Assessment of Microbial Load of Milk Shakes Available in Various Educational Institutes of Lahore

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Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: A milkshake is a delicious and non-carbonated refreshment produced using dairy, frozen flavour related item. The growth of microbes is inevitable in milk shakes when proper hygienic conditions were not maintained. These microbes may produce toxins which are harmful and may lead to food poisoning. This study was established to calculate total plate count (Staphylococcus aureus, Total coliform and Salmonella) present in Apple and Banana milk shakes.

Study Design: The samples from 25 different public and private educational institutes were collected to analyse using pour plate method to determinate total microbial load in apple and banana milk shake.

Results and Discussion: Total plate count in August was significantly higher than in November. In August TPC range of banana shake was between 2.3x10⁷ - 7.2x10⁷ cfu/ml and respectively in November range between 2.1 x10⁷ - 6.7 x10⁷ cfu/ml. In August TPC range of apple shake between 2.2x10⁷ - 7.5x10⁷ cfu/ml and respectively in November range between 2.08 x10⁷ - 6.5 x10⁷ cfu/ml. The S. aureus in banana milk shake were positive 19 (76%) in the month of August and 15 (60%) were...
also positive in the month of November. The S. aureus in apple milk shake were found to be positive 18 (72%) during August and 16 (64%) were also positive during November. The Total coliform count in apple milk shake was positive 16 (64%) in August and 14 (56%) were also positive in November. The total positive coliforms were 15 (64%) in August and 14 (56%) were also positive in November.

**Conclusion:** The findings of the present study showed a much higher prevalence of microbial load in banana and apple. We suggested that in most of the samples, the total bacterial load was much higher than recommended by the Gulf standard. So these drinks are not fit for consumption.

**Keywords:** Microbial load; milk shakes; food poisoning; microbial load; Staphylococcus aureus; coliform bacteria.

1. **INTRODUCTION**

A milkshake is a delicious and non-carbonated refreshment produced using dairy, frozen yoghurt and flavour-related item being sold in streets and mostly sailed in educational setups. It is served in disposable glass with a straw or in various serving styles. The milkshake is made blending the apple and banana pulp with milk, sugar in a blender or drinks blender and by including ice at last [1] (Petridou et al. 1997). Natural product juices contain vitamins, and minerals that are necessary for individual nourishment and they play a critical part in the antipathy of heart problems, tumor and diabetes. Natural product juices are essential and good sources of supplements and contain a few vital properties that may lessen the danger of different illness. They contain a lot of cancer prevention agents, vitamins C and E, and have charming taste and fragrance [2].

In developing countries, the 916 cases were reported for each 100,000 populace. Considering WHO reports could be assessed at one billion dollars, considering therapeutic expenses and profitability [3]. Poor cleanliness practices have been connected with ingenious pathogenic organisms like *Staphylococcus aureus* [4].

Various types of liquid shakes are consumed day by day by a vast number of populations. The majority of these shakes are accessible in shops or canteens. It is additionally noticed that most of the shopkeeper utilises tap water for making juices, which can be the fundamental source of bacterial contamination [5].

2. **MATERIALS AND METHODS**

2.1 **Study Design**

This present research work was designed to calculate TPC (*Staphylococcus aureus, Coliform Count and Salmonella*) in milkshake, sold in 25 major educational institutes of Lahore, Pakistan. Total 100 samples of milkshake were collected from different educational institutes. All samples were collected from all mentioned institutes and sent to the University of Veterinary and Animal Sciences (UVAS), Lahore-Pakistan for further processing.

2.2 **Sample Collection**

Samples of commercially available milkshake were collected from various cafeterias of educational institutes of Lahore.

2.3 **Microbial Analysis**

Microbial analysis was conducted for detection of the total bacterial count in all samples, mainly contains Total *Staphylococcus aureus* (TSC), Total *Salmonella count* (SC) and Total coliform Count (TCC).

2.4 **Sample Preparation**

Before culturing of samples, all the sample of milkshake was stored at 4°C. After thawing, 1 ml sample was taken by using of sterile pipette and transferred to a sterilised test tube which comprises normal saline (9 ml) for making a 10 fold serial dilution. After dilution, 1 ml diluted sample was taken from the first tube and transferred it into the next tube by using of another sterile pipette. This procedure was repeated again and again up to 9th test tube, discarded 1 ml from the 10th test tube for obtaining the desired dilution.

