Biological Forum – An International Journal **15(3): 242-246(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Studies on Microbiological Quality of Khoa Sold in Maharashtra

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(Received: 15 January 2023; Revised: 17 February 2023; Accepted: 24 February 2023; Published: 22 March 2023)
(Published by Research Trend)

ABSTRACT: Milk and milk products are the sources of first class proteins for all the age groups. Khoa is one of the most important traditional Indian dairy products. Owing to its high nutrient and favourable moisture content, khoa can serve as a favourable medium for the growth of a variety of putrefactive microorganisms. The market khoa usually keeps well for about 48 h under usual Indian conditions. But beyond which it deteriorates due to microbial action. These organisms gain access as contaminants from different sources in to the product. The present study was conducted to ascertain the microbial quality of khoa sold in twenty two districts in four zones of Maharashtra during 2021-22. The object is to highlight the main microbial food safety hazards in the dairy chain and to propose appropriate preventive and control measures. Fifteen khoa samples were randomly procured during the three seasons from each of the four zones of the State during 2020-21 from various vendors. The data on microbiological quality was categorized season-wise. The counts of aerobic, coliforms, Staphylococci, yeast and moulds, *Escherichia coli*, *Salmonella* and *Listeria monocytogenes* were estimated. It was observed that the highest counts of the test organisms were observed in all the four zones in the samples collected during February to May and the lowest counts were recorded during October to January. It was observed that the highest average aerobic counts were recorded in Western Maharashtra (49.11×10^5 cfu/g) during Feb-May 2022; the highest average coliform counts of 61.18 cfu/g were observed in Western Maharashtra; the highest Staphylococci counts of 21.33 cfu/g, yeast and mould counts of 29.69 cfu/g were reported in Vidarbha. It was observed that the pathogenic organisms, *viz.* Salmonella sp. and *Listeria monocytogenes* was not observed in any of the samples during the study period. The results on the microbiological quality of *khoa* sold in Maharashtra reveals that the microbiological quality of the product is well within the standards specified by the FSSR (2020).

Keywords: Khoa, pathogens, safety, microbiology, Maharashtra.

INTRODUCTION

It is now almost two and half decades that India emerged and maintained its position as the world leader in milk production with a steady and impressive growth in the milk production. Our annual milk production had crossed the land mark of 200 MMT during the previous financial year 2021-2022 while Maharashtra showed an annual production of 12 MMT (Ranjan *et al.*, 2023). Ours is among the world's largest and fastest growing market for milk and milk products as the source of first class proteins especially for children and vegetarians. The milk and milk products supplies most essential elements like calcium and phosphorus along with numerous other essential major and minor substances (Kalyankar *et al.,* 2016).

Indian traditional sweetmeats are very popular inland and worldwide. Several types of sweets are prepared in different parts of country and categorized by different name(s) and taste. *Khoa* and *channa* are used for the preparation of sweets. *Khoa* is also known as *Khoya*, *Kava* and *Mawa.* Conventionally it is prepared by continuous boiling of milk in an open kettle until desirable concentration (normally 65-72% total solids) and texture are achieved. According to one estimate about 5.5 per cent of total milk production is converted into *khoa* (Kulkarni and Hembade 2012). It supplies calcium and phosphorus along with numerous other essential major and minor nutrients. The khoa and khoa-based milk sweets provide a good means of conserving and preserving surplus milk solids. Nearly 50% of total milk produced in India is used in making a variety of traditional milk products.

Khoa has high commercial significance because of its usages in several types of sweet preparations. With a price of Rs. 300 per kg, the *khoa* market size would be worth Rs. 70,000 crore. Uttar Pradesh is the leading khoa producing state having a share of 36% (out of total *khoa* production in India). The estimated market size of

khoa based sweets is INR 840 billion. The popular *khoa*-based sweets include *peda, burfi, kalakand*, milk cake etc. Almost 52% of the milk produced in India is utilized for the manufacture of traditional dairy based sweets (Codex Alimentarius 2019). Khoa is a concentrated milk product. It is highly nutritious food. According to Indian Standard Institute, *khoa* shall not contain more than 28% moisture and fat not less than 26% on dry matter basis. It is a major intermediate base product for a variety of sweets (Karthikeyan and Pandian 2013). As per Protein Foods & Nutrition Development Association of India (PFNDAI), total sweets export from India was INR 750 crores in 2019 of which bulk was traditional sweets. The FAO's global dairy trends predict that as the income increases, the people prefer to spend a higher share of their food budget on animal proteins like dairy products than the food crops. Bovine milk contains roughly about 3% protein, of which 80% is caseins and 20% is whey proteins. The past three decades have witnessed buoyant growth in the consumption of livestock products (Kalyankar *et al*., 2016a).

