

Assessment of microbial load of milk shakes available in various educational institutes of Lahore

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 analyze 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 2.3×10^7 - 7.2×10^7 cfu/ml and respectively in November range between 2.1×10^7 - 6.7×10^7 cfu/ml. In August TPC range of apple shake between 2.2×10^7 - 7.5×10^7 cfu/ml and respectively in November range between 2.08×10^7 - 6.5×10^7 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.

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

29 **INTRODUCTION:**

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

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

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

48

49 **MATERIAL AND METHODS:**

50 **Study design:**

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

56 **Sample collection:**

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

59 **Microbial Analysis:**

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

63 **Sample preparation:**

64 Before culturing of samples, all the sample of milkshake was stored at 4°C. After
65 thawing, 1 ml sample was taken by using of sterile pipette and transferred to sterilized test
66 tube which comprises normal saline (9 ml) for making a 10 fold serial dilution. After dilution,
67 1 ml diluted sample was taken from the first tube and transferred it into the next tube by using
68 of another sterile pipette. This procedure was repeated again and again up to 9th test tube,
69 discarded 1ml from the 10th test tube for obtaining the desired dilution.

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 sterilized 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. For calculation of *Staphylococcus aureus* and *Salmonella* count (CFU/ml). After the process of incubation, all bacteria colonies with distinction with yellow color for *Staphylococcus aureus* and black color for *Salmonella* counted. Total coliform count was carried out on MacConkey Agar counting distinct pinkish colored colonies.

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.

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

Statistical Analysis:

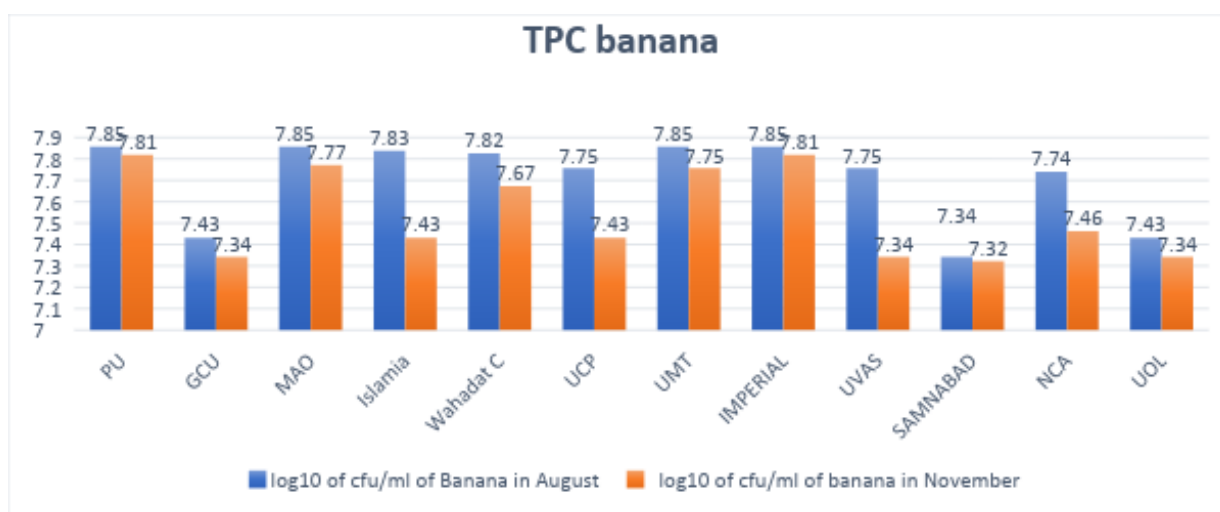
The data were statistically interpreted with descriptive statistics [Analysis of Variance (ANOVA) and t-test] for determined the variation of milk shake samples.

RESULTS:

Total plate count:

92 A 25 apple and 25 banana shake were collected from various educational institute of
 93 Lahore in the month of August firstly. They were collected again in the month of November
 94 from same spots. The results of Banana and Apple range of TPC in August $2.3-7.2 \times 10^7$ and
 95 $2.2-7.5 \times 10^7$ respectively Banana and Apple range of TPC in November $2.1-6.7 \times 10^7$ and
 96 $2.08-6.5 \times 10^7$ respectively. It is revealed that mean of banana milk shake all samples in
 97 month of August which was (5.96×10^7) and relatively (4.25×10^7) in November.

