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# Microbiological assessment of milk based product Khoa marketed from Bhokar region

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## Abstract

Bhokar taluka comprises 80 villages, and milk is collected daily in considerable quantities. People are provided khoa for businesses in the Bhokar region dist. Nanded. The Khoa quality was analysed by MBRT, among used Khoa samples, BKS-01, BKS-02 and BKS-04 were showed good quality and BKS-03 was fairy quality of used milk. The khoa samples used in this study had a high bacterial count. There were numerous contamination sources present during processing of the khoa, posing a risk of spoilage. The results revealed that the four khoa samples used had a high bacterial count. BKS-01 had a total plate count of 23 x $10^5$ CFU/g, BKS-02 had 69 x $10^5$ CFU/g, BKS-03 had 37 x $10^6$ CFU/g, and BKS-04 had 31 x $10^5$ CFU/g. Potato dextrose agar medium, Sabaurdor's Agar, and Czapek-Dox agar were utilized to isolate fungal species. BKS-01 had maximum mycelial growth of up to 60 cfu/g in the sample. No single colony was found on agar medium plates that had been prepared. As a result, freshly prepared khoa should be utilized in the production of khoa-based goods.

***Key words**: Khoa, bacterial count, mycelia count, media, MBRT.*

## 1. Introduction

In the Indian system, a variety of milk products are employed, including Khoa, a partially desiccated milk that is traditionally used in the preparation of a variety of sweets, vegetable curries, and other foods. In India, khoa is prepared by condensing milk and eliminating the water with continual heating. It's a perishable food that has a limited shelf life. The temperature of the milk is raised high enough during the manufacture of khoa to destroy most of the bacteria's vegetative cells.

Because the product is prepared using traditional methods with no concern for the quality of raw materials utilised or hygienic storage, the shelf life of the product is significantly impacted by thermoduric organisms and organisms obtained during storage [1].

Harmful organisms such as *Staphylococcus aureus* and *Bacillus cereus* regularly contaminate Khoa, according to a number of studies done across India. Worker handling and dirty processing utensils are the most likely sources of microbes in Khoa [2]. The goal of this study was to estimate the microbial count of Khoa marketed in Bhokar region, dist. Nanded.

## 2. Materials and Method

**Collection of Khoa from Bhokar**

Total 04 samples of khoa were collected at random in pre-sterilized containers from popular sweet marts of various parts of Bhokar dist. Nanded region and these samples were transported in ice bucket to Department of Microbiology at College. Each sample of khoa was processed under sterile condition by taking 1 g of khoa with 10ml of physiological saline and homogenized by mortar pestle and MBRT test was applied to assess the milk quality [3, 4].

**Isolation of Microorganisms**

**Isolation of Bacterial species**

Inoculation of processed sample was carried on nutrient agar and MacConkey agar (HI Media) after incubation at 37°C for 24 hrs the identification of the colonies grown was made. Performed various biochemical tests of the Obtained isolates were identified (Godbole Suchitra et al., 2013). The used media such as Nutrient agar having (in %), Peptone:0.5, Beef extract:0.3, NaCl:0.5, Agar-Agar:2.0, Distilled water:100, pH:7.2±0.2 also MacConkeys agar (Hi-Media) were used in the present investigation [3, 4].

**Isolation of Fungi**: For the isolation of Khoa fungus, the Khoa culture was serially diluted and disseminated on several selective media such as Potato dextrose agar medium (The medium contains (g/lit) Potato peel: 200.0, Dextrose: 20.0, Agar agar: 20.0, pH: 5.6±0.2), Sabaurdor's Agar (The medium contains (g/lit) Peptone: 10.0, Dextrose: 40.0, agar agar: 20.0, pH: 5.6±0.2), Czapek-Dox agar (The medium contains (g/lit) Sucrose: 30.0, Sodium nitrate: 2.0, Dipotassium phosphate: 1.0, Magesium sulphate: 0.5, Potassium chloride: 0.5, ferrous sulphate: 0.01, agar agar: 20.0, pH: 7), [3, 4, 5].

**Serial Dilution method:** To isolate various microorganisms from collected khoa samples, one g of each sample was mixed in 9 ml of sterile saline solution placed in test tubes, and the suspension was serially diluted up to $10^{-7}$. The diluted suspensions (0.1 ml) of dilutions 3 onwards were inoculated on used selective media and incubated at 35°C for 3 days [3, 4].