According to one estimate about 5.5% of total milk production is converted into *khoa* (Banerjee, 1997). Since the production of various indigenous milk products like *khoa* is in the hands of *'halwais'* mostly residing in rural areas, where unhygienic processing conditions are prevalent, the products are grossly contaminated. The contamination results into a considerable increase in the putrefactive organisms, thereby, lowering the keeping quality of the products. The entry of pathogens which have great significance from the public health point of view is a major concern as the products are consumed by different sections of society including the vulnerable groups.

Bhatnagar *et al.* (2007) analyzed microbiological quality of *khoa* sold in Chambal region of Madhya Pradesh (India) and observed the viable counts from 1.3 × 10^4 to 2.1 × 10^6 cfu/g. Kumar *et al.* (2012) reported that the yeast and mould count of *khoa* in Allahabad city between 21to 29.33cfu/g. Godbole *et al.* (2013) observed that microbial contamination in *khoa* samples sold in different parts of Nagpur city (India) and reported that all the 20 samples showed bacteriological count ranging from 4.88x10^5 to 1.2 × 10^7 CFU/g.

It was reported that the total viable counts of *khoa* samples from local vendors, private manufacturers and organised dairies were ranged from 16 × 10^4 to 2.71 × 10^5, 1.7 × 10^3 to 2.9 × 10^4 and 7 × 10^2 to 4 × 10^3 cfu/g, respectively (Karthikeyan and Pandiyan 2013). Bajaj *et al.* (2013) reported that the *khoa* samples from Nanded city found the yeast and mould count range between 3.1× 10 to 5.3 × 103 cfu/g. Kamble *et al*. (2018) studied the microbiological quality of *khoa* sold in a district from Marathwada. The coliform counts ranged between 3.5 to 8.5 cfu/g. Chaudhary *et al.* (2020) observed the mean total viable counts (standard plate counts) in *khoa* from different markets of Kanpur city (Uttar Pradesh) to be 4.65 cfu/g.

Keeping in view the public health significance as seen from the various studies frequently reported and the strict regulatory bindings, the present investigation was planned and executed to ascertain the microbiological quality of *khoa* sold in 22 districts under the four zones in Maharashtra, *viz.,* Marathwada, Vidarbha, Western Maharashtra and North Maharashtra during 2020-21.

MATERIALS AND METHODS

Procurement of *khoa* samples. Sixty samples of *khoa*, 15 each from each of the four divisions *(*each sample of 50 g) from 22 districts of Maharashtra during three seasons (*Viz.,* October to January, February to May and June to September) were aseptically procured using a simple randomized sampling method from Marathwada, Vidarbha, Western Maharashtra and North Maharashtra during 2020-21. Samples from different brands and locally available popular manufacturers were collected randomly.

Preparation of *khoa* samples for microbiological analysis. Immediately after procurement the samples were aseptically packed, suitably labelled and stored under icepacks and brought to the laboratory prepared under aseptic for its further analysis. A sanitized set of pestle and mortar was taken for macerating the sample. Approximately eleven gram of the *khoa* sample was weighed aseptically in a sterile 100 ml glass beaker and it was transferred aseptically to the sanitized mortar with the help of a sterile stainless steel spatula. The sample was then macerated thoroughly by making a paste using small quantity of previously warmed (45°C) 99 ml of 2% sterile diluents and the contents were transferred to the same conical flask to obtain the first dilution *i.e.* 1:10. Subsequent dilutions were made accordingly.

Microbiological analyses of *khoa*. The microbiological quality of *khoa* samples in terms of the total aerobic counts (SPC) by using the nutrient agar medium (NA), coliform counts by using Violet Red Bile agar (VRBA), yeast and mold counts by using potato dextrose agar (PDA), Staphylococcal counts by using Staphylococcal agar (SA) and *Escherichia coli* counts by using MacConkey's agar (MA), *Salmonella* species by using *Salmonella* agar and the *Listeria* sp. were estimated by using Listeria agar (Himedia). The standard protocol/procedures were adopted as prescribed by Prevention of Food Adulteration Act (2010). The data on microbiological quality of *khoa* was categorized season and zone-wise.

Statistical analysis of the data. The data obtained on the microbial counts in *khoa* during various seasons and the zones was statistically analyzed by using descriptive statistics in this investigation, the Chi-squared test was performed to assess the relationship between the seasonal variation and variation in microbial counts of *khoa* sold in different zones, the *P*-values <0.05 were considered significant.