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



100

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

102

103 The current study was aimed to evaluate pathogens in the milkshakes available in
 104 various public and private sector educational institutes. Our results showed that in the month
 105 of august banana Shake, showed highest TPC of 7.2×10^7 cfu/ml, whereas, average TPC was
 106 5.96×10^7 cfu/ml. TPC value of \log_{10} was between 7.85-7.34 (Fig. 1). Whereas in the month of
 107 November banana Shake, showed highest TPC of 6.7×10^7 cfu/ml. average TPC was $4.25 \times$
 108 10^7 cfu/ml. TPC value of \log_{10} was between 7.82-7.32 (Fig. 1).

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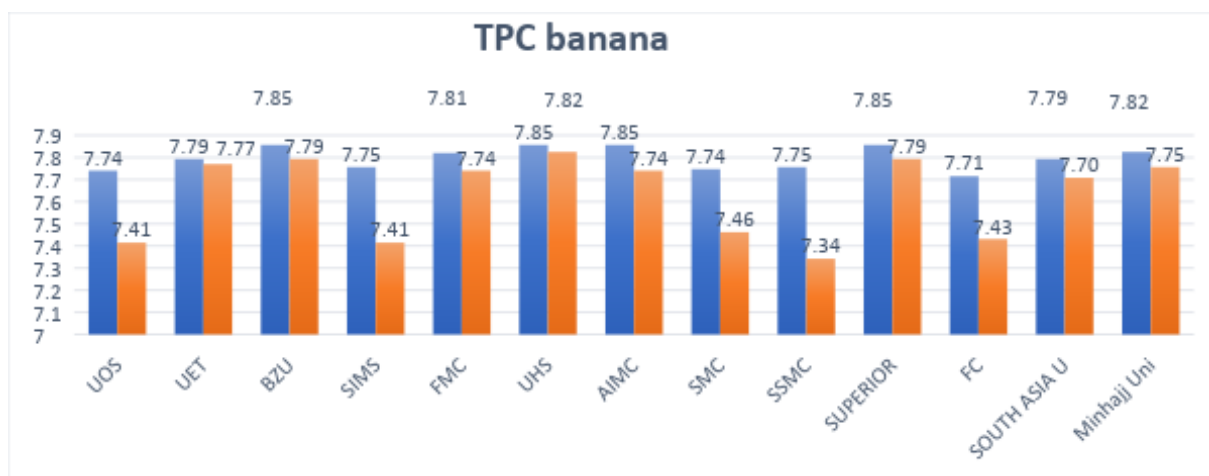
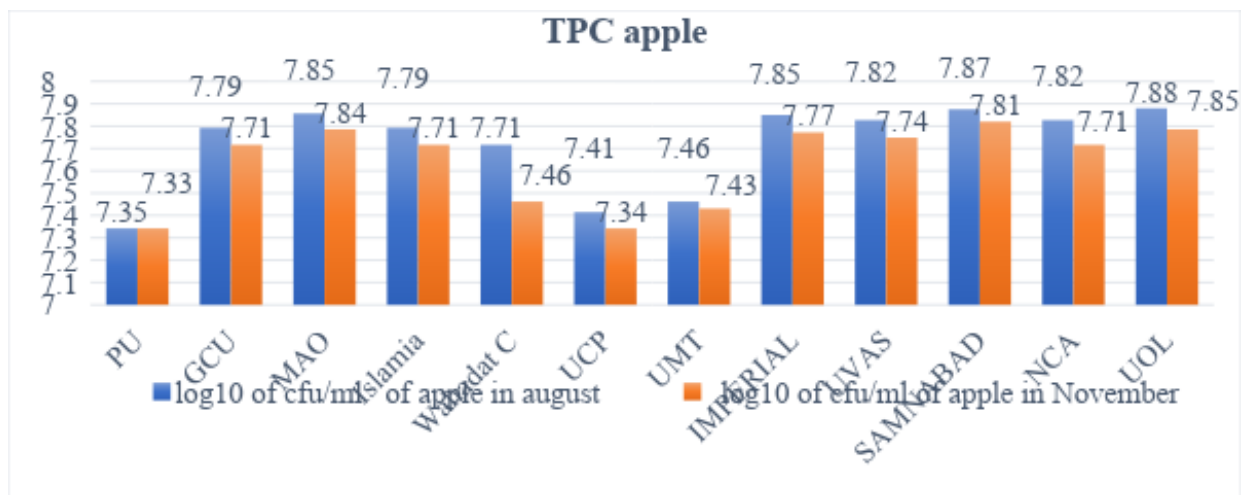


Fig. 1(b): Institute wise comparative analysis of total plate count of banana milk shake

The study shows mean of apple milk shake all samples in month of August which was (5.38×10^7) and relatively (4.26×10^7) in November.

