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Microbial Hazard Analysis of Milk and Khoa in Manual and Mechanical Production Processes in Parbhani City of Maharashtra State of India

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ABSTRACT

The present study was planned for microbial hazard analysis of Khoa production process in and around Parbhani city of Maharashtra State, India. The manual and mechanical methods of Khoa production processes were evaluated. The microbial hazard analysis of identified CCP'S of production process was done. A total of six commercial Khoa production units consisting three each manual and mechanical methods were used during present studies. A total of eight replicates were made for sample collections from each unit. A total of nine critical control points from each method were selected for hazard analysis. A total of 24 samples were collected from each identified CCP in both the methods. Total Viable Count, Total Staphylococcal Count, Total E. coli Count. Total Yeast and Moulds Count to compare microbial quality of raw milk were used in manual and mechanical method of Khoa production. The mean TVC count differ significantly ($p<0.01$) within and amongst groups. A non-significant effect within and amongst groups was observed in raw milk samples in relation to Staphylococci spp. and E. coli. The entire milk sample revealed absent of Yeast and Moulds. TVC counts of all the CCP'S (C1 to C9) in both the methods were compared within and between groups. A significant ($p<0.05$) effect of CCP was observed upon TVC counts. The TVC counts of manual and mechanical method differ significantly ($p<0.05$) amongst production processes. The Khoa production units also had significant effect upon TVC counts .The mean Staphylococcal count differ significantly ($p<0.01$) within and amongst groups. A highly significant ($P<0.01$) effect of Khoa production process was seen in total Staphylococcal counts. The total Staphylococcal count differs significantly ($p<0.05$) amongst production units. A highly significant ($p<0.01$) effect of CCP was seen in within and amongst group was seen in relation to E. coli count. Khoa production process has significant ($p<0.05$) effect upon E. coli count. However the Khoa production did not have effect on E. coli counts. The Yeast and Moulds were absent in both the production process from C1 to C7. The mean Yeast and Moulds count at C8 in manual method were 3.36 ± 0.07 and 2.16 ± 0.33 in mechanical method at C9 were 2.87 ± 0.21, 1.75 ± 0.35 respectively. The mean Staphylococcal count were significantly ($p<0.01$) lower in Khoa produced by mechanical method. However mean total viable count E. coli counts did not differ within and amongst group. The Yeast and Moulds counts also did not differ within and amongst groups.

Key words: *Khoa, Microbial hazard, Manual and Mechanical Production*

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INTRODUCTION

India ranks 1st in respect of buffalo and second in cattle in the world. India has 57% of buffalo world population. Dairy sector provides livelihood and security net to the landless and marginal farmers. India continuous to be largest producer of the milk in the world since15 years. The milk yield was 17 to 22 million tones in 1960 has increased to 163.7 million tones in 2016-2017. The annual growth rate of Indian milk production is 5.53% and global was 2.09% [1].

Milk and its products are considered as major causes of milk borne illness worldwide. It is an ideal growth medium for several microorganisms. Milk and milk products are considered as vehicles for *Staphylococcus aureus* infection to the human. Although heating is likely to be destroy all pathogens there is concern of contamination during processing, storage and packaging[5].

Nanu *et al.*[15] studied quality assurance and public health safety of raw milk and their products and studied that the quality and safety of milk begins with producer. Milk get contaminated at various stages, environment, handling, packaging and storage. The microorganisms which may gain entry to milk can multiply and brings either spoilage or render them unsafe due to potential health hazards. The threat posed by disease spread through contaminated milk is well known as epidemiological impact of such diseases is considerable. The quality of each sample needs to be evaluated by estimating Total Viable Counts (TVC), *Coli* form Counts (CC), *Staphylococcal Counts* (SC), *Yeast* and *Moulds* Counts (YMC).

