4.0 Results

Microwave is a type of electromagnetic waves, which are non-ionizing that generates heat at faster rate. In order to assess the effect of microwave on selected bacterial cultures in milk and milk products such as khoa and paneer, the study was conducted and results of the experiments are presented in this section.

4.1 Characterization of Bacterial Cultures:

Raw milk sample was plated on to different selective media. Depending on the colony morphology, colonies were selected. A total of 26 isolates M1 to M26 were picked up. Out of these 26 isolates, 5 colonies were selected from each of the respective selective media, purified and subjected to biochemical tests.

Tables 1-5 refer to the results of various identification tests carried out on the isolates obtained from raw milk using different selective media and these included *E.coli*, *Salmonella* sp., *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis*. According to Table 1, milk isolate M1 matched with identification key of *E.coli*, while M2, M3, M4 & M5 did not match with identification key due to variation in Indole, Methyl red, VP tests and they were kept as unidentified.

As shown in Table 2, M6 was identified as *Salmonella* sp. based on typical colony formation on SS agar and hydrogen sulphide production in TSI H2S test. M7 to M10 did not match with identification key of Salmonella and hence all of them were grouped as unidentified isolates.

Table 3 denotes the identification of isolates M11-M15 obtained on cetrimide agar. Blue green pigmentation on the cetrimide agar represents the isolate M11 as *Ps. aeruginosa* and remaining isolates were unidentified isolates.

*Table 4 represents the identification of isolates M16-M20 obtained on Mannitol Salt agar. Typical yellow coloured colony of M16 was considered as Staph. aureus. M19 and M20 were identified as Micrococcus spp. where as M17, M18 were unidentified.*

M21 was identified as *Bacillus subtilis* based on selectivity by heating to 80°C/10min, spore staining and red pigmentation. M24 and M25 milk isolates were
identified as heat resistant *B. stearothermophilus*. M22 and M23 did not match with identification key of *Bacillus* spp (Table 5).

4.2 SURVIVABILITY OF MICROWAVE EXPOSED BACTERIAL CULTURES

**IN DIFFERENT MENSTRA:**

It has been established that microwaves cause death of bacteria. In order to determine the minimum exposure period required to bring out complete destruction of bacteria, selected cultures namely *E.coli*, *Salmonella* sp., *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were inoculated separately into broth, skim milk, whole milk, paneer and khoa and exposed upto 60 sec in a microwave oven. At intervals, these samples were drawn and subjected for plating using different media and the number of survivors was determined by standard procedures. As soon as the samples were removed from the microwave oven, the temperature of the samples was also recorded and the temperature ranged from 51°C to 93°C.

4.2.1 Survivability of bacterial cultures in Modified Yeast Glucose (MYG) broth:

The number of survivors of different cultures in MYG broth after microwave exposure to different periods are given in Table 6. All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 6. It may be seen from the Table that *E.coli* log count, which was 7.09 in unexposed sample, reduced to 6.85, 5.14, 3.77 and 0.39 after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However, when selective medium VRBA, was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 6.43, 3.70 and 3.31, nil after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively.

The log count of *Salmonella* sp., which was 6.60 in unexposed sample, reduced to 5.99, 3.07, 1.61 and 0.66 after exposure to 15, 20, 25 and 30 secs respectively on MYGA. However when selective medium SS-agar was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 5.20, 2.90 and 0.62 and nil after exposure to 15, 20, 25 and 30 sec respectively.
**Ps.aeruginosa** showed an initial log count of 6.44 in unexposed sample, it got reduced to 5.78, 5.02, 3.95 and nil count after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However when selective medium CA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 5.23, 4.73, 3.83 and nil after exposure to 15, 20, 25 and 30 sec respectively.

The log count of **Staph. aureus**, which was 6.63 in unexposed sample, reduced to 5.90, 5.41, 4.55 and 0.59 log count after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However when selective medium MSA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 5.34, 4.95, 3.99 & nil after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively.

Spores of **B.subtilis**, showed a log count of 3.69 in unexposed sample, and reduced to 1.77, 1.60, 1.00 and 0.36 counts after exposure to 15, 20, 25 and 30 sec respectively on MYGA. However when selective medium 2% NA was used to determine the number of survivors, it was seen that the number of survivors was less & the counts were 1.70, 1.62, 1.00 & 0.30 after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively.

### 4.2.2 Survivability of bacterial cultures in skim milk:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 7. The numbers of survivors in different cultures in skim milk after microwave exposure to different periods are given in Table 7.

As shown in Table that the log count of **E.coli**, which was initially 6.25 in unexposed sample, reduced to 6.08, 3.73, 0.78 and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However when selective medium, VRBA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 3.57, 0.81, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.
Salmonella sp. showed an initial log count of 5.92 in unexposed sample, reduced to 3.33, 1.54, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However when selective medium SSA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 2.56, 0.62, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

The log count of Ps. aeruginosa, which was 7.08 in unexposed sample, reduced to 4.33, 1.19, nil and nil count after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However when selective medium CA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 3.84, 0.77, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

Staph. aureus showed initial log count of 7.06 in unexposed sample, reduced to 4.37, 1.44, nil and nil count after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However when selective medium MSA was used to determine the number of survivors, it was seen that the number of survivors was less & the counts were 3.70, 1.01, nil & nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

The log count of spores of B. subtilis, which was 3.60 in unexposed sample initially, reduced to 2.44, 1.83, 1.18 and nil count after exposure to 20 sec, 30 sec, 40 secs and 50 secs respectively on MYGA. However when selective medium 2% NA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 2.40, 1.86, 1.00 & nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

4.2.3 Survivability of bacterial cultures in whole milk:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 8. It may be seen in Table 8 that E.coli log count, which was 6.52 in unexposed whole milk sample initially, reduced to 5.41, 3.92, 1.87 and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However when selective medium, VRBA was used to determine the number of survivors, it was seen that the number of survivors was less and the log
counts were 4.07, 2.19 nil, and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