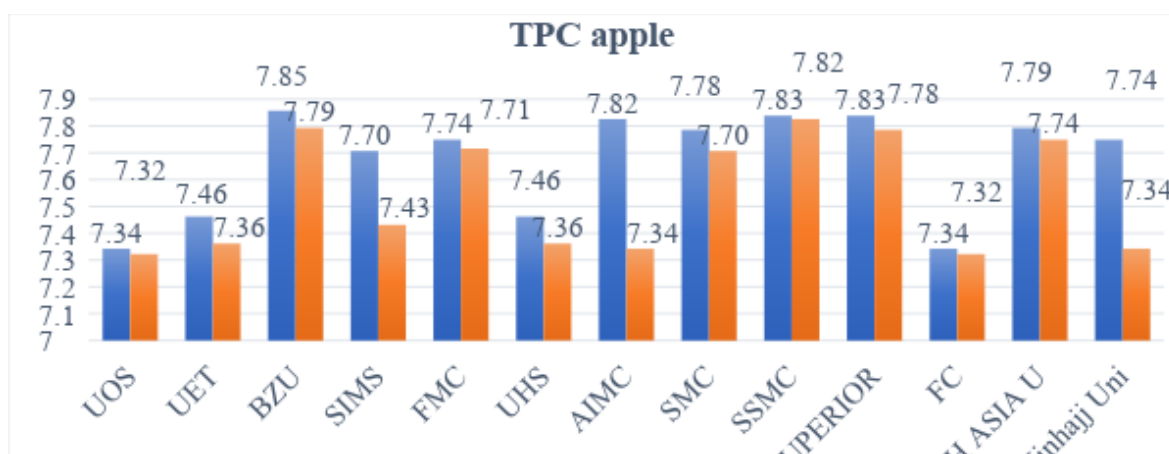
In the month of August apple Shake, showed the highest range of TPC is 7.5×10^7 cfu/ml. whereas average TPC was 5.38×10^7 cfu/ml. TPC value of \log_{10} was between 7.88-7.34 (Fig. 2). Whereas in November Apple Shake, showed highest TPC of 6.5×10^7 cfu/ml, average TPC was 4.26×10^7 cfu/ml. TPC value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

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122

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



124

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

126 **Total *Staphylococcus aureus* count of banana milk shake:**

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

130 Present study shown mean of banana milk shake all samples in month of August
 131 which was (3.54) and relatively (3.49) in November.

132

According to our study was to calculate total *Staphylococcus aureus* count of banana milkshake in the month of August maximum value was 3.7×10^3 cfu/ml and an average value of total *Staphylococcus aureus* count for the banana shake in the month of August was 3.52×10^3 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 logs value for 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 3.9×10^3 cfu/ml and Mean of different samples of banana shake in the month of November was 3.13×10^3 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.