2.5 **Laboratory Analysis**

From 6th and 7th dilution, 1 ml of diluted sample was taken and poured into two separate sterile petri. After the addition of diluted sample into sterilised petri dishes, 15 ml of media (Nutrient
agar) poured down into each petri dish, and allowed for solidification. At the end, the medium was permitted to solidify. All the Samples were cultured on Salmonella Shigella agar (SSA) for calculation of total colonies of Salmonella, for determining the TSC count selective media Mannitol salt Agar is used. At the end, the medium was permitted to solidify. All the Samples were cultured on Salmonella Shigella agar (SSA) for calculation of total colonies of Salmonella, for determining the TSC count selective media Mannitol salt Agar is used. At the end, the medium was permitted to solidify. For calculation of Staphylococcus aureus and Salmonella count (CFU/ml). After the process of incubation, all bacteria colonies with distinction with yellow colour for Staphylococcus aureus and black colour for Salmonella counted. Total coliform count was carried out on MacConkey Agar counting distinct pinkish coloured colonies.

2.6 Colony Counting

After the process of incubation, all colonies either they were aerobic bacteria or anaerobic were counted by using of colony counter. 30-300 colonies on average were counted and results per dilution were recorded. Following formula was applied to calculate total bacterial count.

\[
CFU / ml = \frac{(no. \ of \ colonies \times \ dilution \ factor)}{volume \ of \ culture \ plate} [6]
\]

2.7 Statistical Analysis

The data were statistically interpreted with descriptive statistics [Analysis of Variance (ANOVA) and t-test] for determined the variation of milk shake samples.

3. RESULTS

3.1 Total Plate Count

A 25 apple and 25 banana shake were collected from various educational institute of Lahore in the month of August firstly. They were collected again in the month of November from same spots. The results of Banana and Apple range of TPC in August 2.3-7.2 x 10^7 and 2.2-7.5 x10^7 respectively Banana and Apple range of TPC in November 2.1-6.7 x10^7 and 2.08-6.5 x10^7 respectively. It is revealed that mean of banana milk shake all samples in month of August which was (5.96 x10^7) and relatively (4.25 x10^7) in November.

Fig. 1 (a) (b) were shown all logs value for the comparative analysis of banana shake in August and November which range was between 7.85-7.32.

The current study was aimed to evaluate pathogens in the milkshakes available in various public and private sector educational institutes. Our results showed that in the month of august banana Shake, showed highest TPC of 7.2 x 10^7 cfu/ml, whereas, average TPC was 5.96 x10^7 cfu/ml. TPC value of log_{10} was between 7.85-7.34 (Fig. 1). Whereas in the month of November banana Shake, showed highest TPC of 6.7 x 10^7 cfu/ml. average TPC was 4.25 x 10^7 cfu/ml. TPC value of log_{10} was between 7.82-7.32 (Fig. 1).

The study shows mean of apple milk shake all samples in month of August which was (5.38 x10^7) and relatively (4.26 x10^7) in November.

In the month of August apple Shake, showed the highest range of TPC is 7.5 x 10^7 cfu/ml. whereas average TPC was 5.38 x10^7 cfu/ml. TPC value of log_{10} was between 7.88-7.34 (Fig. 2). Whereas in November Apple Shake, showed highest TPC of 6.5 x 10^7 cfu/ml, average TPC was 4.26 x 10^7 cfu/ml. TPC value of log_{10} was between 7.85-7.32 (Fig. 2). All logs value for the comparative analysis of apple shake in August and November which range was between 7.88-7.32.

![TPC banana](image)

**Fig. 1(a).** Institute wise comparative analysis of total plate count of banana milk shake
Fig. 1(b). Institute wise comparative analysis of total plate count of banana milk shake

Fig. 2 (a). Institute wise comparative analysis of total plate count of apple milk shake

Fig. 2 (b). Institute wise comparative analysis of total plate count of apple milk shake

3.2 Total Staphylococcus aureus Count of Banana Milk Shake

Total 25 sample of banana milk shake collected from different educational institute in the month of August and November. In banana shake Staphylococcus aureus range in August was $3.3 \times 10^3$ to $3.7 \times 10^3$ cfu/ml and $2.2 \times 10^3$ to $3.9 \times 10^3$ cfu/ml in November respectively.