The initial log count of *Salmonella* sp., which was 5.66 in unexposed whole milk sample was reduced to 4.39, 1.93, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, when selective medium SSA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 3.48, 0.39 nil, and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

*Ps.aeruginosa* showed initial log count of 7.20 in unexposed whole milk sample and reduced to 4.71, 2.20, nil and nil count after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, when selective medium CA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 2.99, 1.0, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

The log count of *Staph. aureus*, which was 7.26 in unexposed whole milk sample initially, reduced to 4.40, 1.52, nil and nil count after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, when selective medium MSA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 3.34, 0.30, nil & nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

The spores of *B.subtilis* showed initial count of 3.56 in unexposed whole milk sample and reduced to 2.40, 1.04, 0.30 and nil count after exposure to 20 sec, 30 sec, 40 secs and 50 sec respectively on MYGA. However, when selective medium 2% NA was used to determine the number of survivors, it was seen that the number of survivors was less & the counts were 2.35, 1.00, 0.30 & nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively.

4.2.4 Survivability of bacterial cultures in paneer:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 9. It may be seen in
Table 9 that the initial log count of *E.coli*, which was 6.93 in unexposed paneer sample, reduced to 4.34, 3.00, 0.63 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However when selective medium, VRBA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 2.50, 0.78, nil, and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

The log count of *Salmonella* sp., which was 6.84 in unexposed paneer sample, initially reduced to 4.08, 3.90, 3.90 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium SSA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 2.60, 2.47, after exposure to 30 sec, 40 sec and nil counts when exposed to 50 sec and 60 sec respectively.

*Ps.aeruginosa*, which showed initial count of 6.60 in unexposed paneer sample, was reduced to 4.25, 2.60, 1.30 and nil count after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium CA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 3.78, 2.48, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

The log count of *Staph. aureus*, which was 7.08 in unexposed paneer sample, initially reduced to 3.60, 2.30, 1.60 & nil count after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium MSA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 2.00, nil, nil & nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

The spores of *B. subtilis*, which was 3.54 in unexposed paneer sample initially, reduced to 2.78, 2.18, 1.60 and nil count after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium 2% NA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 2.68, 2.10, 1.60 & nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.
4.2.5 Survivability of bacterial cultures in khoa:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 10. The numbers of survivors in different cultures in khoa after microwave exposure to different periods are given in Tables 10. The initial log count of *E. coli*, which was 6.69 in unexposed Khoa sample, reduced to 2.9, 2.30, 1.30 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium, VRBA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 1.30, nil, nil, and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

The log count of *Salmonella* sp., which was 6.56 in unexposed khoa sample initially, reduced to 2.60, 2.20, nil and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium SSA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 1.48, nil, nil and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec.

*Ps. aeruginosa* showed initial log count of 6.90 in unexposed khoa sample was reduced to 4.25, 2.45, 1.30 and nil count after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium CA was used to determine the number of survivors, it was seen that the number of survivors was less and the log counts were 4.00, 2.20, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

The log count of *Staph aureus*, which was 6.48 in unexposed khoa sample initially, reduced to 2.48, 1.90, 1.32 & nil count after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, when selective medium MSA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 1.78, nil, nil & nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

The spores of *B. subtilis*, which was initially 3.50 in unexposed khoa sample was reduced to 2.45, 1.32, 1.19 and nil count after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.
sec respectively on MYGA. However, when selective medium 2% NA was used to determine the number of survivors, it was seen that the number of survivors was less & the log counts were 2.28, 1.30, 1.00 & nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively.

4.3 INJURED BACTERIAL CULTURES DURING MICROWAVE EXPOSURE FOR DIFFERENT PERIODS IN DIFFERENT MENSTRA:

Exposure of bacterial populations to short periods of microwave may result in a situation where some cells are killed, while the other cells may have various degrees of injury and subsequent repair of the injury to become healthy cells. It has been reported that in the presence 2% salt some of the heat injured cells of certain culture fail to form colonies on agar media. In order to determine the number of injured cells, bacterial cells were exposed to different periods ranging from 15 sec to 60 sec. The exposed bacterial cells were then plated on two different media namely MYGA to get both healthy cells and injured cells and MYGA with 2% salt to get only healthy cells. For this study the following five cultures namely *E.coli*, *Salmonella* sp., *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were inoculated separately into broth, skim milk, whole milk, paneer and khoa and exposed for up to 60 sec in a microwave oven. At intervals, these samples were drawn and subjected for plating using different media and the number of injured cells (salt sensitive) was determined by standard procedures. As soon as the samples were removed from the microwave oven, the temperature of the broth and milk samples was also recorded and the temperature ranged from 51°C to 93°C.

4.3.1 Injured bacterial cultures in Modified Yeast Glucose broth:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 11. The results of both healthy and injured cells (salt sensitive) of *E.coli* when they were exposed to microwave in MYG broth are given in Table 11 and Fig 1. It may be seen that the initial log count of *E.coli* before exposure was 7.09 and this log count reduced to 6.85, 5.14, 3.77 and 0.39 at 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However, the log count of healthy cells was lower and they were 6.52, 4.32, 3.35 and nil after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively when plated on 2% MYGA. Thus the calculated
injured log cells counts (salt sensitive) were 0.33, 0.82, 0.42, and 0.39 at 15 sec, 20 sec, 25 sec and 30 sec respectively.

*Salmonella sp.* showed the initial log count of 6.60 before exposure to microwave and this count reduced to 5.99, 3.07, 1.61 and 0.66 at 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However, the healthy cells log count, got reduced to 4.93, 2.77, 0.74 and 0 after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively when plated on 2% MYGA. Thus the calculated injured log cells counts (salt sensitive) were 1.06, 0.30, 0.71, and 0.66 at 15 sec, 20 sec, 25 sec and 30 sec respectively.

It may seen that the initial log count of *Ps.aeruginosa* before exposure to microwave was 6.44 and this log count got reduced to 5.78, 5.02, 3.95 and nil at 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However, the healthy cells log count, got reduced to 5.46, 4.88, 3.87 and nil after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively when plated on 2% MYGA. Thus the calculated injured log cells counts (salt sensitive) were 0.32, 0.14, 0.08, and nil at 15 sec, 20 sec, 25 sec and 30 sec respectively.

