6.0 SUMMARY

6.1 Milk and many of the indigenous milk products including paneer and Khoa are known to have short shelf life. They contain all the necessary nutrients for the growth of microorganisms leading to spoilage of these items. Several preservation methods such as heat treatment, fermentation etc have been used to increase the shelf life of these products. Microwave ovens are the recent innovation and people use it to reheat and cook the foods. Microwaves are the long wave length radiations that develop heat in the materials by creating friction on polar water molecules. Since heat development is very fast and does not transfer heat to the containers, microwave is slowly gaining interest in the dairy industry also.

6.2 The present study was carried out to investigate the effect of microwave exposure of different menstra inoculated with selected bacterial cultures. The investigation also included the aspects pertaining to the isolation and characterization of bacterial contaminants of raw milk; assessment of injurious effect of microwave on the bacterial cells in different menstra; Determination of the release of DNA and protein from bacterial cells during microwave exposure and studying the effect of storage of microwave treated milks & some indigenous products such as khoa and paneer.

6.3 Raw milk samples were plated on to different selective media such as M-Endo agar for *E.coli*, cetrimide agar for *Ps.aeruginosa*, mannitol salt agar for *Staph.aureus*, Salmonella shigella agar for salmonella, *B. subtilis* was selected by subjecting the sample to $80^\circ$C heating for 10 min. and then plating onto 2% nutrient agar. Depending on the colony morphology five colonies from each selective media were selected and purified accounting for 25 isolates $M_1$ to $M_{25}$. 
6.4 All the isolates were subjected to different identification biochemical tests and of them only five isolates matched with the standard cultures and these five isolates were finally identified as *E.coli* (M₁), *Salmonella* sp (M₆), *Ps.aeruginosa* (M₁₁), *Staph.aureus* (M₁₆) and *B.subtilis* (M₂₁).

6.5 All the five cultures were individually inoculated upto 7 log numbers into MYG broth, skim milk, whole milk, paneer and khoa and these samples were then exposed to microwave for periods of upto 60 sec. At regular intervals, samples were drawn and tested for the number of survivors using MYG agar and selective agar.

6.6 In MYG broth, after 30 sec exposure, except *B.subtilis*, all the remaining four cultures were completely killed when tested using selective agar. However when tested using MYG agar, less than 1.0 log survivors was observed in all the five cultures.

6.7 In respect of both skim milk and whole milk it was observed that for complete destruction of all the test cultures, a maximum exposure time of 50 sec was required as tested with MYG agar and selective agar.

6.8 For complete destruction of all the cultures in paneer and khoa the minimum exposure time required was 60 sec. However at 50 sec except *B. subtilis* and *Ps.aeruginosa*, the remaining cultures were killed when tested using selective agar.

6.9 The number of injured cells formed during microwave exposure of MYG broth, skim milk, whole milk, paneer and khoa containing added test organisms individually was also monitored during exposure period of upto 60 sec. At intervals samples drawn were plated on MYG agar to get both healthy and injured cells and also plated on MYG agar containing 2% salt to get only healthy cells. The difference between counts obtained on MYGA and MYGA with salt was taken as injured cells.
6.10 In MYG broth, the highest number of injured cells was observed at 15 sec in case of both *Salmonella sp.*, and *Ps.aeruginosa*, 20 sec in respect of both *E.coli* and *B.subtilis*, 30 sec in *Staph.aureus*

6.11 In skim milk the maximum number of injured cells was observed at 20 sec in respect of *E.coli*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis*, while in respect of *Salmonella sp.*, it was observed at 30 sec.

6.12 In whole milk at 20 sec the maximum number of injured cells was seen in respect of both *Salmonella sp.*, and *Ps.aeruginosa*, while at 30 sec the maximum number of injured cells was observed in respect of *Staph.aureus* and *B.subtilis* and at 40 sec the maximum number of injured cells was seen in *E.coli*.

6.13 In paneer, the maximum number of injured cells was seen at 30 sec in respect of *B.subtilis*, while at 40 sec in respect of both *Salmonella sp.*, and *Ps.aeruginosa* and at 50 sec in respect of both *E.coli* and *Staph.aureus*.

6.14 In khoa samples, the maximum number of injured cells was determined and it varied from 30 sec in *Salmonella sp.*, *Staph.aureus* and *B.subtilis* to 40 sec in *E.coli* and 50 sec in *Ps.aeruginosa*.

