Results
&
Discussion
RESULT AND DISCUSSION

Khoa is an indigenous product produced by continuous heating of milk with constant stirring and scrapping till a semi solid substance is produced. At Jhansi, Desi, Danedar and Shivpuri variety is sold in a market which in Khoa industry is considered as Dhap, Danedar and Pindi variety. In the present work Danedar and Pindi variety were used for analysis during experimentation. The physical characters of both the varieties were studied and it was found that Danedar variety was white, soft with granular texture, mouldy consistency, loose with some isolated spots and the smell and taste was sweet. The Pindi variety is yellowish in color, hard and steaky in texture, doughy and hardy in consistency, form in appearance, oily in smell and taste and it is not very much liked when taken raw. Our observations are similar to those of Narain & Singh (1979), Jail khani et.al.; (1979), Patel et.al.; (1990).
Mineral Components

The mineral component was measured for both the varieties of Khoa. The ash content was the same, citrate, and chloride, copper, phosphorous and iron content was slightly higher in Danedar Khoa while calcium, magnesium, sodium and potassium were higher in the Pindi variety. The values given in Table - II are mean values of the five readings obtained during the study. Calcium was 621 and 705, magnesium was 63.65 and 65.03, sodium 162 and 176, potassium 336 and 342 in Danedar and Pindi varieties respectively. While phosphorous was 373 and 368, citrate 535 and 503, chloride 327 and 321, copper 0.17 and 0.12 while iron 2.35 and 2.15 in Danedar and Pindi variety respectively. Our finding are similar to those Rajorhia and Srinivasan (1979), Ghatak and Bandyopadhyay (1989), Sindhu (2000). The mineral component observed by the present author was slightly observed by Boghra and Mathur (1990). This might probably due to the quality of milk extinct of desiccation and method of preparation.

Vitamin Contents –

The water-soluble vitamins in buffalo milk and two higher Khoa were studied Thiamine, Riboflavin, Vitamin B₆, Folic acid and Vitamin C. In Danedar Khoa and Pindi variety thiamine was 97.848 and 95.54 μ gm per 100 gm respectively. Riboflavin 392.98 and 388.8 respectively. Vitamin B₆ 72.495 and 70.49 respectively, folic acid 8.84 and 8.40 respectively, vitamin C 9.965 and 7.81 respectively. When compared with the vitamin content of buffalo milk the loss of vitamin in Danedar and Pindi variety were for thiamine 30.236 and 32.545 for riboflavin 67.07 and 71.25 respectively. For vitamin B₆ 19.595 and 21.6, folic acid
4.23 and 4.67 and vitamin c 1.44 to 3.6 μ gm per 100 gm respectively. These losses are more for thiamine and riboflavin and less for vitamin B6, folic acid and vitamin c as compare to those of Rajan Sharma et.al.; (1999) and Sapre et.al.; (1988). These differences may be due to the quality of milk at the two places and preparation of Khoa for milk under the conditions of the producers. Our value for lower losses of vitamin B6, folic acid and vitamin c may be due to the fact that we obtained Khoa in the morning and the effect of fluorescent light and sunlight was less. The higher loss in thiamine and riboflavin may be due to the oxidation of these vitamins as reported by Bossett et.al.; (1991), Sahbaz et.al.; (1993).

**Physico Chemical Properties -**

While studying the physico chemical properties of fresh Khoa it was observed that the pH of both the varieties were same dry matter of Pindi variety were higher with 70.56 % as compared to Danedar variety where the dry matter was 59.78. The moisture content of Danedar variety was slightly higher at 40.22% then Pindi variety with 37%. Protein content was also higher in Danedar variety with 31.55% then compare to Pindi variety with 29.01. The fat percentage of Danedar Khoa was 24.91% which was less when the Pindi variety was 28.70. Ash percentage of Danedar Khoa was less 1.89% and Pindi variety was higher with 2.81%. Aneja (1992) also found variations in moisture, fat, protein and ash content. But the moisture content and protein content were less then found in our studies this difference may be on the quality of milk and the method of Khoa preparation at the end product. The emulsifying capacity of buffalo milk is due to the presence of triglycerides which is present in higher proportions as compared to Sidhu (1996), Diwani and Jai Prakash (2002).
Biochemical Group of Organisms –