The initial log count of *Staph.aureus* before exposure to microwave was 6.63 and this log count got reduced to 5.90, 5.41, 4.55 and 0.59 at 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However, the healthy cells log count, got reduced to 5.59, 5.00, 4.11 and nil after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively when plated 2% MYGA. Thus the calculated injured log cells counts (salt sensitive) were 0.31, 0.41, 0.44, and 0.59 at 15 sec, 20 sec, 25 sec and 30 sec respectively.

*B.subtilis* showed initial log count of 3.69 before exposure to microwave and this log count reduced to 1.77, 1.60, 1.00 and 0.36 at 15 sec, 20 sec, 25 sec and 30 sec respectively on MYGA. However, the healthy cells log count, which was reduced to 1.46, 0.96, 0.60 and nil after exposure to 15 sec, 20 sec, 25 sec and 30 sec respectively when plated on 2% MYGA. Thus the calculated injured log cells counts (salt sensitive) were 0.31, 0.64, 0.40, and 0.36 at 15 sec, 20 sec, 25 sec and 30 sec respectively.

4.3.2 Injured bacterial cultures in skim milk:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 12. The
number of injured cells of different cultures in skim milk, using MYGA with 2% salt after microwave exposure to different periods is given in Table 12 and Fig 2.

_E.coli_ initially showed log count of 6.25 and it got reduced after microwave exposure to 6.08, 3.73, 0.66 and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count was reduced to 5.77, 3.54, and 0.51 after exposure 20 sec, 30 sec, 40 sec and 50 sec respectively on 2% MYGA. Thus the calculated injured log cells counts (salt sensitive) were 0.31, 0.19, 0.15 and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

Initially _Salmonella sp._, showed log count of 5.92 in skim milk and got reduced to 3.33, 1.54, and nil after exposure to microwave for 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count was reduced to 3.02, 1.15 and nil after exposure to 20 sec, 30 sec, 40 sec. and 50 sec respectively when plated on 2% MYGA. Thus the calculated injured log cell counts (salt sensitive) were 0.31, 0.39, nil and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

_Ps.aeruginosa_ exposed to microwave in skim milk got reduced from initial log count 7.08 to 4.33, 1.19 and 0 at 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count was reduced to 4.00, 0.89, and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively when plated on 2% MYGA. Thus the calculated injured log cells counts (salt sensitive) were 0.33, 0.30, nil and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

_Staph.aureus_ initially showed log count of 7.06 and it got reduced after microwave exposure to 4.37, 1.44, nil and nil for 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count was reduced to 3.95,1.05, nil and nil after exposure 20 sec, 30 sec, 40 sec and 50 sec respectively on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.43, 0.39, nil and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

_B.subtilis_ exposed to microwave in skim milk got reduced from initial log count of 3.60 to 2.44, 1.83 and 1.18 and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count was reduced to 1.03, 0.81, 0.26 and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively when plated on 2%
4.3.3 Injured bacterial cultures in whole milk:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 13. The number of injured cells of different cultures in whole milk using MYGA with 2% salt after microwave exposure to different periods is given in Table 13 and Fig 3.

It may seen that the initial log count before exposure was 6.52 and this log count got reduced to 5.41, 3.92, 1.87 and nil after exposure to microwave for 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count got reduced to 5.20, 3.79, 1.33 and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.21, 0.12, 0.54, and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

Initially *Salmonella sp.* had log count of 5.66 in whole milk and after exposure to microwave for 20 sec, 30 sec, 40 sec and 50 sec got reduced in log counts of 4.39, 1.93, nil and nil respectively on MYGA . However, the healthy cells log count was reduced to 3.70, 1.39, nil and nil after exposure to 20 sec, 30 sec and 40 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.69, 0.54, nil and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

*Ps.aeruginosa* showed 7.20 as initial log count in whole milk and exposure to microwave reduced the initial log count to 4.71, 2.20, nil and nil at 20 sec, 30 sec, 40sec and 50 sec respectively on MYGA. However, the healthy cells log count got reduced to 3.18, 1.91, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 1.53, 0.29, and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

Initially *Staph.aureus* showed log count of 7.26 before exposure to microwave and this log count got reduced to 4.33, 1.52, nil and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count got reduced to 4.17, 1.03, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively when
plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.16, 0.49, and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

The initial log count of *B.subtilis* before microwave exposure was 3.56 and this count got reduced to 2.40, 1.04, 0.30 and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively on MYGA. However, the healthy cells log count, got reduced to 2.26, 0.47, nil and nil after exposure to 20 sec, 30 sec, 40 sec and 50 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.14, 0.57, 0.30, and nil at 20 sec, 30 sec, 40 sec and 50 sec respectively.

4.3.4 Injured bacterial cultures in paneer:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 14. The number of injured cells of different cultures in paneer, using MYGA with 2% salt after microwave exposure to different periods is given in Table 14 and Fig 4.

*E.coli* showed initial log count of 6.93 and this count got reduced to 4.30, 3.00, 0.69 and nil when exposed to microwave for 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, the healthy cells log count got reduced to 4.20, 2.80, 0.44 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.10, 0.20, 0.25, and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

Initially *Salmonella sp* had log count of 6.90 and this count got reduced to 4.08, 3.90, 3.90 and nil when exposed to microwave for 30 sec, 40 sec, 50 sec and 60 sec respectively. However, the healthy cells count, was reduced to 4.04, 3.40, 3.48 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.04, 0.50, 0.42 and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

Initially *Ps.aeruginosa* had log count of 6.60 and this count got reduced to 4.25, 2.60, 1.30 and nil when exposed to microwave for 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, the healthy cells log count was reduced to 4.00, 2.30, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when plated
on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.25, 0.30, 0.30 and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

_**Staph.aureus**_ before exposure to microwave in paneer showed log count of 7.08 initially and this count got reduced to 3.60, 2.30, 1.60 and nil after exposure to microwave at 20 sec, 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. Further the healthy cells count, was reduced to 3.48, 2.17, 1.30 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.12, 0.13, 0.30 and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

_**B.subtilis**_ initially showed log count of 3.54 and this count got reduced to 2.78, 2.18, 1.60 and nil after exposure to microwave for 20 sec, 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, the healthy cells log count after exposure to microwave on 2% MYGA was 2.26, 2.00, 1.30 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively. Thus the calculated injured cells log counts (salt sensitive) were 0.52, 0.18, 0.30, and nil at 20 sec, 30 sec, 40 sec, 50 sec and 60 sec respectively.

