatment will add to produce desired quality pickles with prolonged shelf life.