Among the biochemical forms counts were recorded for total organisms, acid producers, proteolytic, chromogenic, thermophillic, lipolytic, spore count yeast and mould count, coliform and enterococcus count. Both Danedar and Pindi variety were analyzed. The total count of Pindi variety was more then Danedar variety. Were the count was 41.7 x 10^3 and 24 x 10^3 respectively. Acid producers in Danedar Khoa was 30.32 x 10^3 were Pindi variety were 23.2 x 10^3 Proteolytic forms were more in Danedar Khoa and were equal to 28.1 x 10^3 as compare to Pindi variety where their population was 21.41 x 10^3. Chromogenic forms were again more in Danedar Khoa that is 27.31 x 10^3 as compared to 17.9 x 10^3. Thermophillic were more in Pindi then in Danedar Khoa and were equal to 0.12 x 10^3 and 0.21 x 10^3 respectively in Danedar and Pindi variety. Lipolytic forms were almost double in the Pindi variety with 16.1 x 10^3 in Danedar variety as compared to 33.43 x 10^3 in Pindi variety. Spore counts were present in large number in Pindi variety where they are 0.41 x 10^3 compared to Danedar with 0.03 x 10^3. Yeast and moulds were almost equal with 0.021 x 10^3 in Danedar Khoa and 0.037 x 10^3 in Pindi Khoa. Coliforms counts were also almost in equal number in both the variety where their number was 0.012 x 10^3 in Danedar and 0.015 x 10^3 in Pindi Khoa Enterococcus were in a very big number in Pindi variety as compared to that of Danedar variety. From the above results it is evident that Danedar Khoa has more acid producers proteolytic forms and chromogenic forms. It may be due to the fact that Danedar Khoa is prepared from slightly acidic milk and have a high protein content. From the higher number of lipolytic forms in Pindi variety it appears that they have a high lipid content and the milk used for Pindi variety is higher in fat content. As it might had been prepared with some higher fat content.
milk. From the overall results it can be said that Danedar Khoa can be placed in a good grade and Pindi Khoa in a fair grade. Our results are higher then those found by Ghodeker (1969), Kumar et.al.; (1975). The market samples were heavily contaminated by acid producers Proteolytic and chromogenic. These results are similar to those of Naidu, Ranganathan (1965). The S.P.C. counts was similar to those of Singh et.al; (1975). The presence of yeast and moulds is objectionable since they produce discoloration and lipolytic defects. Their number shows their post management contamination.

**Microbiological Analysis-**

**Bacteria and Moulds During Storage -**

During this experiment data’s on bacterial count, yeast and mould count and Coliform count for 2, 4 and 8 days were taken and have been shown in Table - V. From this table it will be evident that the number of organism increased, increased in the days of storage. The number of organism was higher in Pindi variety as compared to Danedar Khoa. In Pindi variety the total viable count and bacteria were $42 \times 10^3$, $92 \times 10^3$ and $108 \times 10^3$ after 2, 4 and 8 days respectively. These number are higher as compared to Danedar Khoa on each day of isolation. In Danedar variety the number was $24 \times 10^3$, $40 \times 10^3$ and $64 \times 10^3$ after 2, 4 and 8 days respectively. Yeast and Moulds were less in number as compared to the bacterial forms. In Pindi Khoa the number was 317, 48 and 83 after 2, 4 and 8 days. In Danedar variety its number was 21, 46 and 68 after the same days of isolation. Coliform counts were 15, 18 and 21 in Pindi variety after 2, 4 and 8 days. While in Danedar they were 12, 14 and 19 on the same days of isolation. Bacteriological quality is similar to those found by Anamika and Kalimuddin (2002). The distribution pattern has
also been worked by Rajarajan et.al.; (2007). The trend present in their results was also similar to those found in our studies.