4.3.5 Injured bacterial cultures in khoa:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 15. The number of injured cells of different cultures in khoa, using MYGA with 2% salt after microwave exposure to different periods is given in Table 15 and Fig 5.

_**E.coli**_ showed initial log count of 6.69 and this count got reduced to 2.90, 2.30, 1.30 and nil after exposure to microwave for 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, the healthy cells log count was reduced to 2.60, 1.95, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.30, 0.35, 0.30, and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

The initial log count of _**Salmonella sp.**_, was 6.59 and this count got reduced to 2.60, 2.20, nil and nil after exposed to microwave for 30 sec, 40 sec, 50 sec and 60 sec
respectively on MYGA. However, the healthy cells log count, was reduced to 2.00, 1.78, nil and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log count (salt sensitive) were 0.60, 0.42, nil and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

*Ps.aeruginosa* when they were exposed to microwave in khoa reduced from initial log count of 6.90 to 4.25, 2.45, 1.30 and nil log count, was reduced to 4.00, 2.20, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec thus the calculated injured cells log counts (salt sensitive) were 0.25, 0.25, 0.30 and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

*Staph.aureus* when they were exposed to microwave in khoa had an initial log count of 6.48 and this count got reduced to 2.48, 1.90, 1.30 and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, the healthy cells log count got reduced to 2.00, 1.78, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when exposed to 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.48, 0.12, 0.30 and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

*B.subtilis* initially showed log count of 3.50 and this count got reduced to 2.45, 1.30, 1.30 and nil after exposure to microwave for 30 sec, 40 sec, 50 sec and 60 sec respectively on MYGA. However, the healthy cells log count got reduced to 2.08, 1.00, 1.00 and nil after exposure to 30 sec, 40 sec, 50 sec and 60 sec respectively when plated on 2% MYGA. Thus the calculated injured cells log counts (salt sensitive) were 0.37, 0.30, 0.30, and nil at 30 sec, 40 sec, 50 sec and 60 sec respectively.

4.4 DETERMINATION OF RELEASE OF DNA AND PROTEIN FROM CELLS DURING MICROWAVE EXPOSURE:

It has been reported that bacterial cells upon exposure to microwave would result in the cell wall weakening with consequent release of intracellular contents such as DNA and protein. In this study bacterial cultures such as *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were grown separately in broth medium, centrifuged and cell pellets were dispensed in TRIS buffer in two sets, one set of cultures subjected to heat
treatment at $80^0\text{C} / 30 \text{ Min.}$ and the other set was subjected to microwave exposure. The release of DNA and Protein were estimated by procedures described in materials and methods.

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Tables 16 - 20. The release of DNA and protein from different cultures in MYG-broth after heat treatment and microwave exposure are given in Tables 16 - 20 and Fig 6 - 10.

It may be seen from Table 16 and Fig 6 that in heated broth sample containing $E.\text{coli}$, the release of DNA was 10.40 µg/ml where as protein was 87.33 µg/ml, however in microwave exposed cells the release of DNA was 7.15 µg/ml where as the protein was 111.03 µg/ml.

The release of DNA was 21.95 µg/ml, where as the protein was 44 µg/ml in heated broth sample containing $Salmonella \text{sp.}$, however in microwave exposed cells the release of DNA was 13.73 µg/ml where as the protein was 295 µg/ml (Table 17 and Fig 7).

It may be seen from Table 18 and Fig 8 that in heated broth sample containing $Ps.aeruginosa$, the release of DNA was 22.50 µg/ml where as protein was 194.67 µg/ml, however in microwave exposed cells the release of DNA was 21.60 µg/ml where as the protein was 225.43 µg/ml.

Table 19 and Fig 9 shows that the release of DNA was 6.05 µg/ml where as protein was 165.67 µg/ml in heated broth sample containing $Staph.aureus$, however in microwave exposed cells the release of DNA was 6.55 µg/ml where the protein was 438.33 µg/ml.

The release of DNA was 13.80 µg/ml where as the protein was 166.67 µg/ml in heated broth sample containing $B.subtilis$, however in microwave exposed cells the release of DNA was 13.03 µg/ml where as the protein was 152 µg/ml (Table 20 and Fig 10).

4.5 SHELF LIFE STUDIES OF MICROWAVE TREATED SKIM MILK AND WHOLE MILK:
Microwave treatment causes substantial enhancement of the shelf life of skim milk and whole milk. In order to determine the shelf life of microwave treated skim milk and whole milk, bacterial cultures such as *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* were inoculated separately, subjected to microwave exposure and then stored at room temperature. At intervals of 3 days, 6 days, 9 days, and 15 days the samples were tested for physiological changes and bacteriological profile. Similarly the samples were stored at refrigeration temperature and at intervals of 5 days, the samples were tested for upto 60 days for physiological changes and bacteriological profile.

4.5.1 Skim milk at room temperature:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 21. Skim milk samples were stored for upto 15 days at room temperature and then at intervals of 3 days, the samples were tested for acidity, COB, alcohol alizarin test and viable counts and the results are given in Table 21 and Fig 11. Before microwave exposure all the skim milk samples showed an acidity of 0.14%, COB and alcohol alizarin tests are negative and an initial viable count of all the inoculated samples ranging from 3.68 to 7.20. However after exposure the TA remained unchanged but the log viable counts of the samples were reduced to nil.