According to our study was to calculate total Staphylococcus aureus count of banana milkshake in the month of August maximum value was $3.7 \times 10^3$ cfu/ml and an average value of total Staphylococcus aureus count for the
banana shake in the month of August was $3.52 \times 10^3$ cfu/ml. Average $\log_{10}$ of total *Staphylococcus aureus* count was 3.54. TSC value of $\log_{10}$ was between 3.59-3.5 (Fig. 3). Total of 76% samples was positive for *Staphylococcus aureus* and 24% Negative (Fig. 4). All $\log_{10}$ values for the comparative analysis of banana shake in August and November which range was between 3.34-3.59. The Present study was to calculate total *Staphylococcus aureus* count of banana milkshake in the month of November, it was showed the maximum ranges of samples was $3.9x \times 10^3$ cfu/ml and Mean of different samples of banana shake in the month of November was $3.13 \times 10^3$ cfu/ml. It was showed that Mean of $\log_{10}$ of all the samples was 3.49. TSC value of $\log_{10}$ was between 3.59-3.34 (Fig. 3). Fig. 4 showed that sample of total *Staphylococcus aureus* was positive 60% and 40% were Negative.

Fig. 4 were shown that the *S. aureus* were positive (76%) in the month of August and (60%) were also positive in the month of November.

### 3.3 Total *staphylococcus aureus* Count of Apple Milk Shake

Total 25 sample of apple milk shake collected from different educational institute in the month of August and November. In apple shake *Staphylococcus aureus* range in August was 2.7-4.3 $\times 10^3$ and 3.1 to 3.7 $\times 10^3$ in November respectively.
Fig. 4. Comparative analysis of total *Staphylococcus aureus* count of banana milk shake

The Present study was to calculate total *Staphylococcus aureus* count of apple milkshake in the month of August, it was showed the maximum ranges of samples was $4.3 \times 10^3$ cfu/ml. The Mean of different samples of apple shake in the month of August was $3.57 \times 10^3$ cfu/ml. The study was shown that Mean of log$_{10}$ of all the samples was 3. 55. TSC value of log$_{10}$ was between 3.63-3.43 (Fig. 5). Fig. 6 showed that sample of total *Staphylococcus aureus* was positive 72% and 28% Negative.

Fig. 5 (a) (b) were shown all logs value for the comparative analysis of apple shake in August and November which range was between 3.43-3.63.

Fig. 6 were shown that the *S aureus* was positive (72%) in August and (64%) were also positive in November.

3.4 Total coliform Count of Banana Milk Shake

Total 25 sample of banana milk shake collected from different educational institute in the month of August and November. In banana shake Total coliform range in August was $1.3-3.7 \times 10^2$ and $1.1-3.3 \times 10^2$ in November respectively.

Fig. 7 (a) (b) were shown all logs value for comparative analysis of banana shake in August and November which range was between 2.08-2.61.
Fig. 5 (b). Institute wise comparative analysis of total *Staphylococcus aureus* count of apple milk shake

Fig. 6. Comparative analysis of total *Staphylococcus aureus* count of apple milk shake

Fig. 7 (a). Institute wise comparative analysis of Total coliform count of banana milk shake
Total coliform count of banana milkshake in the month of August showed highest ranges of $3.7 \times 10^2$ cfu/ml and an average of $2.53 \times 10^2$ cfu/ml. Average of $\log_{10}$ of all the samples was 2.39. TCC value of $\log_{10}$ was between 2.61-2.21 (Fig. 7). 64% samples were positive and 36% samples were negative for the Total coliform count (Fig. 8).

Total coliform count of banana milkshake in the month of November was $3.3 \times 10^2$ cfu/ml with highest Total coliform value. Average value was $2.1 \times 10^2$ cfu/ml. Average value of $\log_{10}$ of all the samples was 2.28. TCC value of $\log_{10}$ was between 2.53-2.08 (Fig. 7). The Total coliform count was positive for 56% and Negative for 44% samples (Fig. 8).

Fig. 8 were shown that the total coliform was positive (64%) in August and (56%) were also positive in November.