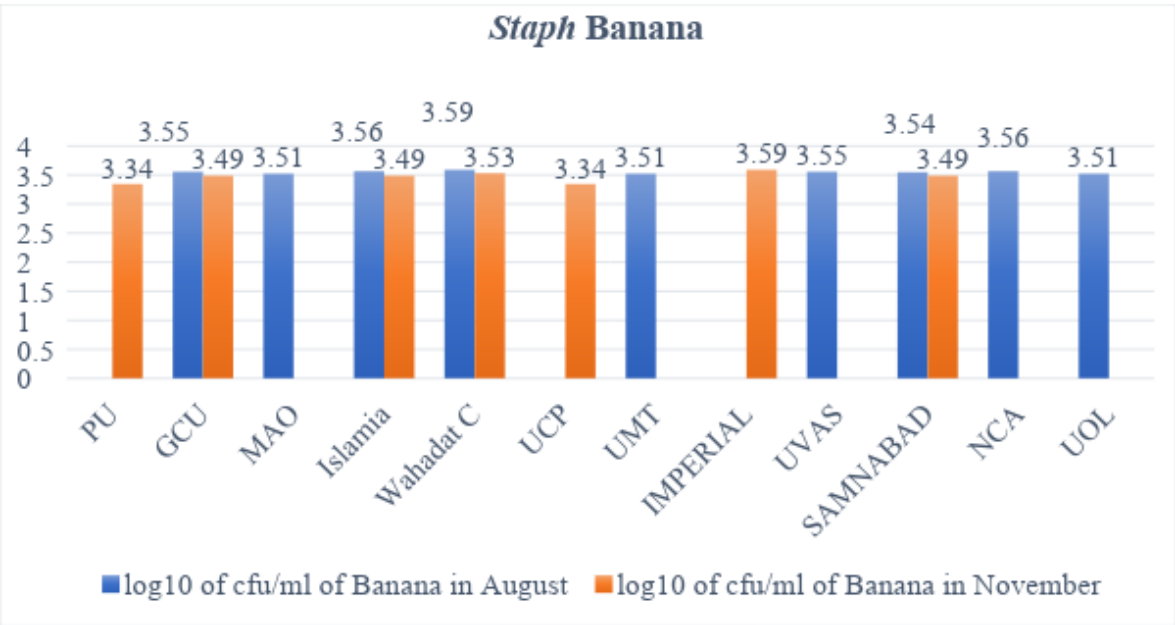


Fig. 3 (a): Institute wise comparative analysis of total *Staphylococcus aureus* count of banana milk shake

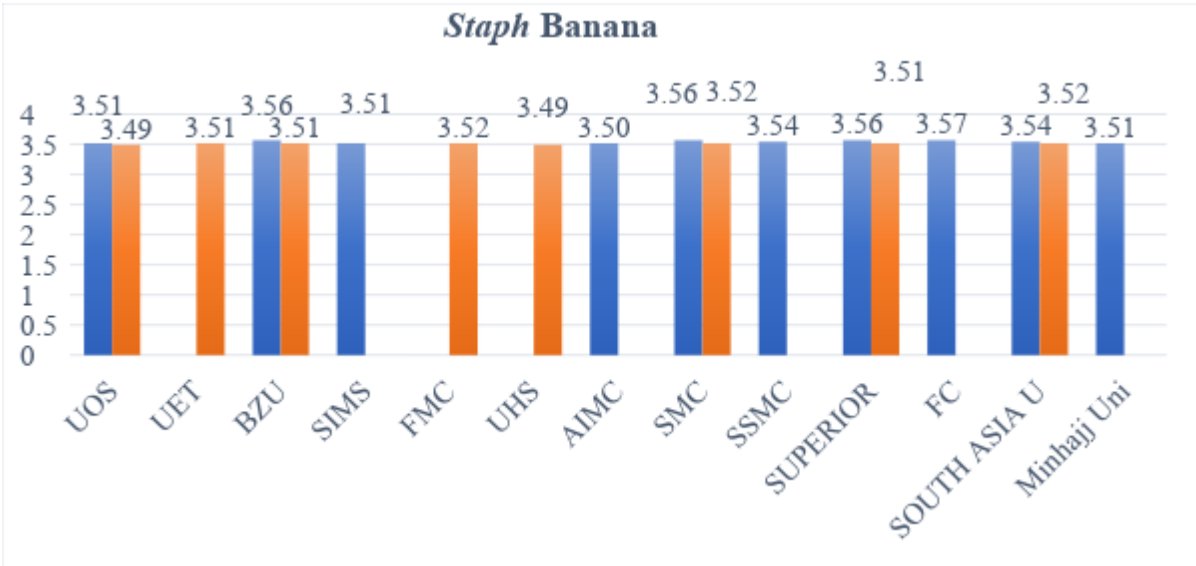


Fig. 3 (b): Institute wise comparative analysis of total *Staphylococcus aureus* count of banana milk shake

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.

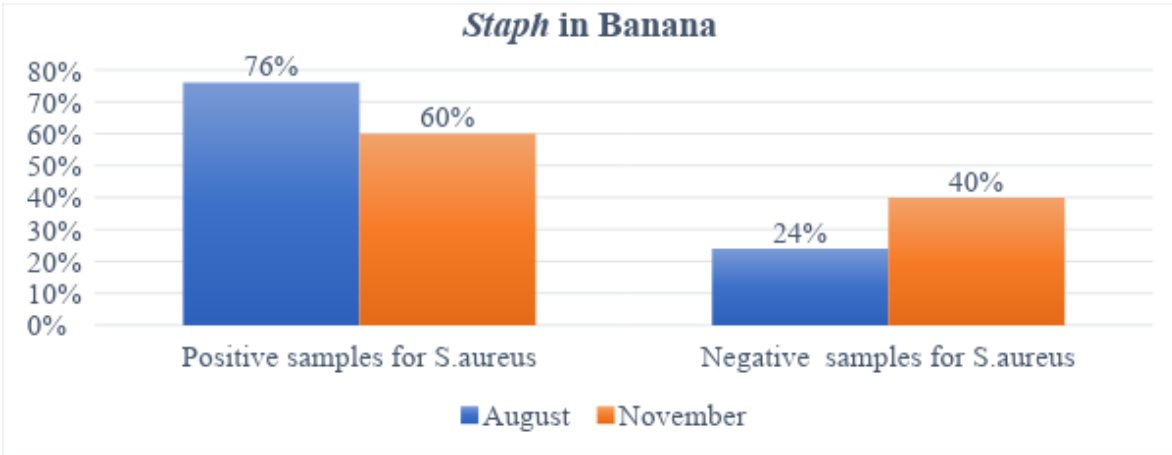


Fig. 4: Comparative analysis of total *Staphylococcus aureus* count of banana milk shake

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 3.7×10^3 in November respectively.