Room temperature storage of microwave exposed skim milk for up to 3 days showed no change in the initial acidity, COB and alcohol alizarin tests where as initial viable count of all the inoculated samples were nil.

After 6 days of room temperature storage, skim milk samples containing *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* revealed a percent of titratable acidity as 0.16, 0.15, 0.16, 0.15, and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, where as log count was 1.46, 1.12, 0.78, 0.16 and 0.82 respectively.

After storage of skim milk for 9 days, the sample containing *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* showed a titratable acidity of 0.20, 0.16, 0.20, 0.16 and 0.16 respectively. However all the skim milk samples inoculated with
Salmonella sp., Staph.aureus and B.subtilis showed negative for both COB & alcohol alizarin test while the other samples containing E.coli and Ps.aeruginosa showed positive for these two tests. The log count of E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis in these samples increased to 5.40, 3.91, 4.89, 3.79 and 3.68 respectively.

After 12 days of storage the skim milk containing E.coli, Salmonella sp., P.aeruginosa, Staph.aureus and B.subtilis showed a percent of titratable acidity as 0.24, 0.16, 0.24, 0.16 and 0.16 respectively. These samples containing Salmonella sp., Staph.aureus and B.subtilis showed negative readings for both COB & alcohol alizarin test while samples containing E.coli and Ps.aeruginosa, were curdled. The log count of E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis of these samples increased to 6.60, 5.08, 6.33, 4.00 and 4.19 respectively.

All the skim milk samples stored for 15 days at room temperature curdled and the log count of E.coli, Salmonella sp., P.aeruginosa, Staph.aureus and B.subtilis increased to 7.79, 6.54, 7.60, 6.62 and 6.38 respectively.

4.5.2 Skim milk at refrigeration temperature:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 22. Skim milk samples were stored for 60 days at refrigeration temperature and then at intervals of 5 days, the samples were tested for acidity, COB, alcohol alizarin test and viable counts and the results are given in Table 22 and Fig 12. Before microwave exposure all the skim milk samples showed an acidity of 0.14%, COB and alcohol alizarin tests were negative and an initial viable count of all the inoculated samples ranged from 3.68 to 7.20. However after exposure, the TA, COB and alcohol alizarin tests remained unchanged but the log viable counts of the samples were reduced to nil.

Refrigeration storage of microwave exposed skim milk for up to 5 days showed no change in the initial acidity, COB and alcohol alizarin tests. The microwave exposed skim milk samples showed the log counts of E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis were 0.83, 1.02, 0.40, 0.71 and 0.23 respectively.
After 10 days of refrigeration storage of skim milk samples containing *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* revealed a percent of titratable acidity as 0.16, 0.15, 0.16, 0.15 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, where as log count was 1.58, 1.37, 1.22, 1.44 and 0.87 respectively.

After storage of skim milk for 15 days in refrigerator, the sample containing *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* showed a titratable acidity of 0.16, 0.15, 0.16, 0.15 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, where as log count was 1.59, 1.70, 1.86, 2.14 and 1.60 respectively.

After 20 days of refrigeration storage of skim milk samples containing *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* showed a percent of titratable acidity as 0.16, 0.15, 0.16, 0.15 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, where as log count was 1.76, 1.92, 1.99, 2.26 and 2.13 respectively.

After 30 days of refrigeration storage the skim milk containing *E.coli, Salmonella sp.*, *P.aeruginosa, Staph.aureus and B.subtilis* showed a percent of titratable acidity as 0.17, 0.16, 0.17, 0.16 and 0.15 respectively. These samples containing *Salmonella sp.*, *Staph.aureus and B.subtilis* showed negative readings for both COB & alcohol alizarin test while samples containing *E.coli and Ps.aeruginosa*, showed COB and alcohol
alizarin tests positive. The log count of *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* of these samples increased to 5.44, 4.61, 5.70, 4.43 and 5.10 respectively.

All the skim milk samples stored for 60 days at refrigeration storage curdled and the log count of *E.coli*, *Salmonella sp.*, *P.aeruginosa*, *Staph.aureus* and *B.subtilis* increased to 6.25, 6.31, 6.52, 5.81 and 5.87 respectively.

4.5.3 Whole milk at room temperature:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 23. Whole milk samples were stored for up to 15 days at room temperature and then at intervals of 3 days, the samples were tested for acidity, COB, alcohol alizarin test and viable counts and the results are given in Table 23 and Fig 13. Before microwave exposure all the skim milk samples showed an acidity of 0.14% TA, COB and negative for alcohol alizarin tests and an initial viable count of all the inoculated samples ranging from 3.80 to 7.02. However after exposure the TA remained unchanged but the log viable counts of the samples were reduced to nil.

Room temperature storage of microwave exposed whole milk for up to 3 days showed no change in the initial acidity, COB and alcohol alizarin tests where as initial viable count of all the inoculated samples were nil.

After 6 days of room temperature storage of whole milk samples containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* revealed a percent of titratable acidity as 0.16, 0.14, 0.16, 0.15, and 0.14 respectively. However all the samples showed negative for both COB & alcohol alizarin test, where as log count was 1.33, 0.97, 1.38, 1.00 and 0.82 respectively.

After storage of whole milk for 9 days at room temperature, the sample containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed a titratable acidity of 0.20, 0.16, 0.20, 0.16 and 0.16 respectively. However all the whole milk samples inoculated with *Salmonella sp.*, *Staph.aureus* and *B.subtilis* showed negative for both COB & alcohol alizarin test while the other samples containing *E.coli* and
*Ps.aeruginosa* showed positive for these two tests. The log count of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* in these samples increased to 5.40, 2.63, 5.00, 2.54 and 2.46 respectively.

After 12 days of room temperature storage the whole milk containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* showed a percent of titratable acidity as 0.24, 0.17, 0.24, 0.16 and 0.17 respectively. These samples containing *Salmonella sp., Staph.aureus and B.subtilis* showed negative readings for both COB & alcohol alizarin test while samples containing *E.coli and Ps.aeruginosa*, were curdled. The log count of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* of these samples increased to 6.70, 4.62, 6.26, 3.98 and 4.25 respectively.