6.15 All the test cultures were suspended in MYG broth at a concentration of $10^7$ cell / ml and then subjected to heat treatment at 80°C / 10 Min or to microwave exposure for 30 sec and the release of DNA and protein from the cells was determined.
6.16 The DNA release was lower in microwave treated cultures compared to heat treated cells while the protein content was higher in microwave treated cells compared to heat treated samples in respect of \textit{E.coli, Salmonella sp.,} and \textit{Ps.aeruginosa.} However in respect of \textit{Staph.aureus,} both DNA and protein were higher in microwave exposed cells. In contrast both DNA and protein were lower in microwave exposed cells compared to heat treated cells of \textit{B.subtilis}

6.17 The shelf life studies of microwave treated skim milk, whole milk, paneer and khoa containing added test cultures individually were carried out at room temperature and refrigeration temperature for up to 60 days. At intervals the samples drawn were examined for titratable acidity and viable count.

6.18 Both skim milk and whole milk samples inoculated with test cultures, treated with microwaves and held at room temperature showed COB positive on 9\textsuperscript{th} day of storage and curdling on 15\textsuperscript{th} day of storage. However, samples held at refrigeration temperature showed COB positive on 50\textsuperscript{th} day and curdling on 60\textsuperscript{th} day of storage.

6.19 Paneer samples inoculated with test cultures, treated with microwave and held at refrigeration temperature had nil viable count on zero and 5\textsuperscript{th} day but from 10\textsuperscript{th} day onwards the count gradually increased and reached a count of more than 6 logs at the end of 60 day storage period. The control samples without microwave treatment spoiled on 7\textsuperscript{th} day of storage. However samples held at room temperature began to show viable count on 6\textsuperscript{th} day of storage and 30\textsuperscript{th} day all the cultures had attained a viable count of more than 6 logs.

6.20 Similarly microwave treated khoa samples and held at room temperature began to show viable count on 6\textsuperscript{th} day of storage and on 30\textsuperscript{th} day the viable count was more than 6 logs. Khoa samples held at refrigeration temperature
began to show viable count on 10\textsuperscript{th} day and on 60\textsuperscript{th} day the count exceeded 6 logs.

6.21 Microwave treated paneer and khoa samples containing added test cultures were packed separately in PET sachet and stored at refrigeration and room temperature and the viable count was monitored during the storage period.

6.22 Treated paneer and khoa samples held at room temperature began to show viable count from 9\textsuperscript{th} day of storage and on 40\textsuperscript{th} day of storage, the count was more than 6.5 logs. However treated paneer and khoa samples held at refrigeration temperature began to show viable count on 15\textsuperscript{th} day of storage and on 70\textsuperscript{th} day of storage the count exceeded 7 logs.
CONCLUSION

The faster dynamic living style of modern world needs fast foods and equipments for quicker warming. The use of microwave offers benefits of short time processing, treatment of viscous or heat sensitive products and heat treatment of products after packing to prevent post processing contamination. The results of the present study indicate that spoilage and pathogenic bacteria present in milk and milk products can be inactivated by exposing them to microwave. Liquid foods would require shorter time of upto 30 sec., while semisolid foods like paneer and khoa require longer exposure time of upto 50 sec. In microwave treated samples, salt sensitive injured cells do exist and they may pose a threat to the shelf life of the milk and milk products. It is important to evolve a suitable procedure to assess the injured cells, as prolonged storage of microwave treated foods are likely to be spoiled in view of the growth and multiplication of injured cells. However, the microwave process appears to be effective for keeping the foods in good condition for short periods of storage.
Future research needs have been identified in the following areas:

- Effects of food formulation on heating patterns.
- Effects of equipment design factors, including frequency (for example, 915 MHz is sometimes proposed instead of the commonly used 2450 MHz for better uniformity of heating).
- Development of variable frequency ovens (although currently more expensive for food applications) for improved uniformity of heating.
- Understanding factors affecting heating patterns, including qualitative changes occurring with frequency changes.
- Monitoring and real-time adjusting for process deviations in microwave and radio frequency processing.
- Assessing the damage caused to microbes and the ability of injured cells to grow and become healthy cells.
- The mechanism of non-heat injury and death caused by microwave in various types of microbes.