**Isolation of Microorganisms by spread-plate technique**

With the help of Marker pen, label the bottom of the agar medium plates with the name of the bacterium to be inoculated. Three plates are to be inoculated. Pipette 0.1 ml of the respective sample onto the center of a various agar plate. Dip the spreader (L-shaped glass rod) into a beaker of ethanol and then tap the rod on the side of the beaker to remove any excess ethanol. Briefly pass the ethanol-soaked spreader through the flame to burn off the alcohol, and allow it to cool inside the lid of a sterile Petri plate. Spread the bacterial sample evenly over the agar surface with the sterilized spreader, making sure the entire surface of the plate has been covered. Also make sure you do not touch the edge of the plate. Immerse the spreader in ethanol, tap on the side of the beaker to remove any excess ethanol, and reflame. Repeat the procedure to inoculate the remaining two plates. Invert the plates and incubate for 24 to 48 hours at room temperature or 30°C. After incubation, measure some representative colonies and carefully observe their morphology [3, 4].

**Morphological Characterization**: Shape, size, elevation, color, opacity, grams nature, and other morphological properties of isolates were studied for characterization [3, 4].

## 3. Results and Discussion

Milk-based product Khoa samples were taken from the different popular sweet marts of Bhokar city. For this study was designated as BKS (Bhokar Khoa Sample). Bhokar taluka has 80 villages, and a large amount of milk is gathered every day in the dairy from these villages. In the city of Bhokar, there are now 12 private milk parlors. People are provided with khoa from four large dairy businesses. Because there is a high risk of contamination during the collection of such khoa from various places due to incorrect handling and a lack of knowledge about microbial contamination, hence it is necessary to investigate contamination present in khoa.

**Methylene Blue Reductase Test (MBRT)**

In the homogenizer, the collected khoa samples were adequately homogenised. The four khoa samples were homogenised and tested using the MBRT method to determine the quality of the milk. The MBRT revealed that milk samples BKS-01, BKS-02, and BKS-04 were of good quality. The BKS-03 revealed that milk quality was fair (Table 1).

The MBRT procedure was carried out in test tubes. Tubes BKS-01, BKS-02, and BKS-04 are blue in colour, also tube containing BKS-03 sample had showed decolorization of methylene blue dye. It is concluded that the longer the methylene blue remains unchanged, the better the milk quality.

The above obtained results were compared to the Reddy et. al (1983), work they studied methylene blue reduction tests revealed that 12 samples were of bad quality and 08 samples were of fair quality during the examination [6]. There have been reports of *E.coli* contamination in sweets ranging from 10 CFU/gm to $1.0x10^2$ CFU/gm, SPC of $5x10^3$ CFU/gm to $2.1x10^5$ CFU/gm in khoa from Hissar market (6), and SPC count of $3x10^5$ CFU/gm to $7.5x10^7$ CFU/gm in Sandesh samples [7].

**Table 1: Results of Khoa samples by MBRT method** (BKS: Bhokar Khoa Sample)

| Sr. No | Sample no | Recorded Results (Milk quality) |
|---|---|---|
| **1** | BKS-01 | Good |
| **2** | BKS-02 | Good |
| 3 | BKS-03 | Fair |
| 4 | BKS-04 | Good |

**Table 2:** colony forming units (CFU) of bacterial, mold and coliforms on media (BKS: Bhokar Khoa Sample)

| **Sample** | **Total plate count (Bacterial) (cfu/g)** | **mold count (cfu/ g)** | ***Coliform* count (cfu/g)** |
|---|---|---|---|
| BKS: 01 | $23 \times 10^5$ | 25 -60 | Nil |
| BKS: 02 | $69 \times 10^5$ | 14-35 | Nil |
| BKS: 03 | $37 \times 10^6$ | 27-52 | Nil |
| BKS: 04 | $31 \times 10^5$ | 34-46 | Nil |

**Table 3:** The nutrient agar plates incubated up to Three days ( BKS: Bhokar Khoa Sample)

| **Sample** | **Total plate count (cfu/g)** | | |
|---|---|---|---|
| | **Day I** | **Day II** | **Day III** |
| BKS: 01 | $23 \times 10^5$ | $27 \times 10^5$ | $5 \times 10^6$ |
| BKS: 02 | $69 \times 10^5$ | $73 \times 10^5$ | $12 \times 10^6$ |
| BKS: 03 | $37 \times 10^6$ | $43 \times 10^6$ | $14 \times 10^7$ |
| BKS: 04 | $31 \times 10^5$ | $40 \times 10^5$ | $37 \times 10^6$ |

**Isolation of Microorganisms**

The collected khoa samples were diluted using the serial dilution method, and $10^{-5}$ to $10^{-7}$ dilutions were collected for the current investigation because there was a possibility of large impurities in the samples. For the isolation of mesophilic and enteric bacteria, the produced dilutions were disseminated over several media employed in the current study, such as nutritional medium and MacConkey agar. Potato dextrose agar medium, Sabaurdor's Agar, and Czapek-Dox agar were utilized in the isolation investigation to isolate fungal species.