All the whole milk samples stored for 15 days at room temperature curdled and the log count of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* increased to 7.57, 6.36, 7.46, 5.79 and 6.10 respectively.

4.5.4 Whole milk at refrigeration temperature:

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 24. Whole milk samples were stored for 60 days at refrigeration temperature and then at intervals of 3 days, the samples were tested for acidity, COB, alcohol alizarin test and viable counts and the results are given in Table 24 and Fig 14. Before microwave exposure all the whole milk samples showed an acidity of 0.14%, COB and alcohol alizarin tests were negative and an initial viable count of all the inoculated samples ranged from 3.80 to 7.02. However after exposure, the TA, COB and alcohol alizarin tests remained unchanged but the log viable counts of the samples were reduced to nil.

Refrigeration storage of microwave exposed whole milk for up to 5 days showed no change in the initial acidity, COB and alcohol alizarin tests. The microwave exposed whole milk samples showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 0.68, 1.05, 0.33, 0.46 and 0.40 respectively.
After 10 days of refrigeration storage of whole milk samples containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* revealed a percent of titratable acidity as 0.16, 0.15, 0.16, 0.15 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, where as log count 1.20, 1.30, 1.20, 1.16 and 0.80 respectively.

After storage of whole milk for 15 days in refrigerator, the sample containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed a titratable acidity of 0.16, 0.15, 0.16, 0.15 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, whereas log count was 1.49, 1.74, 1.81, 2.68 and 1.62 respectively.

After 20 days of refrigeration storage of whole milk samples containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed a percent of titratable acidity as 0.17, 0.16, 0.17, 0.15 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, whereas log count was 1.72, 1.96, 1.95, 1.93 and 1.82 respectively.

After 30 days of refrigeration storage the whole milk containing *E.coli*, *Salmonella sp.*, *P.aeruginosa*, *Staph.aureus* and *B.subtilis* showed a percent of titratable acidity as 0.17, 0.16, 0.17, 0.16 and 0.15 respectively. However all the samples showed negative for both COB & alcohol alizarin test, whereas log count was 2.78, 2.84, 3.12, 3.01 and 2.81 respectively.

After storage of whole milk for 40 days in refrigerator, the sample containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed a titratable acidity of 0.17, 0.16, 0.18, 0.17 and 0.16 respectively. However all the samples showed negative for both COB & alcohol alizarin test, whereas log count was 3.98, 4.10, 4.18, 4.12 and 3.99 respectively.

After 50 days of refrigeration storage, the whole milk containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed a percent of titratable acidity as 0.20, 0.18, 0.20, 0.18 and 0.18 respectively. The samples containing *Salmonella sp.*, *Staph.aureus* and *B.subtilis* showed negative readings for both COB & alcohol alizarin test while samples containing *E.coli* and *Ps.aeruginosa* showed COB
and alcohol alizarin tests positive. The log count of *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* of these samples increased to 5.80, 5.04, 6.02, 4.92 and 5.62 respectively.

All the whole milk samples stored for 60 days at refrigeration storage curdled and the log count of *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* increased to 6.40, 6.28, 7.02, 6.02 and 5.80 respectively.

4.6 SHELF LIFE STUDIES OF PANEER AND KHOA PACKED IN CONICAL FLASK

Indigenous milk products such as paneer and khoa when exposed to microwave cause substantial enhancement of the shelf life. In order to determine the shelf life of microwave treated paneer and khoa, bacterial cultures such as *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* were inoculated separately thoroughly mixed, subjected to microwave exposure and then stored at room temperature. At intervals of 3 days, 6 days, 9 days, 15 days, 20 days and 30 days, the samples were tested for bacteriological profile. Similarly the samples were stored at refrigeration temperature and at intervals of 5 days, the samples were analysed for bacteriological analysis.

4.6.1 Storage of paneer at room temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 25. Paneer samples were stored for 30 days at room temperature and then at regular intervals of 3 days, the samples were tested for viable counts and the results are given in Table 25 and Fig 15. Before microwave exposure all the paneer samples inoculated with *E.coli, Salmonella sp.*, *Ps.aeruginosa, Staph.aureus and B.subtilis* showed an initial viable count of 6.38, 6.41, 7.11, 6.99 and 4.80 respectively. However after microwave exposure of paneer for 60 sec, the log viable counts of the samples were reduced to nil.

The log viable counts of bacterial cultures showed nil after 3 days of room temperature storage of paneer samples.
Room temperature storage of microwave exposed paneer for 6 days showed the log counts of *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* were 1.30, 1.30, 1.48, 1.60 and 1.78 respectively.

After 9 days paneer samples stored at room temperature containing *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* revealed log viable count of as 2.48, 2.41, 2.46, 2.38 and 2.53 respectively.

Room temperature storage of microwave exposed paneer for up to 12 days showed the log counts of *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* were 3.08, 2.95, 2.89, 3.00 and 2.79 respectively.

After 15 days of room temperature storage of paneer samples containing *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* revealed log count of 4.28, 4.08, 4.00, 4.28 and 3.91 respectively.

Room temperature storage of microwave exposed paneer for up to 20 days showed the log counts of *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* were 5.11, 4.76, 4.28, 5.18 and 4.28 respectively.

After 30 days paneer samples stored at room temperature containing *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* revealed log viable count of 6.85, 6.79, 6.72, 6.61 and 6.32 respectively. All the stored paneer samples showed softened, slimy texture and off odour indicating the spoilage of the product.

### 4.6.2 Paneer at refrigeration temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 26. Paneer samples were stored for 60 days at refrigeration temperature and then at intervals of 5 days up to 20 days and later at 10 days interval up to 60 days, the samples were tested for viable counts and the results are given in Table 26 and Fig 16. Before microwave exposure all the paneer samples inoculated with *E. coli, Salmonella sp., Ps. aeruginosa, Staph. aureus and B. subtilis* showed an initial viable count of 6.38, 6.41, 7.11, 6.99 and 4.80 respectively. However after microwave exposure of paneer for 60 sec, the log viable counts of the samples were reduced to nil.
The log viable counts of bacterial cultures showed nil after 5 days of refrigeration storage of paneer samples.