**Sugar as a Limiting Agent on Mycoflora**

During the estimation of the types of fungal and yeast organisms in Khoa. It was thought to study the effect of sugar in reducing the quantity of mycoflora with this objective Khoa was analyzed for the occurrence of individual myco organisms with and without sugar. The data’s are given in Table - VI. From this study five organisms belonging to yeast were isolated comprising of *Saccharomyces cerevisae*, *Candida lipolytica*, *Rhodotorula glutinis*, *Torulopsis* and *Cryptococcus albicus*. In Pindi Khoa highest percentage occurrence was of *Rhodotorula* followed by Saccharomyces and then *Candida lipolytica*. *Cryptococcus* had the least number almost the same trend was found when Khoa was added but their was a mark deduction in a number of organisms. The same organisms of yeast were isolated in Danedar variety and almost with the similar trend. Where also there was a mark deduction in a number of organisms when sugar was added. About eight different genera of moulds were collected these include *Penicillium nigricans*, *Aspergillus niger*, *Geotrichum candidum*, *Mucor mucedo*, *Syncephalastrum racemosum*, *Fusarium sp.*, *Rhizopus nigricans* and *Cladosporium herbarium*. The percentage occurrence of Danedar variety varied from 2.4% to 12.9% while in the presence of sugar their percentage occurrence varied from 2.4 to 17%. In Pindi variety the percentage occurrence varied from 2.1 to 12.6 with sugar it varied from 2.1 to 15.6. The number of individual species were very much reduced when sugar was added.

It appears that due to increase in the acidity produced by the
growth of acid acidity produced by the growth of acid producing bacteria yeast and moulds find a favorable environment for the growth and multiplication as a result of which the quality of these product get very much reduced. Detection of moulds in Khoa suggests contamination from external sources specially after processing. These organisms bring rapid discoloration accompanied by off flavor. Hence the presence of these organisms can be considered as on indication of unhygienic conditions prevailing during manufacture and subsequent storage. Our results are in accordance of results obtained by Ghodaker et.al.; (1980).

**Identification of Bacterial Isolates –**

The bacterial organisms isolated from Khoa were collected and identified on the basis of morphological and biochemical tests. On the morphological basis four types of organisms were found. Seven isolated out of the total sixteen were Gram negative, motile rods which do not produce spores. Four isolated were Gram negative, non-motile rods, 3 isolates were Gram positive, non-motile, spherical form, two were Gram negative, non motile spherical. Their isolate numbers with morphological characters have been observed in Table - VIII.

For chemical parameters tests were performed through which their enzyme producing capabilities were performed through which their enzyme producing capabilities were tested and on the basis of these tests six bacterial organisms were studied. The organisms show positive tests for lactose, catalase, gas forming, indole and negative tests for vogus prausker, citrate, oxidase and H₂S test and hence identified as *E.Coli*.

The isolate number which gave positive test for lactose, catalase, gas forming, vogues prausker and citrate test while showing negative
tests for indole, oxidase and H$_2$S test were identified as *Klebsiella pneumoniae*.

The isolate no. 1 and 3 gave positive test for lactose, catalase, gas forming, Vogues Prausker, citrate and oxidase but gave negative test for indole and H$_2$S test were identified as *Enterobacter*.

Isolate no. 2 and 7 gave positive test for lactose, gas forming, citrate and H$_2$S but gave negative test for vogues prauskar, indole and oxidase were identified as *Citrobacter*.

Those isolates numbered as 11, 13 and 14 give negative tests for lactose, gas forming, Vogues prausker, citrate and H$_2$S but give positive test for catalase and DNAase were identified as *Staphylococcus*.