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 4.3×10^3 cfu/ml. The Mean of different samples of apple shake in the month of August was 3.57×10^3 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.

The Present study was to calculate total *Staphylococcus aureus* count of apple milkshake in the month of November, it was showed the maximum ranges of samples was 3.7×10^3 cfu/ml. The Mean of different samples of apple shake in the month of November was 3.34×10^3 cfu/ml. It was showed that Mean of \log_{10} of all the samples was 3. 52. TSC value of \log_{10} was between 3.56-3.49 (Fig. 5). Fig. 6 showed that sample of total *Staphylococcus aureus* was positive 64% and 36% 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.

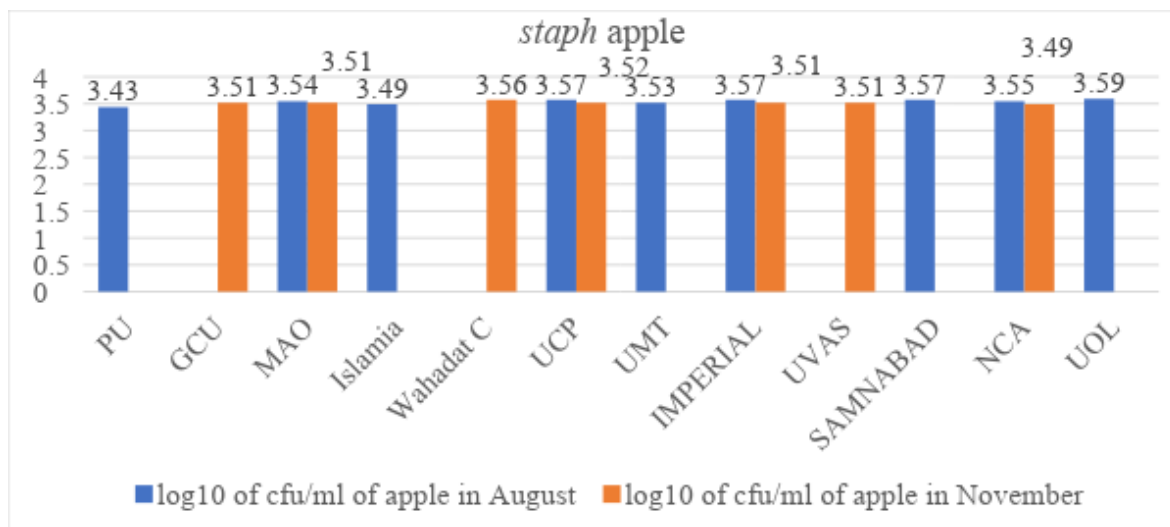


Fig. 5 (a): Institute wise comparative analysis of total *Staphylococcus aureus* count of apple milk shake

