



Research Paper

PREVALENCE OF PATHOGENIC MICROORGANISMS IN KHOA BASED MEETHAI (MALAI PEDHA) SOLD IN PUNE CITY

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Abstract

The prevalence of pathogens in khoa based sweets malai pedha was probed from twenty sweet marts in Pune city along with their packing material used for sale. The keeping quality and microbial load was assessed using suitable parameters. Prevalence of *E.coli* and *S.aureus* in the samples from non certified shop was 31.57% and 78.94% respectively. A high incidence of the pathogen *S.aureus* in samples indicate lack of hygienic practices during manufacture and handling. Poor personal hygiene, poor product handling practices and poor storage facilities contributed to such pathogen prevalence in non certified shops. On the other hand, effective implementation of HACCP plan and use of good hygienic practices help to achieve safety of the same product at a HACCP certified shop. This highlights the need for good hygienic practices and/ or HACCP plan in sweet shops to reduce contamination and to achieve safety of end product since most of the products are made and sold in the unorganized sector.

Key words: *E.coli*, *S.aureus*, Khoa based meethai, HACCP, Pathogen prevalence in milk products.

INTRODUCTION

In India, a variety of sweets are available. Among these, *Khoa* or *mava* based sweets are very popular. Khoa is important product of milk which is manufactured at commercial level as well as at small scale. Two methods are commonly used for khoa production, one is open pan evaporation process (traditional method) and other is use of steam jacketed kettle for evaporation. The principle behind khoa making is to reduce moisture content and increase total solids which results in better shelf stability of product than milk[1]. Once khoa is prepared, it is further used for different types of sweet making like, pedha, burfi, gulab jam and other sweets. In pedha category, malai pedha is popular and it is manufactured regularly. For its preparation khoa is reheated along with ingredients like sugar, essence or flours. Further, this intermediate mixture is shaped in to round balls either manually or by using a pedha making machine. At the final stage, pedhas are garnished with dry fruit pieces and arranged in trays. These trays are

transferred to display cabinets of shop. This malai pedha is consumed commonly during festive seasons, weddings, and during any celebration. It is also distributed as *Prasad* during poojas and religious ceremonies.

Various studies have been conducted in different parts of India to understand the microbial quality of khoa and khoa products. These revealed the presence of pathogens in these products. Bacterial pathogens like *Staphylococcus*, *Streptococcus*, *E.coli*, *Salmonella*, *Pseudomonas*, *Proteus*, *Serratia*, *Enterobacter*, *Klebsiella* species were isolated from 50 khoa samples collected from Gwalior and Morena City of Madhya Pradesh[2]. Fungal pathogens like *Aspergillus flavus* and *A. fumigatus* were found in 27 khoa samples from TamilNadu cities[3]. Whereas pathogens like *E.coli* (60%), *Salmonella* (65%) and *S aureus* (90%) were found in 20 khoa samples from Nagpur city of Maharashtra[4]. Growth of these pathogens is supported by the nutritional properties of khoa namely Proteins (14.6%), Fat (31.2%) and calcium (900mg)[5] and chemical properties like water activity 0.96 and pH 6.3.

In case of pedha and other khoa based products, a study was conducted to evaluate microbial quality of 50 samples of pedha sold in Amarawati. Result showed the predominance of *Pseudomonas aeruginosa* (23.91%) and *Staphylococcus aureus* (17.39%), *Salmonella typhi* (16.30%), *E. coli* (14.13%), *Proteus vulgaris* (7.6%), *Shigella flexneri* (8.69%), *Enterobacter aerogenes* (11.95%)[6]. In Chandigarh, 12 sweet shops were surveyed for determining the hygienic standards in shops along with microbial analysis of plain burfi, milk cake, kala kand, gulab jamun, chaina murgi, gajar barfi and khoa burfi (12 samples of each meethai). Analysis of this study focussed on isolation of *E.coli*. Out of 84 samples, *E coli* was isolated from 75 samples[7]. In Anand (Gujarat), 30 pedha samples were analysed for *S aureus* of which 3 samples were found contaminated with *S aureus* and isolates show antimicrobial resistance to cephalothin, co-trimoxazole, cephalixin, methicillin, gentamicin, ciprofloxacin, oxacillin, streptomycin and ampicillin[8]. Prevalence of multi drug resistant pathogens in khoa based products presents the grim reality of malpractices followed in manufacture of these products or the poor quality of the starting material. Such pathogenic microorganisms can cause food borne diseases like typhoid, Salmonellosis, diarrhoea, food poisoning, etc or be the cause of widespread occurrence of drug resistance in the population. This study was designed to check the prevalence of food borne pathogens in *Malaai Pedha* samples from a HACCP certified shop versus HACCP non certified shops. For this study, 19 non certified sweet shops were selected from 7 localities of Pune City and 1 HACCP certified shop was selected.