The isolate no. 16 give positive test for gas forming, Vogues prausker, indole and H$_2$S but give negative test for lactose, citrate, catalase, indole and oxidase were identified as *Salmonella*. Data's obtained on biochemical parameters are given in Table - IX

The identified organisms with their identified numbers have been entered in Table - X. The percentage occurrence of *E.Coli* and *Klebsiella* was found to be highest with 25% percentage occurrence followed by *Staphylococcus* with 18.75 percentage occurrence then were *Enterobacter* and *Citrobacter* with percentage occurrence 12.5 while *Salmonella* showed the least percentage occurrence of 6.25 percent. *E.Coli*. were also reported from Soomoro et.al.; (2002), from Pakistan. Varadaraj et.al; also found *Staphylococcus aureus* in large quantities in fresh Khoa. Grover (1984) collected them in Khoa samples in Ludhiana. Saxena et.al.; (1993) found *E.Coli, Salmonella, Staphylococcus* and *Coliform* in Khoa from
Nagpur market. The presence of these coliform organisms suggest the presence of unhygienic conditions during manufacturing and storage.

**Staphylococcal Count –**

In the previous experiment staphylococcal were found in Khoa which gives a indication of contamination during storage. Therefore the total count and staphylococcal count were thought to be worked out so that quantitative estimation of contamination could be accessed with this background the present estimation was conducted and the results obtained has been given in Table - XI. Both Pindi and Danedar variety were explored. The number of bacteria per gram of Pindi variety is $41 \times 10^3$, while Danedar variety had $24 \times 10^3$ per gram of Khoa. While the staphylococcal count were 400 and 300 per gram of Khoa in Pindi and Danedar variety respectively. The relationship could be clearly established that in Pindi variety they are 0.9% of the total count while in Danedar variety which is 1.25% of the total count. These staphylococcal count are often found in ice cream and kulfi but the presence of DNAase are *Staphylococci* in Khoa is alarming. Since the heat process product it may be due to post contamination from infected workers handling the product. Therefore the possibilities of enterotoxin cannot be ruled out and their percentage may in hence at high temperature. Their presence in low proportion as observed by the present author may be due to the large number of total count which may inhibit their growth. Secondly lactic acid present in Khoa might also had inhibited the growth of these organisms. From these investigation there is a need to educate the urban masses against hygienic methods and unhealthy environment under which Khoa might have been produced. Similar results have been obtained by Khalon and Grover (1984), but they found the organism in higher
number.

The total number of organisms identified during isolation was 16. Out of which three organisms were *Staphyloccci*, 2 from Pindi variety and from Danedar variety. This comes to the percentage occurrence of Pindi varieties 12.5 and Danedar variety 6.25.