Refrigeration storage of microwave exposed paneer for up to 10 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 1.60, 1.00, 1.30, 1.84 and 2.50 respectively.

After 15 days of refrigeration storage of paneer samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 2.15, 2.45, 2.04, 2.20 and 2.72 respectively.

After 20 days paneer samples stored at refrigeration temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 2.99, 2.91, 2.66, 2.90 and 2.89 respectively.

Refrigeration storage of microwave exposed paneer samples for up to 30 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 3.28, 2.99, 2.69, 2.78 and 2.95 respectively.

After 40 days of refrigeration storage of paneer samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 4.38, 4.23, 4.11, 4.30 and 4.08 respectively.

Refrigeration storage of microwave exposed paneer for up to 50 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 6.25, 6.32, 6.18, 6.11 and 6.41 respectively.

After 60 days paneer samples stored at refrigeration temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 7.08, 6.99, 6.62, 7.07 and 6.79 respectively.

4.6.3 Storage of khoa at room temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 27. Khoa samples were stored for 30 days at room temperature and then at intervals of 3 days, the samples were tested for viable counts and the results are given in Table 27 and Fig 17. Before microwave exposure all the khoa samples inoculated with *E.coli, Salmonella sp.,*
*Ps.aeruginosa, Staph.aureus and B.subtilis* showed an initial viable count of 7.11, 6.11, 7.20, 6.45 and 4.72 respectively. However after microwave exposure of paneer for 60 sec, the log viable counts of the samples were reduced to nil.

The log viable counts of bacterial cultures showed nil after 3 days of room temperature storage of khoa samples.

Room temperature storage of microwave exposed khoa for up to 6 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 1.48, 1.60, 1.60, 1.67 and 1.84 respectively.

After 9 days of room temperature storage of khoa samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 2.80, 2.50, 2.55, 2.59 and 2.58 respectively.

After 12 days khoa samples stored at room temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 3.28, 2.90, 2.81, 2.96 and 2.68 respectively.

Room temperature storage of microwave exposed khoa for up to 15 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 4.23, 4.04, 4.18, 4.18 and 3.98 respectively.

After 20 days of room temperature storage of khoa samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 4.99, 4.36, 4.34, 4.41 and 5.08 respectively.

Room temperature storage of microwave exposed khoa for up to 30 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 6.32, 6.15, 6.41, 6.96 and 6.18 respectively. All the khoa samples showed the spoilage signs of sliminess and off flavour.

4.6.4 Khoa at refrigeration temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 28. Khoa samples were stored for 60 days at refrigeration temperature and then at intervals of 5 days, the samples were tested for viable counts and the results are given in Table 28 and Fig 18.
Before microwave exposure all the khoa samples inoculated with E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis showed an initial viable count of 7.11, 6.11, 7.20, 6.45 and 4.72 respectively. However after microwave exposure of khoa for 60 sec, the log viable counts of the samples were reduced to nil.

The log viable counts of bacterial cultures showed nil after 5 days of refrigeration storage of khoa samples.

Refrigeration storage of microwave exposed khoa for up to 10 days showed the log counts of E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis were 1.30, nil, 1.48, 1.48 and 2.55 respectively.

After 15 days of refrigeration storage of khoa samples containing E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis revealed log count of 2.32, 2.17, 1.48, 1.95 and 2.78 respectively.

After 20 days khoa samples stored at refrigeration temperature containing E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis revealed log viable count of 2.83, 2.65, 2.70, 2.58 and 2.93 respectively.

Refrigeration storage of microwave exposed khoa samples for up to 30 days showed the log counts of E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis were 3.15, 2.92, 2.78, 2.62 and 2.98 respectively.

After 40 days of refrigeration storage of khoa samples containing E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis revealed log count of 5.32, 5.20, 5.04, 5.15 and 4.59 respectively.

Refrigeration storage of microwave exposed khoa for up to 50 days showed the log counts of E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis were 6.08, 6.08, 6.08, 6.00 and 6.45 respectively.

After 60 days khoa samples stored at refrigeration temperature containing E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis revealed log viable count of 6.25, 6.38, 6.08, 6.36 and 7.15 respectively. All the stored khoa samples showed the spoilage signs of sliminess and off flavour.

4.7 SHELF LIFE STUDIES OF PANEER AND KHOA PACKED IN
POLY ETHYLENE THERAPHTHALATE (PET)

Indigenous milk products such as paneer and khoa when exposed to microwave cause substantial enhancement of the shelf life. In order to determine the shelf life of microwave treated paneer and khoa, bacterial cultures such as *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were inoculated separately, packed in PET sachets, subjected to microwave exposure for 60 sec and then stored at room temperature. At intervals of 3 days, 6 days, 9 days, 15 days, 20 days, 30 days and 40 days the samples were tested for bacteriological profile. Similarly the samples were stored at refrigeration temperature and at intervals of 5 days upto 20 days and later at 10 days interval upto 70 days, the samples were analysed for bacteriological profile.

4.7.1 Storage of PET packaged paneer at room temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 29. Paneer samples were stored for 40 days at room temperature and then after 3 days, 6 days, 9 days, 15 days, 20 days, 30 days and 40 days, the samples were tested for viable counts and the results are given in Table 29 and Fig 19. Before microwave exposure all the paneer samples inoculated with *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed an initial viable count of 6.32, 6.45, 6.79, 6.52 and 4.54 respectively. However after microwave exposure of paneer for 60 sec, the log viable counts of the samples were reduced to nil.

The log viable counts of bacterial cultures showed nil after 3 days and 6 days of room temperature storage of paneer samples in PET.

Room temperature storage of microwave exposed paneer for up to 9 days showed the log counts of *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were 1.60, 1.30, 1.30, 1.48 and 1.90 respectively.