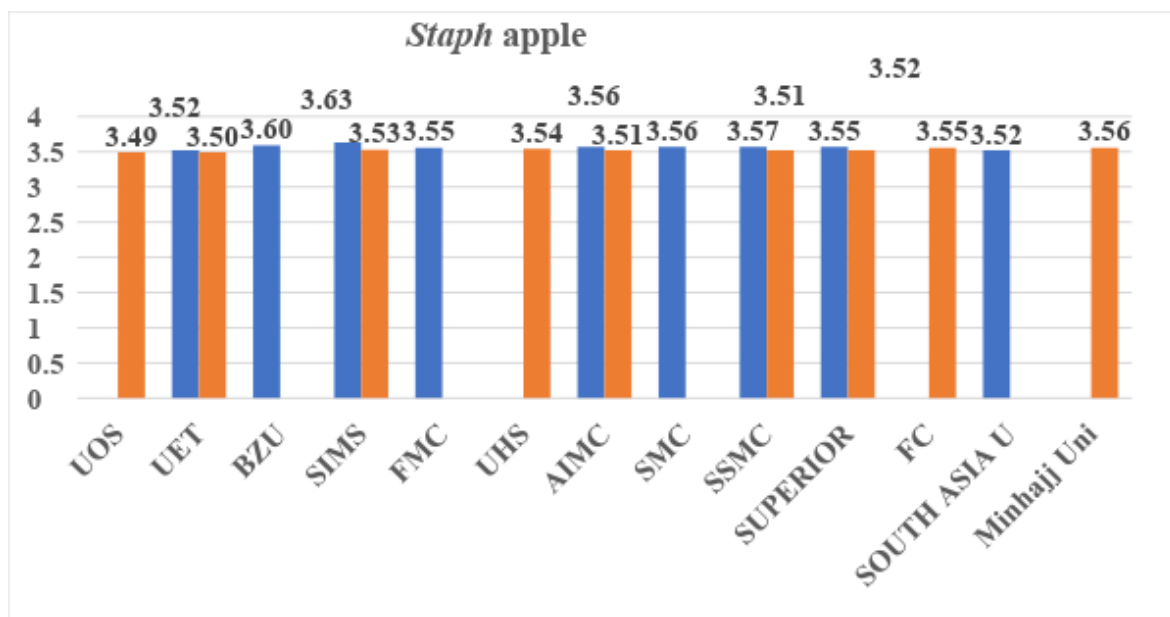


Fig. 5 (b): Institute wise comparative analysis of total *Staphylococcus aureus* count of apple milk shake

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

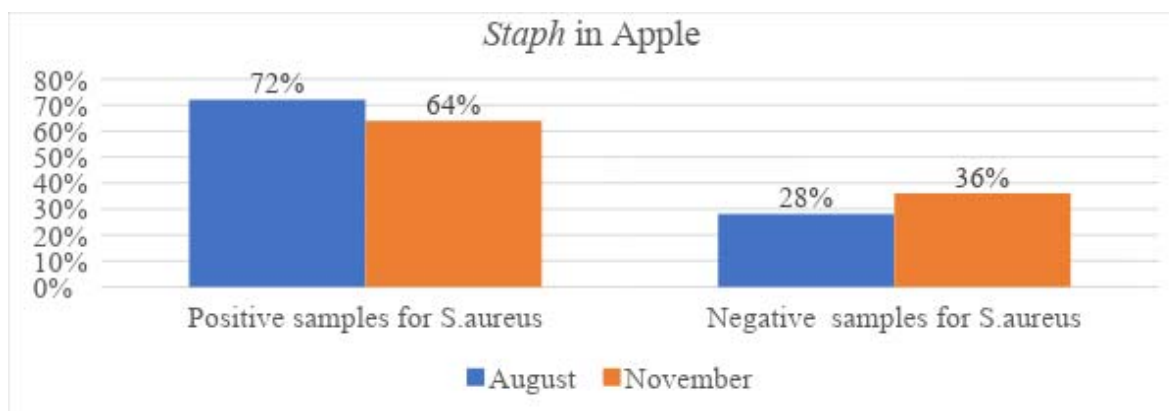


Fig. 6: Comparative analysis of total *Staphylococcus aureus* count of apple milk shake

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.