**Growth Viability of S. aureus in Khoa –**

In this experiment the viability and growth of sources in Khoa was observed under $35^0\text{C}$ and $5^0\text{C}$ temperature with varying moisture levels under these conditions the growth of *Staphylococcus aureus* was observed after 24 and 48 hrs with and without sugar content. The moisture content of 26 to 28, 38 to 42% and 45 to 48% were adjusted by adding sterile water. The organism isolated was inoculated in each flask with $4 \times 10^3$ CFU/gm of Khoa. The suspension of organisms was prepared in peptone water. The data’s obtained have been entered in the Table XIII. From these data’s it can be observed that *S. aureus* grew well during storage. Both at $35^0\text{C}$ and $5^0\text{C}$ but their growth was markedly reduced at $5^0\text{C}$ as compared to $35^0\text{C}$. The increase in moisture level also resulted in increasing the population of *staphylococcus*. At $35^0\text{C}$ temperature and 36 to 28% moisture the number of organisms were $4 \times 10^5$ after 24 hrs. With the same duration at 45 to 48% moisture the number was $6 \times 10^5$ after 48 hrs this number in hence to $5 \times 10^7$ and $7 \times 10^7$ respectively. At $5^0\text{C}$ temperature the number was decreased. At 26 to 28% moisture they were $4 \times 10^5$ after 24 hrs had already stated above but at $5^0\text{C}$ their number reduced to $4 \times 10^5$, similarly at 48 to 32% moisture at 24 hrs. they are $5 \times 10^5$ but at $5^0\text{C}$ the number reduced to $5 \times 10^3$. At 45 to 48% moisture at $35^0\text{C}$ temperature their number was $6 \times 10^4$ which
reduce to $4 \times 10^4$ at $5^0$C temperature. Similar were the results after 48 hrs of storage but their number increased to what were observed after 24 hrs. In the presence of sugar their number decreased both after 24 hrs and 48 hrs at 35 and $5^0$C temperature. At $5^0$C temperature after 24 hrs at 26 to 28% moisture level their number was $4 \times 10^3$ but with sugar the number reduced to $2 \times 10^3$ after 48 hrs their number was $3 \times 10^3$ but to the addition of sugar their number reduced to $3 \times 10^3$ the result showed that the number of organisms were reduced when sugar was added with Khoa. Which further reduced with increase in the storage period. This might be due to the less availability of moisture content with storage together with the rising concentration. Bhatt *et.al.;* (1948) have showed that Khoa serves as an excellent medium for microbial growth. Takabashi *et.al;* (1959), Gandhi *et al;* (1973) reported the production of enterotoxin by *S. aureus* in cheese. In variability enterotoxin productions corresponds to growth of *S. aureus*. The unhiginic practices and lack of sanitation offer ample scope for post process contaminations by *Staphylococci*. Generally the time gape between preparation and consumption of Khoa is 2 to 3 days during which the product is stored in bamboo baskets one above the other wrapped in sacks with no cooling facilities. This enables the rapid multiplication of *Staphylococcus*. Therefore to evaluate the post processing contamination the rise in population was estimated at $35^0$C temperature. Bhatt *et.al.;* have also evaluated the group of test organisms in relation to moisture test organisms in relation to moisture level. They also found that the growth of the organisms was better in partially sterilized sample then in warm sample. In the present study also analysis was made on the fresh Khoa samples. It could be considered as partially sterilized. During processing casein of milk gets coagulated taking up most of the moisture and releasing the availability of nutrients. One possibility of the growth of *Staphylococci* in Khoa may be that during
preparation the chances of forming intermediate compounds due to interaction between casein and lactose under the influence of heat. Varadhraj et.al.; (1984) reported that there are certain growth promoting factors present in Khoa which enable the increased in staphylococcal count.

The growth of *S. aureus* and its consumption in Khoa is a serious concerned and may result in public health hazard. Therefore it is essential to take strict and enforcing of hygienic conditions to avoid *Staphylococcus* contamination in Khoa during storage, handling and transportation. There should be necessary cooling facilities to ensure safe Khoa product for human consumption.

**Bacteria and Moulds Growth During Storage at Varying Temperature** –

This experiment was conducted to study the effect of varying temperature during storage on the number of bacteria and moulds. The study was conducted on Pindi and Danedar Khoa. In Pindi variety at 37⁰C temperature the bacterial count was 60 x 10³ after 2 days which increased to 118 x 10³ after 8 days Moulds were 48 after 2 days and 101 after 8 days at 30⁰C temperature. Bacterial counts were 41 x 10³ after 2 days which increased to 108 x 10³ after 8 days whereas moulds were 47 x 10³ after 2 and 8 days respectively. At 22⁰C temperature bacteria were 30 x 10³ after 2 days and 70 x 10³ after 8 days moulds were 17 and 50 after 2 and 8 days respectively at 5 to 7⁰C temperature the bacterial number were 10 x 10³ which increase to 18 x 10³ after 8 days which increase to 18 x 10³ after 8 days while moulds were 12 and 28 after 2 and 8 days respectively. This shows that with the increase in temperature and days of
storage the number of microorganisms increased. Similar trend was found in Danedar variety. However the number obtained was less as compared to Pindi variety. Hussain et.al.; (2005) also observed microorganisms in milk products Ghodekar et.al.; also found similar trend in microbial count during storage but the number of organisms obtained by them were much more in number as compared to those obtained by the present author. This might be the reason while Khoa turns sour earlier at higher temperature. The presence of yeast and moulds show the correspondent increased in acidity this acid production is actually due to the growth of acid producing bacteria according to Naidu and Ranganathan (1965). due to which yeast and mould find the favorable environment for the growth and multiplication as a result of which the keeping quality of these product is very much reduced. Detection of moulds suggests contamination from external source indicating unhygienic conditions prevailing during manufacture and storage.