Even though Khoa is prepared under hygienic condition they are prone for microbial contamination. It may gain entry right from farms till it reaches final consumers, at the time of transportation, storage and packaging. A preventive measure needs to be evaluated at every stage which will influence microbial quality by applying HACCP principles[12].

Sohal *et al.*[19] reported survival of *E.coli* and *Staphylococcus aureus* during production of Khoa. *S. aureus* recovered when milk is heated at 63˚C containing 6.5 % fat. The reduction of *E.coli* or *S. aureus* during storage was at 6-7˚C as less than one log cycle, regardless of moisture. The potential of pathogen to survive in Khoa during processing should be taken into consideration while production protocol.

HACCP is proven to be more effective when used parallel with quality management system and slandered operating procedure such as GMP and GHP. The food safety management system (FSMS) was introduced by ISO for food safety. Hazard identification, Establish critical control points and critical limits are an important component of the system.

Implementation of FSSAI rules and regulations as per Food Safety Standard Act, 2006 for Khoa production is a challenging task from the public health point of view. The most of the Khoa production business is being done in traditional way with the help of available local resources. In recent times, due to high urbanization the demand for Khoa increased resulting into use of mechanized processing for Khoa production. Little information is available about scientific evaluation of Khoa production processes in unorganized sector.

The present study was planned with objective to study microbial hazard analysis of Khoa production process in manual and mechanical methods from unorganized sector to identify CCP for effective implementation of HACCP.

MATERIAL AND METHODS

Microbial analysis:

Total viable count

For evaluating total viable count (TVC), standard pour plate technique was followed [3].

Determination of differential count:

For the isolation of *Staphylococcus aureus, E.coli,* and *Yeast* and *Moulds* selective media were used. For the Isolation of *E.coli, Staphylococcus aureus, and Yeast & Moulds* was done as per the method described by Bacteriological Analytical Manual[4].

Statistical analysis

Comparison of means of all microbial counts was made within and between groups. The Analysis of Variance (ANOVA) was used for comparison of means between and amongst groups using SYSTAT® software.

RESULTS AND DISCUSSION

Flow chart of Khoa production process

The CCP were identified separately for manual and mechanical Khoa production processes. The flow chart for manual Khoa production process is given in Fig.1, and for mechanical process in a Fig. 2.

Raw milk **CCP1 (C1)**
↓
Milk Can **CCP2 (C2)**
↓
Washing And drying Iron pan**(C3)** and stirrer**(C4)** by water**(C5)**
(CCP3,CCP4,CCP5)
↓
Pouring of raw milk by milk can
↓
Continious boiling
↓
Vigorous strring(with iron ladle)
↓
Simultenious Scrapping of pan bottom & slides by Personnel **CCP6 (C6)**
↓
Semi-solid mass down at bottom of pan
↓
Cooled at room temperature
↓
Khoa **CCP7(C7)**
↓
Kept in Storage vessel **CCP8 (C8)**
↓
Packed in Polythene bags or musline cloth **CCP9(C9)**

Figure 1: Manual Khoa Production Process

Raw milk **CCP1 (C1)**
↓
Milk Can **CCP2 (C2)**
↓
Washing And drying Stainless steel pan**(C3)** with blade **(C4)** By water **(C5)**
↓
Pouring of raw milk by milk can
↓
Continious boiling
↓
Vigorous strring
↓
Simultenious Scrapping of pan bottom & slides by personnel **CCP6 (C6)**
↓
Semi-solid mass down at bottom of pan
↓
Cooled at room temperature
↓
khoa **CCP7 (C7)**
↓
Kept in Storage vessel **CCP8 (C8)**
↓
Packed in Polythene bags or musline cloth **CCP9 (C9)**

Figure 2: Mechanical Khoa Production Process

Microbial analysis of raw milk

Raw milk is good medium for growth of many microorganisms including pathogenic organisms; the number and type of microorganisms in milk are dependent upon factors like animals, environment hygiene. presence of pathogenic bacteria often emergent as a major public health concern .In milk processing quality of products also depend upon microbial quality of raw milk used for processing[14].