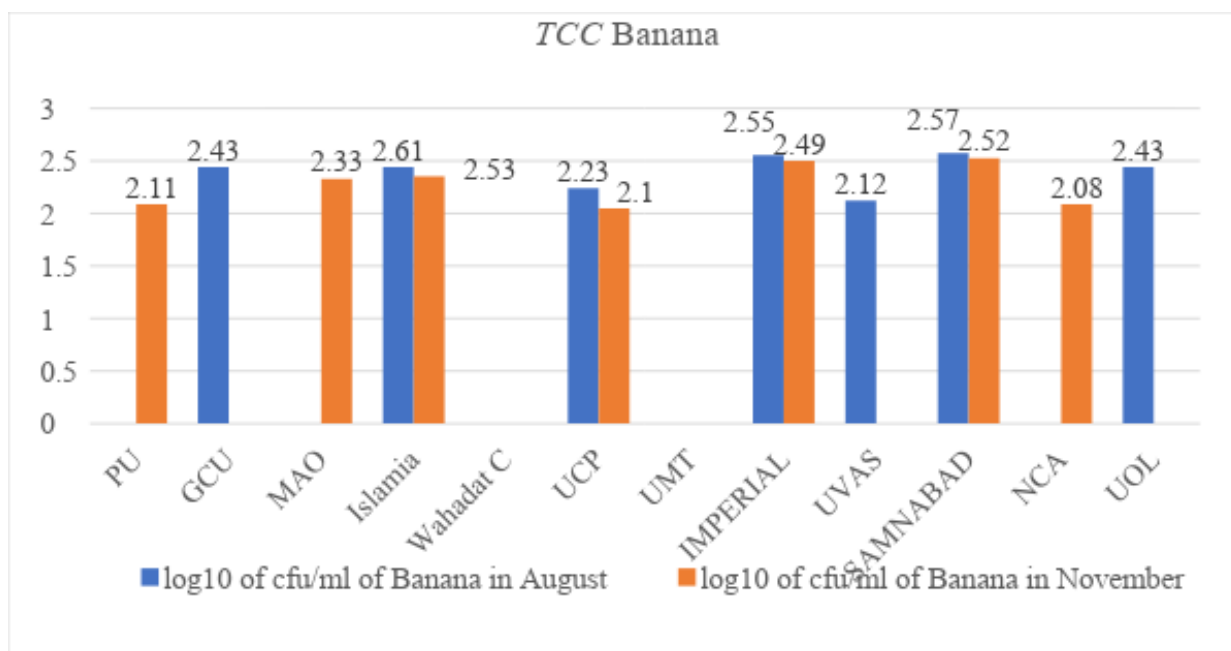


Fig. 7 (a): Institute wise comparative analysis of **Total coliform** count of banana milk shake

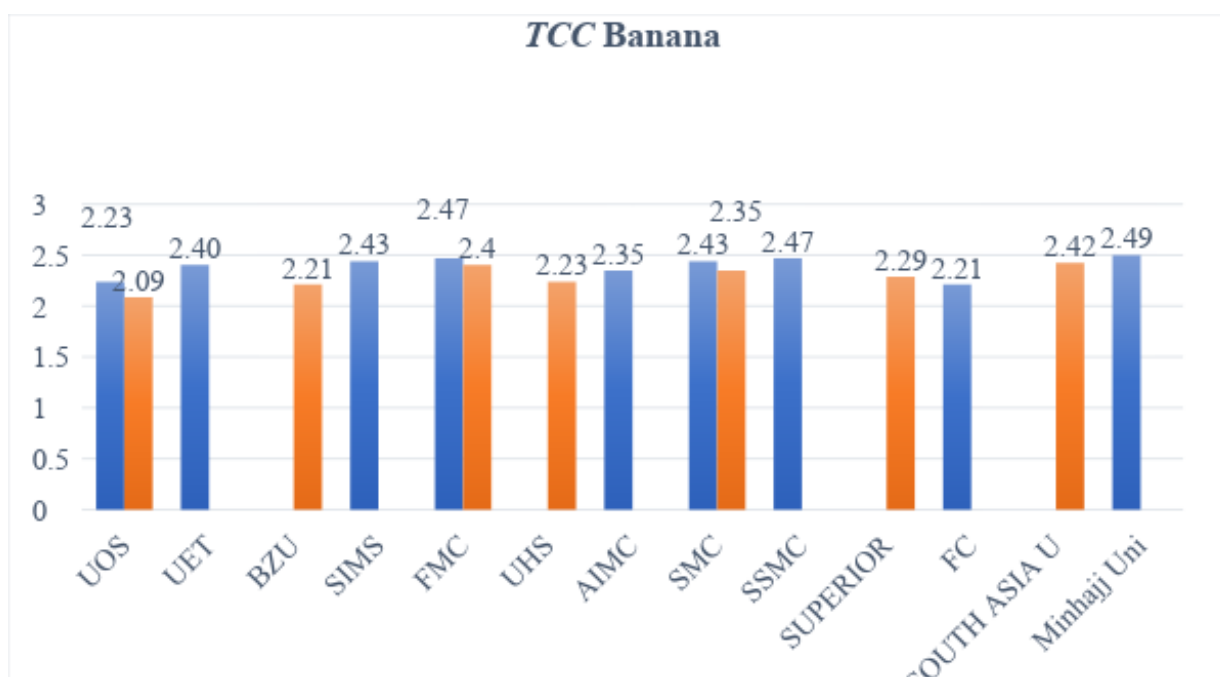


Fig. 7 (b): Institute wise comparative analysis of **Total coliform** count of banana milk shake

Total coliform count of banana milkshake in the month of August showed highest ranges of 3.7×10^2 cfu/ml and an average of 2.53×10^2 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 3.3×10^2 cfu/ml with highest **Total coliform** value. Average value was 2.1×10^2 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).

