Milk has been used as an article of food since ancient times in India. It plays an important role in the diet. In India, the share of milk and its products is the largest after cereals, and it accounts for 16% of the total food expenditure. According to United Nation's Food and Agriculture Organization, India has become the single largest milk producing nation in the world, surpassing USA. India has shown impressive growth in the milk production, achieving an annual production of 132.4 million tonnes in the year 2012-13 which is about 15% of the total milk production in the world. India is among the world's largest and fastest growing market for milk and milk products. The average annual growth rate of milk production has been 4% during the past decades.

Khoa is a heat coagulated, partially dehydrated milk product. It is obtained by heat desiccation of whole milk to 65% to 70% milk solids without the addition of any foreign ingredients, mostly in private and unorganized sectors of India. Annual production of khoa is estimated to be about 9,00,000 tonnes and the value of this is around Rs. 45,000 million, which on value addition on its conversion in to khoa based sweets becomes almost double. It has been reported that khoa developed a flat taste in seven days during storage at 5-7°C but laboratory prepared samples remained in good condition for 14 days and which might be due to its high nutritional value and high water activity (0.96) is conducive for the growth of bacteria.

Bashir et al. found quality deterioration of khoa due to development of rancidity which adversely affected its storage life. Antioxidants are substances which prolong the shelf-life of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity, colour changes and loss of nutrient value. Even though many natural and synthetic compounds have antioxidant properties, only a few of them have been accepted as 'generally recognised as safe (GRAS)' substances for use in food products. Use of antioxidants may prevent the breakdown of fat.

Hence, the present study was designed to enhance the shelf life of khoa using certain antioxidants like tocopherol acetate and sodium ascorbate packed in LDPE pouch under refrigeration condition (5±2°C). In the present study, the shelf life of khoa was extended to 14 days by adding antioxidant combination like tocopherol acetate and sodium ascorbate at the level of 15 ppm and 200 ppm, respectively.

**MATERIALS AND METHODS**

Fresh milk (cow) was obtained from dairy farm of VCRI, Namakkal, Tamil Nadu and was standardized to 6 per cent of fat. Chemicals of analytical reagents (AR) grade procured from Himedia were used for various analysis and preservation studies. Khoa was prepared as per the procedure of De and was mixed with different concentrations of tocopherol acetate (10, 15 and 20 ppm) and sodium ascorbate (400, 600 and 800 ppm) and combination of both. It was packed with LDPE pouches with 65 µm thickness and was stored at 5±2°C. Different analysis were carried out at different concentration of...
antioxidants during storage period of zero day, seventh day, fourteenth day and twenty first day. Six trails were carried out for each treatment.

Khoa samples were evaluated for sensory attributes like colour and appearance, taste, flavour, body and texture and overall acceptability by using 9-point hedonic scale as per the procedure of Amerine et. al.11. The TBA number of the khoa was determined by following the method of Tarladgis et. al.12.

Total viable count, Coliform count were conducted as per standard methods for examination of dairy products13. The procedure adopted for the enumeration14 of yeast and mould count was as per IS: 3507:1966.

RESULT AND DISCUSSION

Preliminary trials were conducted using three levels of tocopherol acetate (10, 15 and 20 ppm) and sodium ascorbate (400, 600 and 800 ppm). The best concentration for further studies was selected on the basis of sensory evaluation. It was 15 and 600 ppm for tocopherol acetate and sodium ascorbate, respectively. The combination of both was again subjected to sensory evaluation and further analysis was carried out compared with the other treatments.

The sensory evaluation revealed that there were significant differences noticed between different parameters like colour and appearance, taste, flavour and body and texture of khoa at different levels of treatments. Tocopherol acetate (15 ppm), sodium ascorbate (600 ppm) and their combinations showed significantly higher (p<0.05) values compared to other treatments. Combination of tocopherol acetate (15ppm) and sodium ascorbate (600ppm) treated samples were found to be the best with overall acceptability 8.00±0.00 on zero day and were significant until 14th day of storage with 6.17±0.17 and lowers significantly on 21st day (2.33±0.21) storage period compared to control which shows 8.67±0.21, 7.00±0.00, 4.17±0.17and 2.0±0.00 on zero, 7th, 14th and 21st day of storage period, respectively.

These observations are in similar to the reports of Kumar et. al.4, who reported combination of sodium ascorbate and tocopherol acetate are best in overall acceptability in khoa and Yadav et.al.15, where potassium sorbate, tocopherol acetate and combination (tocopherol acetate + sodium ascorbate) treated samples were found to be best and equally acceptable in peda.

It was recorded that the sensory score of overall acceptability on 21st day was very low and it could be due to the presence of mould and yeast growth on the khoa and are on accordance with the reports of Kulkarni and Hembade16 who inferred that the samples were acceptable for two days at room temperature and 10 days at 5°C.

The results are in concurrent with the Yadav et.al.15, who observed combination (tocopherol acetate + potassium sorbate) and combination (tocopherol acetate + sodium ascorbate + potassium sorbate) treated samples were found best and equally effective in checking the increase in TBA value of peda.

The Total viable count in the khoa treated with selected three treatments during storage period is given in Table-2. There were increase in the total viable count are observed in all the treatments during storage period. Minimal increase in the total viable count was recorded in the treatment with sodium ascorbate and its combination compared to control and tocopherol acetate. This may be due to the antimicrobial properties of tocopherol acetate and sodium ascorbate. These findings are in accordance with Yadav et.al.15, who recorded Potassium sorbate, sodium ascorbate and combination (tocopherol acetate + sodium ascorbate + potassium sorbate) treated samples were found best. The present study revealed that there was no coliform count observed in any trails with different concentrations during different storage period.