MATERIALS AND METHODS

Sample selection: For this study, 19 sweet shops were randomly selected from 7 localities i.e. Bibavewadi, Sahakar nagar, Hadapsar, Karve nagar, Kondhawa, Balaji nagar and Swargat-Parvati area of Pune. In addition to these 19 shops, one HACCP (Hazard Analysis and Critical Control Point) certified sweet shop which has a chain of outlets was also selected.

Procurement of Pedha: From each shop, 3 samples of 100 g malai pedha were collected on 3 different days. Samples were collected in same packing material which is normally given to the consumer. This was done considering the quality of packing material and packing practices followed across the counter. After collection of sample, labelling was done and samples were sent for analysis in the Poultry Diagnostic and Research Centre (PDRC) laboratory within 2 hours after of collection in container with ice bag to maintain temperature.

Sample preparation: 10 g of test sample was added in 90 ml sterile 0.85% physiological saline i.e. 1:10 dilution was made. This dilution was used for further analysis.

Microbial Analysis: Each sample was examined for following:

1. Total Plate Count (TPC): was done by IS method[9].
2. *Escherichia coli*: IS method[10] was used for detection.
3. *Staphylococcus aureus*: was detected by IS method[11].
4. *Salmonella*: This pathogen was detected by using IS method[12].
5. Yeast and Moulds: were detected by using ISO method[13].

Data analysis: Microbial counts were represented as log 10 CFU/g. Mean and standard deviation was calculated. Mean value per shop is determined from 3 values obtained from 3 independent samples.

RESULTS

Microbial counts of 19 sweet shops without HACCP certification:

Total plate count: Table 1 represents the total viable count of samples from shops without HACCP certification. The values were ranging from 0.03 to 335.3x10⁵ CFU/g with mean (+/-SD) of 66.1x10⁵+/-107.71 CFU/g. There is no standard set for microbial quality of pedha in Food Safety and Standard Act, 2006 India (FSSAI)[14]. But FSSAI has a microbial standard for khoa where the acceptable level of TPC is set at 50,000/g. The values obtained from these samples were much higher than specification.

Yeast and mould count: Yeast and Mould counts of pedha samples from non HACCP sweet shop ranged from 2.66 to 3.66x10¹ CFU/g with the mean (+/-SD) 0.455x10¹+/-1.08CFU/g..Yeast and mould counts in samples were found from only 3 shops as given in Table1.

Table 1: Total plate count and Yeast & mould count of samples from 19 shops without HACCP certification

Sampling area	Sample code	TPC CFU/g	Y&M CFU/g
Bibavewadi	S1	202.01x 10 ⁵ +/- 344.67	3.33 x 10 ¹ +/- 5.77
	S2	0.03 x 10 ⁵ +/- 0.005	—
	S3	2.01 x 10 ⁵ +/- 3.45	—
Sahakar nagar	S4	0.09 x 10 ⁵ +/- 0.092	—
	S5	3.66 x 10 ⁵ +/- 0.57	—
	S6	68.02 x 10 ⁵ +/- 114.31	2.66 x 10 ¹ +/-4.61
Hadapsar	S7	13.44 x 10 ⁵ +/- 22.96	—
	S8	6.93 x 10 ⁵ +/- 11.31	—
	S9	0.014 x 10 ⁵ +/- 0.02	—
swarget- parvati area	S10	82 x 10 ⁵ +/- 103.59	2.66 x 10 ¹ +/- 4.61
	S11	293.5 x 10 ⁵ +/- 440.43	—
	S12	335.33 x 10 ⁵ +/- 413.93	—
Karve nagar	S13	0.096 x 10 ⁵ +/- 0.11	—
	S14	26.73 x 10 ⁵ +/- 22.93	—
Balaji nagar	S15	1.66 x 10 ⁵ +/- 2.02	—
	S16	2.02 x 10 ⁵ +/- 3.44	—
	S17	20.2 x 10 ⁵ +/- 34.4	—
Kondhawa	S18	200 x 10 ⁵ +/- 346.0	—
	S19	0.04 x 10 ⁵ +/- 0.005	—