In present study total viable count ,total *Staphylococcal* count, total *E.coli* count, total *Yeast* and *Mould*s count estimated using selective media. All 24 Samples from each group of manual and mechanical production units were analysed, the results are depicted in Table 1.

The mean TVC counts observed in raw milk where $\log_{10}$ 5.76 ± 0.02 and mechanical units were 5.38 ± 0.05.Comparison of group amongst group revealed significant ($p<0.01$) differences in TVC counts, this may be due to differences in sources of raw milk. Earlier, Subramanian and Ora[21] also successfully used TVC counts for evaluation of microbial quality of raw milk and reported that presence of bacteria in raw milk were due to contamination from various sources. The microorganisms not only contaminate the milk also multiply and grow if cooling facilities are not available.

Varadraj and Nambudripad[22] found various *Staphylococcus* spp. in raw milk used for Khoa production. The *Staphylococcal* count found were 1×10^6 cfu/ml of milk. In present study the *Staphylococcal* count of $\log_{10}$ 3.36 ± 0.05 were seen in milk samples of manual group and 2.88 ± 0.43 in mechanical group. The mean *Staphylococcal* count within and between groups did not differ statistically (Table 1).

The mean *E.coli* count of raw milk in manual groups was 3.20 ± 0.52 and 3.68 ± 0.36 in mechanical groups. The mean *E. coli* count within and amongst groups did not differ significantly ($p<0.05$). Earlier workers also reported similar finding[2, 6, 10].

FSSAI[7] standards stipulate *Yeast* and *Mould*s count of various milk and milk products counts. The *Yeast* and *Mould*s counts are indicators of contamination of raw milk. However, in present study *Yeast* and *Mould*s count could not be detected in all 48 milk samples tested from manual and mechanical Khoa processing units.

Table 1: Results of microbial analysis of milk sample used for Khoa production

Sr. No.	Test performed	Khoa production process (Mean ± S.E.) ($\log_{10}$=cfu/ml)	
		Manual (Mean ± S.E.) (n=24)	Mechanical (Mean ± S.E.) (n=24)
1	Total Viable Count	5.76±0.02[ab]	5.38±0.05[ab]
2	Total *Staphylococcal* Count	3.36±0.05	2.88±0.43
3	Total *E. coli* Count	3.20±0.52	3.68±0.36
4	Total *Yeast and Moulds* Count	ND	ND

a-($p<0.01$)
b-($p<0.05$)
ND- Non detected

Microbial analysis of Khoa production process TVC counts of CCP in Khoa production process

All the 9 CCP of manual and mechanical Khoa production units were analysed for TVC counts. The mean TVC counts were compared within and amongst group. The results are given in Table 2. The mean TVC counts differ significantly ($p<0.05$) at each CCP, from C1 to C9 in both methods of Khoa production. It was interesting to note that the TVC counts started decline from Raw milk (C1) to utensils (C4) and them increased in Khoa production. It was clearly evident from this observation that reduction in TVC counts from raw milk up to end product may be due to heat processing. The high TVC counts from water, hands of personnel, storage vessel, Khoa and packaging material clearly indicate role of contamination of utensils, water, hands of personnel. The observations were similar in both manual and mechanical groups. However significantly ($p<0.05$) lower TVC counts were observed in mechanical processing than manual processing. This may be due to minimized handling and lower environmental contamination. The Khoa production unit and replicates also showed significant ($p<0.05$) effect on mean TVC counts.

Karthikeyan and Dhanalakshmi[11] evaluated hygienic quality of Khoa in and around Chennai. The presence of high TVC counts during processing was found to be due to poor bacterial quality of raw milk, contamination during storage, unsatisfactory sanitation and unsuitable temperature. The results of present study are on similar lines.