RESULTS AND DISCUSSION

The samples of *khoa* were procured randomly during the three seasons from various market outlets in 22 districts under the four zones in Maharashtra. The summary of findings on the microbiological quality of *khoa* is presented in Table 1.

Aerobic counts: The results on the aerobic microbial

counts presented in this table reveals that the average counts during June-Sept were 47.23×10^3 cfu/g in Marathwada, 21.94×10^2 in Vidarbha, 87.56×10^4 in Western Maharashtra and 63.04×10^4 in North Maharashtra. These counts during Oct to January were 29.27×10^2, 77.33×10^3, 83.51×10^3 and 17.61×10^3 in Marathwada, Vidarbha, Western Maharashtra and North Maharashtra, respectively. These counts in the summer months i.e. February to May were 84.41×10^4, 98.09×10^4, 49.11×10^5 and 68.92×10^4 in Marathwada, Vidarbha, Western Maharashtra and North Maharashtra, respectively. It was observed that the lowest counts of all the five groups in the four zones were observed during the Sept-Jan. These results are in close conformity with earlier findings (Goyal *et al*., 2007; Jadhav, 2016).

Coliform counts: It could be seen from the content of Table 1 that the highest coliform counts (61.18 cfu/g) were recorded in North Maharashtra during Summer months. However the lowest counts of 2.84 cfu/g in Marathwada during winter months. The data further reveals that these indicator organism counts never exceeded the prescribed limits of 1×10^2 cfu/g during any of the study period slots. It is further that the coliform average counts in Marathwada during June-Sept were 34.11cfu/g, Oct. –January 2.84 and Feb-May 57.68. The overall highest average counts during all the three sampling slots were recorded to by 48.34 cfu/g in North Maharashtra.

***Staphylococcus aureus* counts:** Milk and dairy products like *khoa* are considered vehicles o*f Staph. aureus* for infection, especially food poisoning in humans. *Staph. aureus* is an important food borne pathogen and causes a wide variety of diseases in humans and animals, ranging in severity from a mild skin infection to more severe diseases, such as pneumonia and septicemia. In view of this the Regulatory authorities proposed the maximum acceptable limit 1×10^2 cfu/g of *khoa*. The data presented in Table 1 reveals that the highest average counts of Staphylococci in *khoa* of 26.62 cfu/g in Western Maharashtra during Summer months followed by 21.33 cfu/g in Vidarbha, 8.74 cfu/g in Marathwada and 11.78 cfu/g in North Maharashtra. It appears that the guidelines suggested by Bhatt *et al.* (2000) for improving the microbiological quality of indigenous dairy products are followed by the *khoa* manufacturers and handlers in the twenty two districts of Maharashtra under the study reported in this publication. Unlike reported by Godbole *et al.* (2013), the study of these workers showed a high degree of bacterial contamination in *khoa* sold in Nagpur city. However, the counts of these organisms are well within the limits prescribed by FSSAI (2020) in almost all the *khoa* samples. It can be attributed to the practice of preparing and post preparing handling and storage of the product by following strict hygienic conditions. The findings on microbial counts of these food poisoning microbes in this study is not in agreement with earlier findings that most of the times the vendors and workers in the shop have no knowledge of the practices and probable dangers if the food safety procedures and standards are not followed. The application of HACCP to identify the critical control points for coliforms and *Staphylococcus spp*. has indicated that the contamination is attributed to the product handlers (Yadav *et al.,* 2009).

Yeast and mould counts: The detection and enumeration of microorganisms in *khoa* is an integral part of any good quality assurance program and reflect the effectiveness of sanitation practices, processing and distribution schemes of local, private manufactures and organized dairies. Hence, the yeast and mould counts were included in this study. As per the FSSAI (2020), the maximum limit of 50/g of yeast and moulds was prescribed. Since the mould spores are transmitted through air, they are ubiquitous in nature. It is well established that the hygienic aspects of dairy products are linked with transportation, preservation and handling. Spoilage of dairy products like *khoa* by moulds is of frequent occurrence in India due to the prevailing tropical climate and high humidity especially in coastal areas. The data obtained in this study and presented in Table 1 reveals that maximum counts of 29.69 cfu/g were observed in Vidarbha while the lowest of 3.32 cfu/g in the *khoa* samples procured from North Maharashtra.