Sampling area	Sample code	TPC CFU/g	Y&M CFU/g
	Mean of observed value	66.19 x 10⁵ +/- 107.71	0.455 x 10¹ +/- 1.08

Presence of pathogens

***Escherichia coli*:** Presence of *E.coli* is the measure of hygienic conditions followed in processing area and water quality in relation with fecal contamination. Pedha samples from 6 shops were found to be contaminated with *E.coli* out of 19 shops i.e. 31.57% samples were contaminated. This count ranged from 0.133 to 13.33 x 10² CFU/g with mean +/-SD 1.14 x 10² +/-3.14 CFU/g. FSSAI specification for khoa is less than 10/g. Obtained values are higher than the specifications set for khoa. Presence of *E. coli* in 31.57% samples was also more than 14.13%. *E.coli* contamination in pedha samples from Amaravati [6].

***Salmonella*:** This pathogen can come in contact with food from the environment, contaminated water and from cold blooded and warm blooded animals. This is commonly found in food contaminated by feces from animal origin. In this study, *Salmonella* was absent in all samples collected from 19 shops in Pune. Whereas in studies conducted in Amaravati prevalence of this pathogen was seen in 16.3% of samples [6]. As per FSSAI specification [14], *Salmonella* shall be absent in 25 g khoa sample.

***Staphylococcus aureus*:** This pathogen acts as an indicator of personal hygiene of workers or food handlers. Samples from 15 shops out of 19 were found contaminated with this pathogen i.e. 78.94% samples were contaminated. Values ranges from 0.66 to 66.66 x 10³ CFU/g with the mean +/-SD 6.01 x 10³ +/-15.32 CFU/g showed in Table 2. If khoa is purchased based on microbial specification stated in FSSAI, then it will help to reduce prevalence of pathogen in khoa products.

Table 2: Pathogen count of samples from 19 shops without HACCP certification

Sampling area	Sample code	E. coli CFU/g	S aureus CFU/g	Salmonella CFU/g
Bibavewadi	S1	—	2.6 x 10 ³ +/- .30	—
	S2	—	—	—
	S3	—	—	—
	S4	—	1.33 x 10 ³ +/- 1.15	—
	S5	4 x 10 ² +/- 3.46	2.76 x 10 ³ +/-2.13	—
Sahakar nagar	S6	—	1.33 x 10 ³ +/-1.15	—
Hadapsar	S7	0.133 x 10 ² +/- 0.23	0.66 x 10 ³ +/- 1.15	—
	S8	—	13.33 x 10 ³ +/- 11.52	—
	S9	—	—	—
swarget- parvati area	S10	—	16 x 10 ³ +/- 20.78	—
	S11	1.33 x 10 ² +/- 1.15	1.4 x 10 ³ +/- 2.25	—

Sampling area	Sample code	E. coli CFU/g	S aureus CFU/g	Salmonella CFU/g
Karve nagar	S12	$0.266 \times 10^2 \pm 0.23$	$2.66 \times 10^3 \pm 2.30$	—
	S13	—	$1.4 \times 10^3 \pm 2.25$	—
	S14	—	$1.33 \times 10^3 \pm 2.30$	—
Balaji nagar	S15	$13.33 \times 10^2 \pm 11.54$	$2 \times 10^3 \pm 2$	—
	S16	—	—	—
Kondhawa	S17	$2.66 \times 10^2 \pm 2.30$	$0.66 \times 10^3 \pm 1.15$	—
	S18	—	$66.66 \times 10^3 \pm 115.47$	—
	S19	—	$0.133 \times 10^3 \pm 0.115$	—
	Mean of observed value	$1.14 \times 10^2 \pm 3.14$	$6.01 \times 10^3 \pm 15.322$	—

Microbial counts of the shop with HACCP certification

Total plate count: TPC of pedha samples from the HACCP certified shop was 14.8×10^6 CFU/g ± 218.32 . Table 3 presents the value of sample.