The results revealed that the four khoa samples used had a high bacterial count. BKS-01 had a total plate count of 23 x $10^5$ colony forming units per g, BKS-02 had 69 x $10^5$ colony forming units per gramme, BKS-03 had 37 x $10^6$ colony forming units per g, and BKS-04 had 31 x $10^5$ colony forming units per g. The enormous bacterial count found on nutritional agar plates was determined based on these findings. There were numerous contamination sources present during the processing of the khoa, posing a risk of khoa spoilage [8, 9, 10, 11].

The media employed to study the mould count was potato dextrose agar (PDA plates). BKS-01, BKS-02, BKS-03, and BKS-04, respectively, exhibited 25-60, 14-35, 27-52, and 34-46 cfu/gm. In comparison to the other three samples, sample no. BKS-02 demonstrated the least amount of fungal development. BKS-01 had maximum mycelial growth of up to 60 cfu/g in the sample. When the samples were compared to the MacConkeys agar medium to look for enteric bacteria, there was no growth on the plates. It confirms that the enteric microbe was not found in any of the khoa samples examined (Table 2).

The nutrient agar plates were kept at a regulated temperature (35±02°C) for up to three days of incubation. The plates displayed the microbial cells growing expansion in the form of colony forming units. After two days of incubation, the plates revealed that the samples BKS-01, BKS-02, BKS-03, and BKS-04 had 27 x $10^5$, 73 x $10^5$, 43 x $10^6$, and 40 x $10^5$ cfu/g, respectively. BKS-01, BKS-02, BKS-03, and BKS-04 had 5 x $10^6$, 12 x $10^6$, 14 x $10^7$, and 37 x $10^6$ cfu, respectively, after the third day of incubation (Table). According to the findings, if infected samples are held for more than three days, they are more likely to spoil the khoa and cause infection in humans, hence fresh khoa should be sold as soon as possible.

According to Godbole Suchitra et al., (2013) they studied total of 20 samples were analyzed to determine the bacteriological quality. A total viable count (TVC) ranging from 4.90 x $10^5$ to 1.2 x$10^7$ CFU/gm of sample was observed. After 24hrs of incubation the colonies were identified from their colony characteristics and growth on different specific media. Out of 20 samples, 55% samples showed the presence of *E.coli*, 65% of samples were contaminated with *Salmonella* sp., and 90% of samples were found to be contaminated with *Staphylococcus* sp. (1).

The study reveals the problem of contamination of khoa samples sold in Nagpur city. Heavy bacterial contamination was found in all samples. This can be attributed to the practice of preparing large bulk of products and storage of products at room temperature for long duration. However considering the impact of consumption of such contaminated products on public health, the HACCP should be applied during the manufacturing process. The analysis from raw material to final product indicate that, though microbiological quality of khoa may be satisfactory at the time of production, it deteriorated by the time it is available for sale in the market [1, 12, 13]. *Staphylococcus species* are found in almost 90% of samples studied. The reports also suggest that *Staphylococcus sp.* is most frequently occurring organism in sweet based milk products such as khoa, rabri, gulabjamun etc [9, 12, 14, and 15]. Millions of people are affected by food borne illness resulting from ingestion of toxin produced by food associated *Staphylococcus* [16, 17].

## 4. Conclusion

According to the findings of the current analysis, the obtained khoa samples from Bhokar city were polluted by mesophilic bacterial and fungal pollutants found in

the air during the research. The most crucial finding was that no single colony could be seen on the MacConkey agar medium plates that had been prepared. As a result, intestinal microbial contamination was not found. Another finding of the study was that increasing the number of days incubated increases the pollutants, indicating that the khoa sample may not be able to remain in the favorable condition for much longer. As a result, freshly prepared khoa should be utilized in the production of khoa-based sweets.

**Conflicts of interest:** The author stated that no conflicts of interest.

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