### 3.5 Total Coliform Count of Apple Milk Shake

Total 25 sample of apple milk shake collected from different educational institute in the month of August and November. In apple shake Total coliform range in August was $1.1-3.9 \times 10^2$ and 1.1 to $3.3 \times 10^2$ in November respectively.

Total coliform count of Apple milkshake in the month of August exhibited highest ranges of $3.9 \times 10^2$ cfu/ml and mean of $2.2 \times 10^2$ cfu/ml. Mean of $\log_{10}$ of all the samples was 2.32. TCC value of $\log_{10}$ was between 2.59-2.04 (Fig. 9). In (Fig. 10) showed the Total coliform count was positive for 64% and Negative for 36%.

Fig. 9 (a) (b) were shown all logs value for the comparative analysis of apple shake in August and November which range was between 2.04-2.98.
Fig. 9(a). Institute wise comparative analysis of total coliform count of apple milk shake

Fig. 9(b). Institute wise comparative analysis of total coliform count of apple milk shake

Fig. 10. Comparative analysis of total coliform count of apple milk shake
Total coliform count of Apple milkshake in the month of November showed the highest ranges of 3.3x $10^2$ cfu/ml and also showed the mean of total samples as 1.7x $10^2$ cfu/ml. Mean of log$_{10}$ of all the samples was 2.22. TCC value of log$_{10}$ was between 2.98-2.04 (Fig. 9). Total coliform count of 56% positive and 44% Negative samples (Fig. 10).

Fig. 10 were shown that the total coliform were positive (64%) in the month of August and (56%) were also positive in the month of November.

4. DISCUSSION AND CONCLUSION

Our study showed a much higher prevalence of contamination as compared to Kim et al. [7], who documented that milkshake was contaminated with E. coli, Staphylococcus aureus and Salmonella. The E. coli was a basic source of water, handler hands, nose and clothe are the major source of contamination. Food poisoning occurred due to Staphylococcus aureus, salmonella and coliform. The study was done in costa Rica the 65 all samples of homemade milk shake were examined in this study we found the total fecal coliforms, E. coli, and Salmonella was examined using pour plate culture method, in the 37.1% of samples of homemade milk shakes and 20% of commercial homemade milk shakes did not meet int. standards of Total coliform as designated of my research all of my samples of banana and apples was free from salmonella but all the samples of banana and apples is to improve the bacterial quality to meet the bacteria standard like TPC (Staph and Total coliform).

According to Verma and Gaur [8] the most probable number of samples (Total coliform) the range of Total coliform s from 9.5 MPN/100ml to greater than 2400 MPN. It was observed that all the juices were with coliforms.

Our findings showed a much higher prevalence of microbial load in banana and apple as compared to Ahmad et al. [9]. Who suggested that in most of the samples, the total bacterial load was much higher than recommended by the Gulf standard. It was observed that in strawberry, banana and apple were highest microbial load as it is for banana 9.3 x 108 and for Apple, it was 7.3 x 109.

According to Nma and Ola [10] findings were according to the set standards of ICMSF. Comparatively our findings had much higher prevalence. In another study by Tortora et al. [11] apple juice was contaminated with (11%) S. aureus, (33%) and E. coli. Thus apple juices were positive for these strains.

In a study conducted by Al-Jedah and Robinson (2002) in Qatar fresh juices available on retail outlets contained TPC in apple equal to 6.6 X $10^6$ cfu/ml and Total coliform was 1.4X $10^5$ cfu/ml. Whereas banana had TPC of 2.2 X $10^6$ cfu/ml and Total coliform were 3.2 X $10^5$ cfu/ml. Thus these results were in accordance to our findings.

Study conducted by Khan et al. [12] on different fruity juices, results exhibited high prevalence of microbes. The microbial load and Total coliform s were (7.7 x $10^5$ - 9 x $10^5$ cfu/ml and 210–1100 cfu/100 ml) very high. Among the various bacteria, E. coli were also involved in contamination, prolonged use without refrigeration, insanitary surroundings, raw materials, chemical properties, equipment were the main sources for microbes. These findings are in agreement with our study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

5. Babu BR, Rastogi NK, Raghavaran KSM. Mass transfer in osmotic membrane distillation of phycocyanin colorant and