Yeast and mould count: Yeast and Mould counts of pedha samples from this category was $15.0 \times 10^2 \pm 217.02$ which is comparatively higher than obtained from pedha samples of non certified shops (Refer table 3).

Presence of pathogens

As the results showed in Table 4, samples collected from this shop were not contaminated with any of these pathogens i.e. *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. This kind of results indicates the effective implementation of HACCP system in processing area.

Table 3: TPC and Yeast & Mould counts of samples from one shop with HACCP certification

Sampling area	Sample code	TPC CFU/g	Yeast & mould CFU/g
Sahakar nagar, Bibavewadi, Karve nagar	SH 1	$148 \times 10^5 \pm 218.32$	$150 \times 10^1 \pm 217.02$

Table 4: Pathogen counts of samples from one shop with HACCP certification

Sampling area	Sample code	E. coli CFU/g	S. aureus CFU/g	Salmonella
Sahakar nagar, Bibavewadi, Karve nagar	SH 1	—	—	—

DISCUSSION

In present study, HACCP certification was chosen as criteria to check and compare prevalence of pathogen in samples collected from non certified shops and samples collected from certified shop. Table 2 and 4 reported that *Staphylococcus aureus* and *E.coli* were present in samples taken from 19 non certified shops, whereas sample from the certified shops was free from

pathogen contamination indicating a high level of hygiene maintained during manufacture and higher microbial quality control of the start material. A similar result was reported by J bandekar[15] when a comparison was made of the quality of pedha obtained from B grade and A grade shops where B grade shops showed a very high prevalence of various kinds of pathogens like *Listeria*, *Shigella*, and *Salmonella*.

Figure 1 describes a predominance of *Staphylococcus aureus* i.e. 78.94% in non certified shops which was higher than study carried in Anand (10%)[8] and Amaravati (17.39%)[6]. FSSAI specification[14] of *S.aureus* for khoa is 50/g. This bacterium causes food poisoning to humans. This kind of food poisoning is caused due to the unhygienic food handling practices by food handlers when the bacterium is shed from bare skin or contact with body parts. Practices like handling of sweets with bare hands, non-usage of aprons, and absence of hair covering devices and handling of money during serving lead to such contaminations. These practices might contribute to poor hygienic conditions during manufacture of khoa or during the manufacture of the final product. Such systems could pose favourable conditions for bacterial contamination [7] and similar handling practices were also observed in this study during sample collection. Enterotoxin (A) produced by *S.aureus* is responsible for the manifestations of staphylococcal food poisoning. This toxin is relative heat stable, resisting 100° C for 10 to 40 minutes depending on concentration of the toxin and nature of the medium. Illness can cause by ingestion of as little as 20 mg of enterotoxin. Therefore to reduce probability of *Staphylococcus aureus* food poisoning, personal hygiene and overall hygienic conditions need to be maintained in all sweet marts because meethai products are consumed most frequently by huge populations. Presence of *E. coli* in 6 samples i.e. 31.57% shows poor water quality, poor personal hygiene and fecal contamination. A similar prevalence was found of *E. coli* in more than 14.13% of pedha samples sold in Amaravati[6]. *E.coli* is a important coliform bacterium used as indicator of fecal pollution[17] and its infections can cause fever, gas, abdominal cramp, diarrhoea, nausea, fatigue and vomiting (in rare cases). To improve hygienic conditions, FSSAI [14] schedule 4 provides guidelines which are as follows,

1. Only potable water, with appropriate facilities for storage and distribution shall be used as an ingredient in processing and cooking.
2. Water storage tanks shall be cleaned periodically.
3. Water tanks shall be in closed conditions.