Counts of Salmonella and *Listeria monocytogenes* in khoa: The renewed interest in the occurrence of salmonellae in dairy products, prompted mainly because of their recovery from a wide variety of dairy products. The 1,200 or more serotypes of these Gram-negative, facultative, asporogenous, rod-shaped bacteria are all considered to be human pathogens. In man they can cause enteric fevers (i.e., typhoid fever and related ailments), gastroenteritis, and septicemias. Treatment of salmoncllosis is often difficult, and a limited number of patients continue to shed the organisms for extended periods even though they appear to be recovered. *Listeria monocytogenes*, as a dairy pathogen is responsible for serious diseases in both humans and animals. *Khoa* is among the main sources and therefore its contamination with *L. monocytogenes*, could lead to life threatening infections in a large population of people. Rapid and accurate detection of *L. monocytogenes* in dairy products is needed to prevent its dissemination through the food chain. The incidence of both of these pathogenic organisms in *khoa* was studied in the present investigation and presented in Table 1. It was observed that none of the *khoa* samples procured from the 22 districts in Maharashtra shown the presence of these pathogens. In a nutshell, it can be safely said from the data presented hereinabove that a high-flying and highly important finding emerged out of this study is the fact that by and large all the microbial counts recorded in the present investigation are well in conformity with the standards specified by the Regulatory Authorities and presented in Table 2.

Table 1: Studies on microbiological quality of khoa sold in Maharashtra.

Regions under study	Sampling months	Aerobic Plate Counts* (cfu/g)	Coliform Counts* (cfu/g)	*Staphylococcus aureus* counts* (cfu/g)	Yeast and Mold Counts* (cfu/g)	*Escherichia coli** (cfu/g)	Salmonella sp. (cfu/g)	*Listeria monocytogenes* (cfu/g)
		2.5×10^4 7.5×10^4	50/g 1×10^2	10/g $1x10^2$	10/g 50/g	<10/g NA	Ab/25g NA	AB/g NA
Marathwada	June-Sept	47.23×10^3	34.11	8.27	8.21	AB	AB	AB
	Oct-Jan	29.27×10^2	2.84	8.27	8.21	AB	AB	AB
	Feb-May	84.41×10^4	57.68	18.74	22.00	9.96	AB	AB
Vidarbha	June-Sept	21.94×10^3	49.32	6.08	5.53	6.73	AB	AB
	Oct-Jan	77.33×10^3	44	21.33	29.69	6.12	AB	AB
	Feb-May	98.09×10^4	54.44	09.21	7.76	12.31	AB	AB
Western Maharashtra	June-Sept	87.56×10^4	21.39	4.22	10.00	5.37	AB	AB
	Oct-Jan	83.51×10^3	31.33	6.49	17.41	9.9	AB	AB
	Feb-May	49.11×10^5	48.03	26.62	9.13	7.33	AB	AB
North Maharashtra	June-Sept	63.04×10^4	41.65	3.32	6.72	4.31	AB	AB
	Oct-Jan	17.61×10^3	42.12	9.87	13.48	3	AB	AB
	Feb-May	68.92×10^4	61.18	11.78	12.65	10.27	AB	AB

**Averages of twenty samples analyzed from each of the regions during each of the sampling slot of the season.*

Table 2: Prescribed microbial specifications/requirements for *khoa*.

Aerobic plate count (cfu/g)		Coliform Count		*Staph. aureus* count		Y&M Count		*E. coli* count		Salmonella Sp.	
m	M	M	M	m	M	m	M	M	M	m	M
2.5×10^4/g	7.5×10^4/g	50/g	1×10^2 /g	10/g	1×10^2/g	10/g	50/g	<10/g	NA	Ab/25g	NA
Test methods	IS 5402/ ISO:4833	5401 Part 1/ ISO:4832		IS5887:Part 2 or IS5887 Part 8		IS:5403 or ISO:6611		IS5887:Part-I or ISO:16649-2		IS5887:Part-3/ ISO:6579	

Source: FSSAI (2020)-Product categories specified in Version-XII (08.09.2020).

CONCLUSIONS

On the basis of the data generated during this investigation it can be concluded that the microbial counts of the target eight groups of organisms reported in the *khoa* samples procured randomly from twenty two districts under four zones of Maharashtra during three seasons are well within the prescribed limits by the Regulatory Authorities. Our scientific findings in this investigation can be attributed to the long term planning and tireless efforts in effective implementing the awareness campaigns about the importance of hygienic practices through academia and industrial and government authorities resulted into significant improvement in the quality of *khoa* sold in the State of Maharashtra.

Acknowledgements. I would like to thank co-authors for their ideas, valuable suggestions and contribution during this work. I would also like to thank our Institute for providing support during this study.
Conflict of Interest. None.

REFERENCES

Bajaj, S. J., Deshmukh, Y. D., Shirfule, A. L., Deshmukh, K. P. and Satav, P. D. (2013). Quality assessment of *khoa* marketed in Nanded city. *Int. J. Green Herbal Chem., 2*, 660-664.

