CHAPTER – 6

SUMMARY AND CONCLUSIONS

6.1 Summary:

Pickles have been an integral part of our heritage and diet from time immemorial. Diversity in Indian culture is aptly reflected in the diversity of pickles throughout the country. The current study has been only a ‘curtain-raiser’in the vast food microbiology arena that can be explored systematically. Pickle-making is both, an art and science. However, our culture has heavily depended on the art part of the process. With the onset of commercialization of the process, on a large scale production by a large number of industrial houses along with medium and small scale establishments and as a cottage industry, the time has come to bring in fresh ideas and know-how to be introduced in the making of pickles. Also, with an increasing awareness of the harm caused by additives/chemical preservatives in order to enhance palatability and shelf life, this is an appropriate time to re-evaluate and ponder on the production processes, to reintroduce “Organic” concepts, from where we have knowingly or unknowingly deviated.

The approach of current study has based on applying basic microbiological principles, Sensu strict, cut down on chemical additives and encourages “Wholesomeness” with an additional benefit of improved organoleptic quality as well as shelf-life.

Keeping above aspects in mind in the present study the work was carried out with respect to qualitative and quantitative aspects of yeasts, molds and bacteria from main raw materials and other ingredients of pickles, from pickles in process, desired quality pickles and spoiled pickles. The desired quality and spoiled pickles were differentiated on the basis of their microbiological, biochemical and organoleptic characteristics. The microbial isolates were characterised morphologically, culturally and biochemically preferably up to genus level. The undesirable property of food i.e. genotoxicity,
of different pickles was studied using bacterial mutagenicity test. Using selected and safe lactic acid bacterial isolates and antifungal bacteria a consortium was prepared and used as starter mix to optimise the pickling process and using 12 different types of pickle raw materials. Various microbial activities as well biochemical changes therein were studied with respect to pH, titrable acidity, aroma and flavouring compounds (diacetyl) which ultimately decide organoleptic qualities of the final product. The novel method of growth and preservation of selected starter bacteria in the mango seed endocarp was successfully attempted. The starter mix bacterial isolates were identified by using advanced method of 16S r RNA sequencing. Organoleptic properties and shelf life studies were carried out using Test sets (using prepared starter mix) and by comparing with Control sets (without use of starter mix) of pickles.

Based on the preceding research, the following findings are observed:

A) Out of a total 510 pickle samples, 144 samples (28.23%) were found spoiled.

B) In spoiled pickles LAB count was considerably lower than NLAB count. The spoilage causing organisms found were bacteria, yeasts and molds. The bacterial isolates identified from spoiled pickles were *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus*, *Bacillus* sp. and pathogens like *E. coli*, *Salmonella*, *Klebsiella*, *Proteus* and *Pseudomonos* sp. The common fungal isolates were *Aspergillus*, *Penicillium* and *Candida* sp. Presence of pathogens is a pointer towards unhygienic preparation and handling of pickles and the consequent diminished safety of such food product.

C) A wide variety of raw materials are used for pickle preparation which includes mango, lemon, green chilli, amla, turmeric, garlic, karonda, bhokare, minemola, idlimbu, bitter gourd and drumstick. Spoilage causing organisms and pathogens originated from main raw materials and ingredients.
D) Amongst LAB isolated from desired quality pickles, main raw materials and ingredients, the three isolates (*Enterococcus casseliflavus*, *Enterococcus durans*, *Lactobacillus plantarum*) fullfilled the criteria for starter culture. A *Bacillus* sp. (*Bacillus licheniformis*) with manifested antifungal activity is included in starter culture consortium.

The selected four isolates used in starter preparation are identified by 16S r RNA sequencing method and are found to be *Enterococcus casseliflavus*, *Enterococcus durans*, *Lactobacillus plantarum* and *Bacillus licheniformis*.

E) The starter mix prepared from equal proportions of all the four isolates was found to produce desirable quality pickles than any one of combination of these.

F) The starter preservation studies are performed for individual isolates as well as starter mix of above four isolates using glass vials and a novel method of preservation in the mango seed endocarp. Mango seed endocarp studies showed not only good preservation of these organisms but also supported the growth of them up to certain extent. These methods showed good viability and preservation up to one year.

G) The dose of starter mix for pickle making was optimised on dry weight basis to 200 mg/kg of pickle for 12 different raw materials of pickles.

H) Quantity of common salt which is used as natural preservative in preparation of pickles was reduced from 20 to 5% to produce hypo-salt pickles. To provide the preservative effect, the reduced salt content was compensated by starter mix added to pickle. The hypo-salt pickles with starter mix showed improved organoleptic qualities like taste, color, flavour and texture.

I) Hypo salt pickles prepared with low salt content and with starter mix showed decreased time for ripening and increased shelf life up to 18 months.
J) Taking into account the antimutagenic activities of LAB, study was performed to test antimutagenic ability of prepared starter culture mix. Study was also performed to screen out different pickles (branded and unbranded) available in market and homemade pickles for their genotoxicity using Ames’ bacterial test. The food adulteration due to undesired chemicals may cause genotoxicity. It was found that mutagenicity and anti-mutagenicity (neutralising) effects were shown by packed as well as loosely sold market pickles and some homemade pickles. All the laboratory made pickles which were hypo-salt and were containing starter mix showed no mutagenic effects but showed anti-mutagenic (neutralising) effects.

6.2 Conclusions -

1) Use of starter culture (starter mix/consortium) dispenses away use of chemical preservative(s) as the starter itself acts as a biopreservative.

2) Hypo-salt pickles can be produced by reducing salt concentration even as low as 5% (w/w). Use of selected starter culture for hypo-salt pickles decreases time of ripening, improves organoleptic qualities of pickles and increases shelf life. The starter mix prepared from a mixture of Enterococcus casseliflavus, Enterococcus durans, Lactobacillus plantarum and Bacillus licheniformis is very suitable for different types of hypo-salt pickles. Use of the lactic acid bacterial starter with antimutagenic ability should be promoted so as to reduce risk of genotoxicity due to use of ingredients, with unknown adultration, in pickle preparations.

Such hypo-salt pickles with selected starter cultures are suitable for hypertensive and diabetic persons and which are consumer friendly. Their commercial production may be seriously contemplated.

3) Sources of pathogenic microorganisms and spoilage causing microorganisms in pickles were found to be main raw materials i.e. fruits
and vegetables and other ingredients along with improper handling and unhygienic conditions during preparation and storage of pickles. Therefore by following Good Manufacturing Practices like proper sanitary, hygienic and aseptic conditions during handling of raw materials, preparation and storage of pickles, chances of contamination of pickles by pathogenic and spoilage causing microorganisms can be reduced which will increase shelf life as well as quality of pickles. This leads us to a stricter HACCP regime that may be imposed upon all kinds of commercial establishments engaged in pickle manufacture. This is in fact in the domain of food regulatory authorities.

4) Preservation of starter mix in mango seed endocarp is novel method of preservation of bacteria. It is cheap and the concept can be extended to other cultures for preservation and processes.