This research finding is also highlights the importance of following legislations. Microbial results of samples from the HACCP certified shop were TPC (149×10^5 CFY/g), yeast and mould counts (15×10^2 CFU/g) and absence of pathogens as compared to uncertified shops. This shows effectiveness of implemented HACCP system while processing. HACCP includes personal hygiene, monitoring, verification, validation and most important specifications for raw material and their risk analysis. Therefore HACCP can ensure manufacturing of microbial safe food products. The procurement of khoa fulfilling the FSSAI[14] specifications and rigid implementation of HACCP rules will help to maintain constant quality and safety of end product and help in keeping food borne infections at bay. However total viable count and yeast & mould count were found to be higher in the case of certified shops indicating that the start material used for manufacture as not as fresh as the uncertified shops or the product was subjected to longer storage.

Fig 1: Percent prevalence of pathogens in samples from 19 shops without HACCP certification

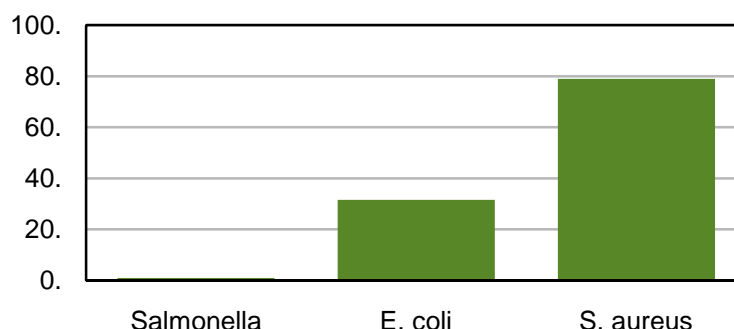
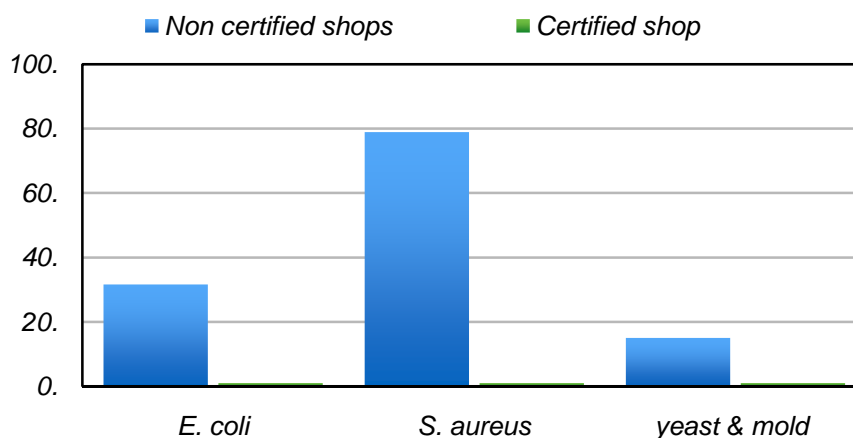


Fig 2: Comparison of percent prevalence of pathogens and microbial quality in samples from non certified shops v/s certified shop



CONCLUSION

A comparison of microbial quality of pedha and prevalence of pathogen in samples from non certified shops and sample from acertified shops is shown in figure 2. It clearly indicates that pathogens were more prevalent in samples from non certified shops than the certified shop. Malpractices like handling of *pedha* with bare hands, non usage of uniforms and insufficient storage practices were observed in non certified shops which may have contributed to the contamination. Whereas samples from the certified shop were free from pathogens which was rigours following of practices like use of hand gloves, wearing uniforms while product handling and good storage conditions. This highlights the need of proper post process handling of *pedha* and personal hygiene at all sweet shops because *pedha* is consumed directly without any heating or a cooking step. However TPC and yeast & mould counts were higher in a certified shops indicating slower movement of the product from these shops to the consumer. Thus, establishment of a HACCP plan or use of basic guidelines stated in FSSAI [14], 2006 schedule IV is a crying need in all kind of sweet shops to ensure food safety for restricting or reducing pathogens. This will help in combating food poisoning and food borne infections. The findings of this study also suggest the need of establishment of a microbial specification for *pedha* and similar products for achieving and maintaining uniform quality and ensuring consumer safety.

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