Chemical Properties Variation During Storage Under Refrigeration –

During the previous experiment it was observed that at high temperature microbial population increases resulting into spoilage of Khoa. Therefore the chemical properties on pH, Dry matter, moisture protein, fat and ash% was studied at 5°C temperature. Both the varieties of Khoa were analyzed and the data’s obtained have been given in the Table - XV and XVI. In both the varieties the pH were almost the same and decreased after storage it was 6.7 and 6.8 in Danedar and Pindi variety after storage for 30 days it reduce to 6.1 and 6 respectively. The dry matter of Danedar Khoa when fresh was 59.78, 58.20 after 3 days, 57.20 after 21 days and 56.99 after 30 days. The dry matter was higher in
the Pindi variety where when fresh was 70.56% and after 30 days 68.34. The moisture percentage was 40.22 in Danedar Khoa which was increased to 43.01 but in Pindi Khoa the moisture percentage was 40.44 which reduce to 36.34 after 30 days, Protein content remained almost the same 31.55 in fresh Danedar Khoa and after 30 days it was 31.18. In Pindi Khoa its percentage was slightly lower with 29.01 when fresh and 29.93 after 30 days. Fat percentage was almost at the same level in both the varieties with 24.91 and 24.70 when fresh after 30 days 24.24 and 24.12 in Pindi and Danedar variety respectively. Ash content was also found at the same level during all the stages of storage 1.89 Danedar and 1.81 in Pindi variety. No correlation could be established between fat percentage of milk and that in Khoa. The value of ash content was found to be less then those observed by other workers like Ghodekar et.al.; (1974), Kumar et.al.; (1975). Might be due to the presence of some alum or coagulants. The percentage moisture was found to be higher then those reported by other workers like Srinivasan and Anantakrishnan (1964), Ghodekar (1969), The standard for Khoa under the prevention of food adulteration act (1954), required that it should not contain more then 28% moisture. The dealers of Khoa complain that such low moisture levels was not practical in the trade since good quality sweets could not be prepared from such an over desiccated product. The All India Mawa Vypar Sangh questioned the quality standards of Khoa on this it was decided to further examined by the government to India through the central committee of food standards in 1970. Then it was decided that pending further instructions from the central ministry of health no prosecution was launched or pannel action was taken on this act provided the sample of Khoa contained a minimum of 20% milk fat irrespective of its moisture content. The fat content of Khoa in our studies was 24.91, which is quite agreeable. The variations found by other workers may be
due to the level of fat in milk and the degree of concentration effected. The protein content and ash contents depend upon the type of milk used extinct of desiccation and addition of adulterants. The yield of Khoa was influenced by total solids in milk and moisture contents.