After 12 days of room temperature storage of paneer samples containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* revealed log count of 2.56, 2.30, 2.49, 2.43 and 2.70 respectively.
After 15 days paneer samples stored at room temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 2.93, 2.63, 2.59, 2.71 and 3.08 respectively.

Room temperature storage of microwave exposed paneer for up to 20 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 3.30, 3.19, 3.24, 3.20 and 3.37 respectively.

After 30 days of room temperature storage of paneer samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 5.16, 4.92, 4.86, 4.88 and 5.30 respectively.

Room temperature storage of microwave exposed paneer for up to 40 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 6.91, 6.55, 6.63, 6.51 and 7.27 respectively. All the stored paneer samples showed softened, slimy texture and off odour indicating the spoilage of the product.

4.7.2 Storage of PET packaged paneer at refrigeration temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 30. Paneer samples were stored for 60 days at refrigeration temperature and then at intervals of 5 days up to 20 days and later at 10 days interval up to 70 days, the samples were tested for viable counts and the results are given in Table 30 and Fig 20. Before microwave exposure all the paneer samples inoculated with *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* showed an initial viable count of 6.32, 6.45, 6.79, 6.52 and 4.54 respectively. However after microwave exposure of paneer for 60 sec, the log viable counts of the samples were reduced to nil.

The log viable counts of bacterial cultures showed nil after 5 days and 10 days of refrigeration storage of paneer samples.

Refrigeration storage of microwave exposed paneer for up to 15 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 1.48, 1.30, 1.48, 1.48 and 1.84 respectively.
After 20 days of refrigeration storage of paneer samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 2.25, 2.48, 2.00, 2.08 and 2.69 respectively.

After 30 days paneer samples stored at refrigeration temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 2.99, 2.82, 2.84, 2.69 and 3.07 respectively.

Refrigeration storage of microwave exposed paneer samples for up to 40 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 4.31, 4.11, 4.04, 4.00 and 4.45 respectively.

After 50 days of refrigeration storage of paneer samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 5.31, 5.20, 5.28, 5.08 and 5.36 respectively.

Refrigeration storage of microwave exposed paneer for up to 60 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 6.96, 6.80, 6.72, 6.93 and 7.01 respectively.

After 70 days paneer samples stored at refrigeration temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 7.37, 7.28, 7.23, 7.20 and 7.43 respectively. All the stored paneer samples showed softened, slimy texture and off odour indicating the spoilage of the product.

4.7.3 Storage of PET packaged khoa at room temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 3. Khoa samples were stored for 30 days at room temperature and then at intervals of 3 days, 6 days, 9 days, 15 days, 20 days, 30 days and 40 days, the samples were tested for viable counts and the results are given in Table 3 and Fig 2. Before microwave exposure all the khoa samples inoculated with *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* showed an initial viable count of 7.15, 6.48, 6.64, 6.50 and 4.58 respectively. However after microwave exposure of paneer for 60 sec, the log viable counts of the samples were reduced to nil.
The log viable counts of bacterial cultures showed nil after 3 days and 6 days of room temperature storage of khoa samples.

Room temperature storage of microwave exposed khoa for up to 9 days showed the log counts of *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were 1.86, 1.77, 1.61, 1.48 and 1.77 respectively.

After 12 days of room temperature storage of khoa samples containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* revealed log count of 2.60, 2.47, 2.56, 2.23 and 2.65 respectively.

After 15 days khoa samples stored at room temperature containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* revealed log viable count of 4.24, 3.98, 3.88, 3.84 and 3.93 respectively.

Room temperature storage of microwave exposed khoa for up to 20 days showed the log counts of *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were 4.91, 4.22, 4.10, 4.18 and 4.27 respectively.

After 30 days of room temperature storage of khoa samples containing *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* revealed log count of 6.03, 5.96, 5.92, 5.71 and 5.90 respectively.

Room temperature storage of microwave exposed khoa for up to 40 days showed the log counts of *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* were 7.00, 7.96, 6.43, 6.96 and 6.64 respectively. All the khoa samples showed the spoilage signs of sliminess and off flavour.

4.7.4 Storage of PET packaged khoa at refrigeration temperature

All the results were analyzed using ANOVA, critical differences were computed and significance of the mean differences are indicated in the Table 32. Khoa samples were stored for 60 days at refrigeration temperature and then at intervals of 5 days upto 20 days and later at 10 days interval upto 70 days, the samples were tested for viable counts and the results are given in Table 32 and Fig 22. Before microwave exposure all the khoa samples inoculated with *E.coli*, *Salmonella sp.*, *Ps.aeruginosa*, *Staph.aureus* and *B.subtilis* showed an initial viable count of 7.15, 6.48, 6.64, 6.50 and 4.58
respectively. However after microwave exposure of khoa for 60 sec, the log viable counts of the samples were reduced to nil.

The log viable counts of bacterial cultures showed nil after 5 days and 10 days of refrigeration storage of khoa samples.

Refrigeration storage of microwave exposed khoa for up to 15 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 1.60, 1.48, 1.78, 1.30 and 1.90 respectively.

After 20 days of refrigeration storage of khoa samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 2.48, 2.60, 2.38, 2.43 and 2.68 respectively.

After 30 days khoa samples stored at refrigeration temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 2.86, 2.90, 2.78, 2.74 and 2.97 respectively.

Refrigeration storage of microwave exposed khoa samples for up to 40 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 4.19, 4.10, 4.01, 4.02 and 4.30 respectively.

After 50 days of refrigeration storage of khoa samples containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log count of 5.15, 5.14, 5.10, 4.95 and 5.32 respectively.

Refrigeration storage of microwave exposed khoa for up to 60 days showed the log counts of *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* were 6.30, 6.04, 6.01, 5.91 and 6.42 respectively.

After 70 days khoa samples stored at refrigeration temperature containing *E.coli, Salmonella sp., Ps.aeruginosa, Staph.aureus and B.subtilis* revealed log viable count of as 7.40, 7.05, 7.08, 6.99 and 7.45 respectively. All the stored khoa samples showed the spoilage signs of sliminess and off flavour.