Table 2: Microbial TVC counts of CCP in Khoa production process

Sr. no.	CCP	Mean Total Viable Count (log_{10}=cfu/ml)	
		Khoa production process	
		Manual (Mean ± S.E.) (N=24)	Mechanical (Mean ± S.E.) (N=24)
1	C1	5.76±0.02[a]	5.38±0.05[a]
2	C2	4.64±0.04	4.22±0.05
3	C3	3.95±0.18	2.86±0.35
4	C4	2.41±0.42	3.81±0.20
5	C5	3.94±0.24	4.05±0.04
6	C6	3.61±0.23	2.55±0.38
7	C7	4.39±0.20	4.09±0.08
8	C8	4.71±0.05	4.25±0.06
9	C9	4.57±0.20	4.16±0.19

ANOVA

Sr. No.	Source	df	Sum of square	Mean-square	F-ratio
1	CCP	8	92.266	11.533	114.840**
2	Khoa production process	1	11.481	11.481	114.323**
3	Khoa production unit	2	1.425	0.713	7.096**
4	Replicates	7	2.326	0.332	3.308**
5	Error	380	38.163	0.100	

**- $p<0.01$

Mean *Staphylococcal* counts of CCP in Khoa production process

The unhygienic conditions at production units lead to contaminations of products with different types of microorganisms leading to low shelf life of finished products[20]. The *Staphylococci* spp. is an important food borne bacteria of public health significance. These bacteria are also resistant to antibiotics. Milk products often contaminated with *Staphylococcus* spp[6]. The results of mean *Staphylococcal* counts of CCP in both manual and mechanical process are given in Table 3.The analysis of variance clearly indicates that the total *Staphylococcal* counts high significant ($p<0.01$) revealed from C1 to C4 in manual Khoa production process. The *Staphylococcal* counts from C1 to C3 in mechanical production process are highly significant ($p<0.01$) effects of Khoa production process was seen amongst manual and mechanical process. A significant ($p<0.05$) effects of Khoa production units and replicates also was seen in both production processes. Presence of *Staphylococcal* contamination during Khoa production was also reported earlier by Godbole *et al.* [9]. The unhygienic conditions during production were found to be responsible for the same. The *Staphylococcal* count were observed between 1.3×10^4 to 2.1×10^4 cfu/ml. Ghodekar *et al*. (1974) also reported that presence of *Staphylococci* spp. in during Khoa production process. Similar observations were also made during study.

Table 3: Mean *Staphylococcal* counts of CCP in Khoa production process

Sr. no.	CCP	Total *Staphylococcal* Count (log_{10}=cfu/ml)	
		Khoa production process	
		Manual (Mean ± S.E.) (n=24)	Mechanical (Mean ± S.E.) (n=24)
1	C1	3.36±0.050	2.88±0.43
2	C2	2.15±0.41	1.83±0.36
3	C3	2.70±0.36	1.17±0.34
4	C4	1.60±0.39	2.37±0.39
5	C5	2.39±0.39	0.28±0.19
6	C6	2.42±0.30	1.80±0.33
7	C7	3.82±0.19	2.04±0.33
8	C8	4.45±0.07	3.86±0.09
9	C9	4.025±0.26	3.85±0.19

ANOVA

Sr. no.	Source	df	Sum of square	Mean square	F-ratio
1	CCP	8	50.969	6.371	30.765**
2	Khoa production process	1	13.916	13.916	67.196**
3	Khoa production unit	2	1.549	0.775	3.147*
4	Replicates	7	3.412	0.487	2.353*
5	Error	269	55.708	0.207	

*-p<0.05, **- p<0.01

Mean *E. coli* counts of CCP in Khoa production process

The total *E.coli* counts of all CCP'S in both manual and mechanical methods of Khoa production process enumerated. The results are given Table 4. A highly significant (p<0.01) effect of CCP was seen within and amongst groups. The Khoa production process also significant (p<0.05) effect of Khoa production units was not seen on *E. coli* counts.