**Sugar Content Verses Microbial Population During Storage**

This experiment was conducted to study the effect of sugar on the population of organisms during storage. The quantity of sugar added was 30%, 40% and 50% and the data’s on bacterial count and yeast and mould count were obtained after fresh 3 days, 6 days, 9 days and 12 days. When fresh without sugar to controlled had $31.70 \times 10^3$, $41.70 \times 10^3$ bacterial organisms and 37 yeast and mould count. This increased in 30% sugar to $43.3 \times 10^3$ after 3 days, bacterial count was $45.36 \times 10^3$, after 9 days the count gradually increased to $47.03 \times 10^3$, after 12 days the number was $48.37 \times 10^3$ in 40% sugar concentration at the initial stage the number noted was $43.2 \times 10^3$. This shows that with the increase in concentration of sugar the number of bacterial count decreased. In 40% sugar after 12 days of storage the number of bacterial count gradually increased to $40.11 \times 10^3$ at 50% concentration on the initial population of $25.3 \times 10^3$ the count increased to $38.66 \times 10^3$ after 12 days. On every occasion that is after 3, 6, 9 and 12 days it was found that with the increase in concentration the total count gradually increased with increase in the days of storage. Similar was the trend found in yeast and moulds. The initial count of moulds were 37 per gm of Khoa, which in 30% concentration was 32.10 in 40 % sugar the mould count was 23.36. These values after 12 days of storage were 78.56, 70.17 and 50.35 respectively. From the overall studies it appears that sugar increases the concentration of Khoa and therefore place and inhibitory role in the growth of microorganism
and it can be used as a growth inhibitory substance for preserving Khoa. The results of the present investigation closely agreed with the result of Kumar *et al.*; (1975) from the Table results obtained indicate that after the storage of Khoa increases the growth of yeast and moulds increased more rapidly than the growth of bacterial organisms. This type of behavior could be related due to the reduction in available water during storage. As the growth period increased available water decreased this restricted the growth of bacteria reducing the competition of yeast and moulds which then shows faster growth as they could grow at reduced available water. Beuchat (1983) indicated that water influences the activity of growth metabolisms and survival of yeasts and moulds and attributed the faster growth of fungi due to the fact that and produce available water competition from bacteria becomes less. We and Ray (1953) also reported that addition of sugar at 60% concentration improves the keeping quality of Khoa four times. In the present study 40 and 50% sugar was able to improve the keeping quality four times. It can be concluded addition of 30, 40 and 50% sugar in Khoa reduced the available water significantly and increase the shelf life 3, 4 and 5 times respectively. Vijaylakshmi *et al.*; (2001) suggested majors for decreasing the microbiological quality for increasing the shelf life of Khoa. Sarkar *et al.*; (2001) also give importance for sugar addition for storage. On the same line the work of Narwade (2007).

**Role of Antifungal Agents On Storage of Khoa –**

In this experiment four antifungal substances were used to study the effect on storage at 30 and 5°C storage. These were potassium sorbate (0.2%), potassium sorbate (0.3%), boric acid (1%), gentamycin (0.2%) and streptomycin (0.2%). The results obtained are given in the Table -
XVIII. Controls were done without the use of any antifungal substance so that comparison could be made on the number of fungal organisms isolated during different days of storage. At 30°C storage was observed up to 21 days when 0.2% potassium sorbate was used. The numbers of fungal organisms were found to be less of control at every stage of isolation. Both at 30°C and 5°C temperature. At 0.3% concentration the fungal organism were 27 which increased to 123 after 21 days in comparison to 250 of control. At 5°C after 7 days the number was 18 which increased to 67 after 28 days of storage. In 1% Boric acid at 30°C the initial number was 35 which gradually increased to 133 after 21 days and at 5°C the number was 19 after 7 days and after 28 days it increased to 75. In 0.2% gentamycin the initial number was 35 which increased to 125 after 21 days of storage at 30°C temperature. At 5°C temperature the number was 20 which increase to 70 after 28 days. Streptomycin when used at 0.2% concentration showed an initial population of 34 which increased to 135 after 21 days at 30°C temperature. At 5°C the number was 25 which increased to 113 after 28 days. From the overall study 3 substances could be selected potassium sorbate 0.3%, boric acid 1% and gentamycin 0.2% which could be used for increasing the shelf life of Khoa by reducing the fungal population. It can be conclude from the above studies that addition of these antifungal agents of Khoa can be maintained the consumers acceptability up to 12 days at 30°C and 21 days at 5°C temperature. These substances at the same time help in reducing aflotoxin production of fungal organisms