Banerjee, A. K. (1997). Processes for commercial production. *Dairy India 5th edn*. Published by PR Gupta, New Delhi, India, pp 387.

Bhatnagar Preeti, A. A. Khan, Manisha Jain, Shuchi Kaushik and S. K. Jai (2007). Microbiological study of khoa sold in Chambal region (Madhya Pradesh): A case study. *Indian Journal of Microbio.*, *47*, 263–266.

Chaudhary, A, Singh, R, Singh, V, Kumari, S, and Upadhyay, R. (2020). Assessment of Microbial Quality of Khoa, *Ind. J. Pure App. Biosci., 8*(2), 104-107.

Codex Alimentarious Commission (2019). Discussion Paper for Development of a Codex Standard for Indian Traditional Dairy Based Sweets, *21st Session Goa, India* 23 - 27 September 2019.

FSSAI (2020). The Prevention of Food Adulteration Act, 2006. Pro *FSSAI-Product categories specified in Version-XII (08.09.2020).*

Godbole Suchitra, Pranoti Dabholkar and Ashwini Pachbudhe (2013). Evaluation of bacteriological quality of Indian cheese (*Paneer*) sold in Nagpur city. *J. Global Biosci.*, *2*(2), 53-56.

Goyal, R. K., Singh, P. K. and Goyal, S. K. (2007). Studies on quality evaluation of market Paneer. *International J. Agric. Sci., 3*(1), 165-167.

Jadhav, B. A. (2016). Effect of storage on microbial quality of different types of milk blend Paneer at room temperature without and with packaging material. *J. Bio. Innov.*, *5*(4), 472-479.

Kalyankar, S. D., Deshmukh, M. A., Chopde S. S., Khedkar, C.D., Lule, V. K. and Deosarkar, S. S. (2016). Milk Powder. In: Caballero, B., Finglas, P., and Toldrá, F.(eds.) *The Encyclopedia of Food and Health,* Vol. 3, pp. 724-728. Oxford: Academic Press.

Kalyankar, S. D., Khedkar, C. D., Patil, A. M. and Deosarkar, S.S. (2016a). Milk: Sources and Composition. In: Caballero, B., Finglas, P., and Toldrá, F. (eds.) *The Encyclopedia of Food and Health, 3,* pp. 741-747. Oxford: Academic Press.

Kamble, N. S., Londhe, G. K. and Narwade, S. G. (2018). Studies on microbiological properties of *khoa* prepared in major *Khoa* producing areas of Marathwada region. *International J. of Chem. Studies, 6*(5), 3426-3429.

Karthikeyan, N. and Pandiyan, C. (2013). Microbial quality of khoa and khoa based milk sweets from different sources, *International Food Res. J., 20*(3), 1443-1447.

Kulkarni, R. V. and Hembade, A. S. (2012). Shelf life of *khoa* prepared in Beed district of Maharashtra, *J. of Dairying, Foods and Home Sci., 31*(3), 173-175.

Kumar, R, Shrivastava, S. L. and Mishra, H. N. (2012). Optimization of khoa and tikhur mix for preparation of khoa-jalebi sweet. *Open Access Scientific Reports. DOI: 10.4172/Scientific Reports*.469.

Prevention of Food Adulteration Rules (2010). PFA Rules, 1954 (amended up to 2009) *New Delhi: Universal Law Publishing Company Pvt Ltd*; 2010. pp. 165–166.

Protein Foods & Nutrition Development Association of India (PFNDAI) (2019). Status Report on Export of Dairy Products from India. Pp47-59.

Ranjan, B. Yedatkar, Vasant V. Niras, Anand R. Sarode, Shital S. Deosarkar, Shrikant D. Kalyankar and Chandrapakash D. Khedkar (2023). Studies on Microbiological Quality of Paneer Sold in various Zones of Maharashtra. *Biological Forum – An International Journal, 15*(1), 392-395.

Yadav, Y. N., Singh C., Dwivedi, B. R. and Gupta, M. P. (2009). Effect of various coagulants on sensory, chemical and microbiological quality of Paneer. *J. of Rural and Agril. Res., 9*(1), 11-14.

How to cite this article: Ranjan B. Yedatkar, Vasant V. Niras, Anand R. Sarode, Shital S. Deosarkar, Shrikant D. Kalyankar and Chandrapakash D. Khedkar (2023). Studies on Microbiological Quality of Khoa Sold in Maharashtra. *Biological Forum – An International Journal, 15*(3): 242-246.