E. coli counts of log_{10} cfu/ml to 1.0×10^2 cfu/gm were reported by Godbole *et al* [9]. The *E. coli* contamination of milk and milk products is due to human factor and unhygienic conditions. *E. coli* contamination is indicator of faecal pollution generally in insanitary condition of water, raw milk and dairy products. Presence of *E. coli* in present study may be due to water, utensils and personnel contamination.

Table 4: Mean *E. coli* counts of CCP in Khoa production process

Sr. no.	CCP	Total *E.coli* Count (log_{10}=cfu/ml)	
		Khoa production process	
		Manual (Mean ± S.E.) (n=24)	Mechanical Mean ± S.E.) (n=24)
1	C1	3.20±0.52	3.68±0.36
2	C2	2.34±0.42	2.68±0.35
3	C3	2.82±0.31	2.37±0.36
4	C4	2.53±0.37	1.53±0.39
5	C5	3.17±0.31	3.02±0.30
6	C6	2.31±0.35	2.23±0.34
7	C7	3.32±0.28	2.98±0.30
8	C8	3.70±0.30	1.53±0.39
9	C9	4.05±0.09	3.75±0.18

ANOVA

Sr. No.	Source	df	Sum of squre	Mean-square	F-ratio
1	CCP	8	41.578	5.197	16.165**
2	Khoa production process	1	4.636	4.636	14.419*
3	Khoa production unit	2	1.260	0.630	1.960
4	Replicates	7	12.097	1.728	5.375*
5	Error	311	99.988	0.322	

*-p<0.05, **- p<0.01

Mean *Yeast and Moulds* counts of CCP in Khoa production process

Spoilage of dairy products by *Yeast* and *Mould*s is of frequent occurrence of in India due to tropical climate and high humidity especially in coastal areas. The contamination with *Yeast and Moulds* is linked to hygienic aspects of dairy products in storage, transportation and handling [11]. *Yeast* and *Moulds* was reported in Khoa produced in Tamilnadu. The percentage of occurrence was found to be from 4.342 to 26.08 was seen[12].

In present study the results of *Yeast* and *Mould*s counts inoculation of samples on Sabouraud Dextrose Agar given in Table 5. It is interesting to note that all the samples screened from all CCP' S did not show presence of *Yeast* and *Mould*s. However *Yeast* and *Moulds* were observed only at storage vessel (C8) and packaging material (C9) in both the production processes. The counts at storage vessel were 3.36 ± 0.07 in manual method where as mechanical method showed the counts of 2.16 ± 0.33. The presence of moisture due to improper drying of vessels might have responsible for *Yeast and Moulds* Packaging material plays important role in maintenance of hygiene of milk products by reading environmental contamination. However in the present study mean *Yeast* and *Mould*s counts of 2.87 ± 0.20 in manual method and 1.75 ± 0.35 in mechanical method indicates contaminated packaging material. This may be harmful from public health point of view. The packaging material used for Khoa wrapping in and around Parbhani city comprises of muslin clothes and sometime polythene bags. This material would have been source of *Yeast* and *Moulds* contamination. Earlier Karthikeyan and Dhanalaxmi[11] reported use of polythene bags for a Khoa packaging material resulted in to *Yeast* and *Mould*s contamination. The results of present study are on similar lines.

Table 5: Mean *Yeast* and *Moulds* counts of CCP in Khoa production process

Sr. no.	CCP	Total *Yeast* and *Moulds* Count (log_{10}=cfu/ml)	
		Khoa production process	
		Manual (Mean ± S.E.) (n=24)	Mechanical (Mean ± S.E.) (n=24)
1	C1	ND	ND
2	C2	ND	ND
3	C3	ND	ND
4	C4	ND	ND
5	C5	ND	ND
6	C6	ND	ND
7	C7	ND	ND
8	C8	3.36±0.07	2.16±0.33
9	C9	2.87±0.20	1.75±.035