**Effect of Antimicrobial Agents on Chemical Constituents—**

In this experiment three antifungal agents which were found to have promising results during the previous experiment were used to study
their effect in chemical constituents during the days of storage upto 21 days. Analysis for alteration in pH, dry matter, moisture, fat, protein and ash percentage were done after every week and the results obtained are shown in the Table - XIX from the data’s obtained it will be evident that ash content remain almost the same during the coarse of analysis. pH, moisture and fat, gradually reduced during storage while the dry matter and protein slightly increased. pH was 6.7 was reduced to nearly 6, moisture from 42.8 gradually reduced to 39, 35, fat from 24.9 gradually reduced to 20 or 22% while the dry matter increased from 60 to 64%, protein from 29.3 increase to almost 30%. These results indicate by using these substances the chemical constituents are not markedly affected as such they can be used for preservation. Rajarajan et.at.; (2006) studied the effect of antifungal agents keeping quality of Khoa. But there observations were restricted to proteolysis and lipolysis and evaluation were made on physical characters of Khoa.

**Inhibitory Effect of Antibiotic on Bacterial Organisms –**

In this study antibiotics used were penicillin, ampicillin, streptomycin, chlorphenicol, erythromycin, gentamycin and tetracycline. The organisms screened were *E.coli, Klebsiela pneumoniae, Enterobacter, Citrobacter, Staphylococcus* and *Salmonella*. From this study streptomycin, chlorphenicol and gentamycin were found to have most promising results while against penicillin and erythromycin most of the organisms were resistant followed by ampicillin and tetracycline. In streptomycin the maximum inhibitory zone was 16 mm found against *Enterobacter* and minimum 10 mm found against *E. coli*. Against chlorphenicol maximum inhibition of 18 mm was observed against *Klebsiella* and minimum of 10.5 against *Salmonella*. In gentamycin
maximum inhibition of 22.2 was observed against *Klebsiella* and minimum of 11 mm against citrobacter. The gradation of these substances could be gentamycin > chlorophenical > streptomycin. Gentamycin in the previous experiment was also found to be good in preventing fungal growth and here against bacteria therefore will be a good option for using it in increasing the shelf life. Guha *et al.*; (1983). Used some antibiotic against coliform organisms and also found that most of the strains were resistant to penicillin and erythromycin end sensitive to streptomycin and chlorophenical. They have not used gentamycin our results inhibits the same trend.

**M.I.C. of effective Antibiotic** –

The MIC concentration of streptomycin, chlorophanical and gentamycin were evaluated against the bacterial isolates from Khoa the results obtained have been given in the Table - XXI. In this study again gentamycin reproduced the least minimum concentration required for inhibition of the organism. Its concentration ranged from 0.5 to 3 μ gm per mm. While that of chlorofenicol was 1.7 to 7 and streptomycin it ranged from 2 to 5 from these studies the antibiotic substances could be used for preservation of microorganisms.

demonstrated the prevalence of food borne micro organisms in milk product, Rehman et.al.; (2006) used antioxidants in storage, Puneet et.al.; (2003) used the microwave treatment for increasing the shelf life, Champak et.al.; (2005) demonstrated the use of sugar to increase the shelf life Ventakesh et.al.; (2005) used the effect of packing material on increasing the shelf life of Khoa. In accordance with the object of these workers the present work was done on the same lines to improve the shelf life of Khoa. In accordance with the object of these workers the present work was done on the same lines to improve the shelf life of Khoa. The use of packing to improve the shelf life of Khoa has been explained to another worker in the lab. While this work was restricted to other methods for increasing the shelf life.