The findings of yeast and mould counts are presented in the Table-3. It was recorded from the present study that there was prominent growth or count of yeast and mould were observed in all different treatments of antioxidants during different storage.
Table-1. Effect of Different level of antioxidants on Thiobarbituric Acid (mg malonaldehyde/kg) value of khoa at different storage period

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TA</th>
<th>SA</th>
<th>TA+SA</th>
<th>Storage mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero day</td>
<td>0.65±0.01</td>
<td>0.64±0.008</td>
<td>0.642±0.01</td>
<td>0.652±0.008</td>
<td>0.65±0.01</td>
</tr>
<tr>
<td>7th day</td>
<td>0.71±0.004</td>
<td>0.69±0.003</td>
<td>0.68±0.002</td>
<td>0.67±0.004</td>
<td>0.69±0.007</td>
</tr>
<tr>
<td>14th day</td>
<td>0.81±0.003</td>
<td>0.80±0.004</td>
<td>0.712±0.002</td>
<td>0.71±0.003</td>
<td>0.76±0.02</td>
</tr>
<tr>
<td>21st day</td>
<td>0.9±0.003</td>
<td>0.89±0.003</td>
<td>0.8±0.02</td>
<td>0.73±0.01</td>
<td>0.83±0.03</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.77±0.04</td>
<td>0.76±0.04</td>
<td>0.71±0.03</td>
<td>0.69±0.01</td>
<td>0.73±0.03</td>
</tr>
</tbody>
</table>

abcd means bearing same superscript column wise and row wise was not significant
TA: Tocopherol acetate - 15 ppm SA: Sodium ascorbate - 600 ppm
TA+SA: Tocopherol acetate - 15 ppm + Sodium ascorbate - 600 ppm

Table-2. Effect of Different level of antioxidants on Total Viable Count (log10 CFU/g) of khoa at different storage period

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TA</th>
<th>SA</th>
<th>TA+SA</th>
<th>Storage mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero day</td>
<td>2.33±0.21</td>
<td>2.33±0.21</td>
<td>2.0±0.25</td>
<td>2.33±0.33</td>
<td>2.23±0.24</td>
</tr>
<tr>
<td>7th day</td>
<td>3.0±0.25</td>
<td>2.83±0.17</td>
<td>2.17±0.17</td>
<td>2.5±0.22</td>
<td>2.62±0.24</td>
</tr>
<tr>
<td>14th day</td>
<td>3.8±0.31</td>
<td>3.5±0.22</td>
<td>3.0±0.37</td>
<td>3.5±0.22</td>
<td>3.46±0.29</td>
</tr>
<tr>
<td>21st day</td>
<td>4.83±0.30</td>
<td>4.5±0.22</td>
<td>4.0±0.37</td>
<td>3.0±0.26</td>
<td>4.08±0.4</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.5±0.47</td>
<td>3.29±0.39</td>
<td>2.74±0.43</td>
<td>2.83±0.31</td>
<td>3.10±0.41</td>
</tr>
</tbody>
</table>

abcd means bearing same superscript column wise and row wise was not significant
TA: Tocopherol acetate - 15 ppm SA: Sodium ascorbate - 600 ppm
TA+SA: Tocopherol acetate - 15 ppm + Sodium ascorbate - 600 ppm

Table-3. Effect of Different level of antioxidants on Yeast and Mould of khoa at different storage period

<table>
<thead>
<tr>
<th>Days (Range)</th>
<th>Control (Avg)</th>
<th>TA (Avg)</th>
<th>SA (Avg)</th>
<th>TA+SA (Avg)</th>
<th>Storage Avg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero day</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>7th day</td>
<td>2.2×10^4</td>
<td>8.1×10^3</td>
<td>6.4</td>
<td>2.5×10^4</td>
<td>1.66×10^4</td>
</tr>
<tr>
<td>14th day</td>
<td>8.2×10^3</td>
<td>6.3×10^3</td>
<td>4.82×10^3</td>
<td>9.4×10^3</td>
<td>5.2×10^3</td>
</tr>
<tr>
<td>21st day</td>
<td>1.74×10^3</td>
<td>4.34×10^2</td>
<td>4.82×10^3</td>
<td>9.4×10^3</td>
<td>5.3×10^3</td>
</tr>
<tr>
<td>Treatment (Avg)</td>
<td>1.58×10^3</td>
<td>2.3×10^3</td>
<td>2.2×10^3</td>
<td>2.8×10^3</td>
<td>10×10^3</td>
</tr>
</tbody>
</table>

TA: Tocopherol acetate - 15 ppm SA: Sodium ascorbate - 600 ppm
TA+SA: Tocopherol acetate - 15 ppm + Sodium ascorbate - 600 ppm
period and the count were increased significantly (p<0.05) from 7th day of storage to 21st day of storage. The Combination of antioxidants incorporated khoa showed slightly lower yeast and mould count on 14th day compared to other treatments and there was higher count observed on 21st day of storage even in tocopherol acetate and sodium ascorbate treated khoa. These results are in accordance with the Vijayalakshmi et al. [17], who studied microbiological quality of sweet khoa during storage at 6-8°C and reported that the total yeast and mould count of sweet khoa at 3,4,5 and 6 days of storage were increased significantly.

The increase in growth of yeast and mould count during storage period might be due to favourable climatic conditions for the growth of fungal organisms or faulty practices of handling, processing and storage of milk products were the possible sources of contamination of yeast and mould in indigenous milk products.

CONCLUSION

It can be concluded from the present study that the combination of antioxidants like tocopherol acetate and sodium ascorbate at the level of 15 ppm and 600 ppm, respectively can significantly reduce TBA value, TVC and coliform count of khoa with significantly higher sensory properties and can be used to increase the shelf life of khoa to 14 days of storage period at 5±2°C under LDPE package.

REFERENCES