ND-Non Detected

Microbial analysis of Khoa

The high nutritive value and high moisture of Khoa is conducive to growth of bacteria. Microbial contamination produces undesirable effects like change in colour, odour, taste and texture of products, besides contamination with pathogenic bacteria results in outbreak of gastrointestinal infections. The contamination of Khoa takes place during cooling or handling after cooling and during storage[9]

In present study Total viable counts, total *Staphylococcal* count, total *E. coli* counts and total *Yeast* and *Mould*s count were estimated from all the samples of Khoa by manual method (n=24) and mechanical method (n=24). The results are given in Table 6.

The average TVC counts observed in manual method of Khoa were 4.39 ± 0.20 and in mechanical method 4.09 ± 0.08. A non significant difference of TVC counts was observed amongst manual and mechanical methods of Khoa production. TVC counts of 3.76±0.062 to 4.08±0.121 were observed by Londhe and Dharam pal (2008). The FSSAI, 2015 microbiological standards stipulates TVC counts of minimum 25×10^3/gm and maximum 75×10^3/gm. The results of present study are on similar lines.

Pathogenic organisms such as *Staphylococci* spp. gains entry into Khoa due to improper heading of workers and contaminated utensils used during processing's. Strains of *Staphylococci* spp. can cause gastroenteritis via heat stable enterotoxins[6]. Total *Staphylococcal* count of $\log_{10}$ 3.82 ± 0.19 in Khoa prepared by manual and 2.04 ± 0.33 in mechanical method was observed. Comparison of means amongst both the methods reveals that Khoa produced by mechanical method was having significantly higher ($p<0.01$). *Staphylococcal* count in Khoa production process by mechanical method was less than manual method. The FSSAI Standards 2015 prescribe minimum 10 cfu/gm and maximum 1×10^2cfu/gm. *Staphylococcus* aureus coagulase positive bacteria. Earlier Godbole[9] reported *Staphylococcal* counts within limits from Khoa samples sold in Nagpur city. The results in present study are on similar lines.

E. coli is frequently contaminating organism of Khoa. It is viable indicator of fecal pollution generally in insanitary condition of water, food, milk and dairy products[20]. In present study total *E. coli* counts of 3.32 ± 0.28 where seen in manually prepared Khoa and 2.98 ± 0.03 in mechanically prepared Khoa samples. A non-significant difference of mean of *E. coli* was seen amongst the group. The *E. coli* counts were within limits of FSSAI, Standards, 2015. The source of *E. coli* might be from water, hands of personnel's, storage vessels and packaging material.

Yeast and *Mould*s counts give an idea of contamination of Khoa during storage. It is interesting to note that *Yeast* and *Mould*s were not observed in any of total 48 Khoa samples screened during study period. The FSSAI, standards, 2015 has stipulated a minimum of 10 cfu/gm and maximum during present study may be because of environmental condition of Parbhani. High summer temperature during study period might have resulted into non-conducive environment for growth and multiplication of spores of *Yeast* and *Mould*s.

Table 6: Results of microbial analysis of Khoa

Sr. no.	Microbial count	Production type (Mean ± S.E.)	
		Manual (n=24)	Mechanical (n=24)
1	Total Viable Count	4.39±0.20	4.09±0.08
2	Total *Staphylococcal* Count	3.82±0.19[a]	2.04±0.33[a]
3	Total *E. coli* Count	3.32±0.28	2.98±0.30
4	Total *Yeast and Moulds* Count	ND	ND

CONCLUSION

The following conclusion were drawn from the study

1) The milk used for Khoa production was of good microbial quality.
2) Microbial contamination in relation to TVC count, *Staphylococcal* count *and E.coli* counts during process was seen through raw milk water, hands of personnel, storage vessel and packaging material.
3) The *Yeast* and *Mould*s contamination was seen during storage of Khoa and on packaging material.

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