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

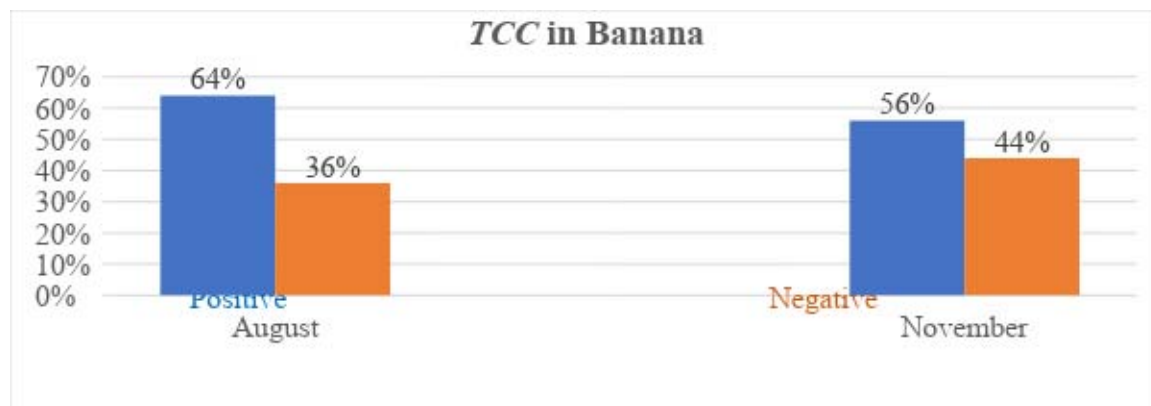


Fig. 8: Comparative analysis of **Total coliform count of banana milk shake**

Total coliform count of apple milk shake:

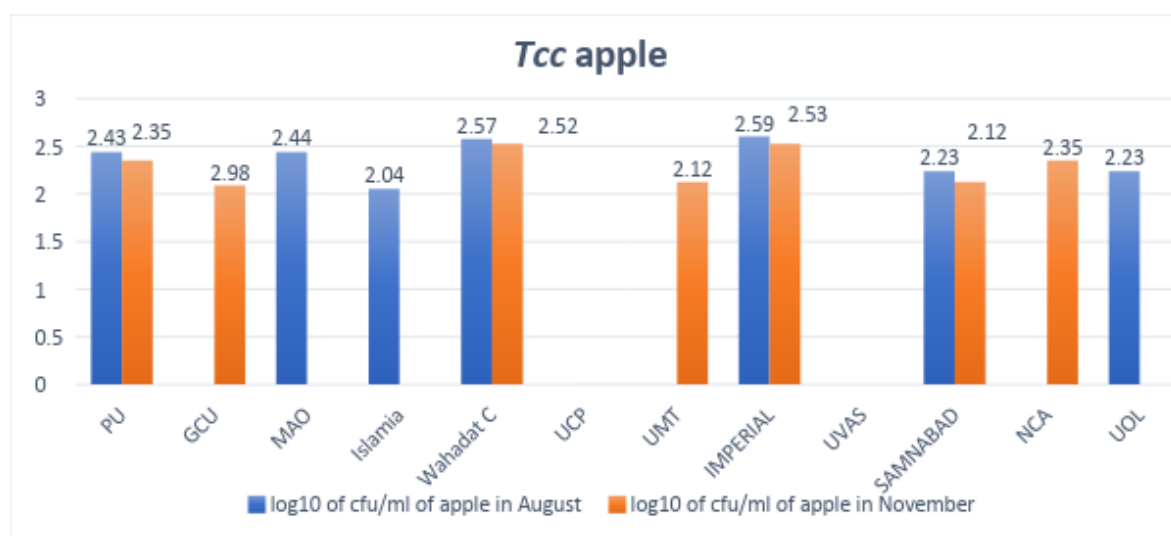
Total 25 sample of apple milk shake collected from different educational institute in the month of August and November. In apple shake **Total coliform** range in August was $1.1-3.9 \times 10^2$ and 1.1 to 3.3×10^2 in November respectively.

Total coliform count of Apple milkshake in the month of August exhibited highest ranges of 3.9×10^2 cfu/ml and mean of 2.2×10^2 cfu/ml. Mean of \log_{10} of all the samples was

223 2.32. *TCC* value of \log_{10} was between 2.59-2.04 (Fig. 9). In (Fig. 10) showed the Total
 224 coliform count was positive for 64% and Negative for 36%.

225 Fig. 9 (a) (b) were shown all logs value for the comparative analysis of apple shake in
 226 August and November which range was between 2.04-2.98.

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228

229 **Fig. 9(a): Institute wise comparative analysis of Total coliform count of apple milk**
 230 **shake**

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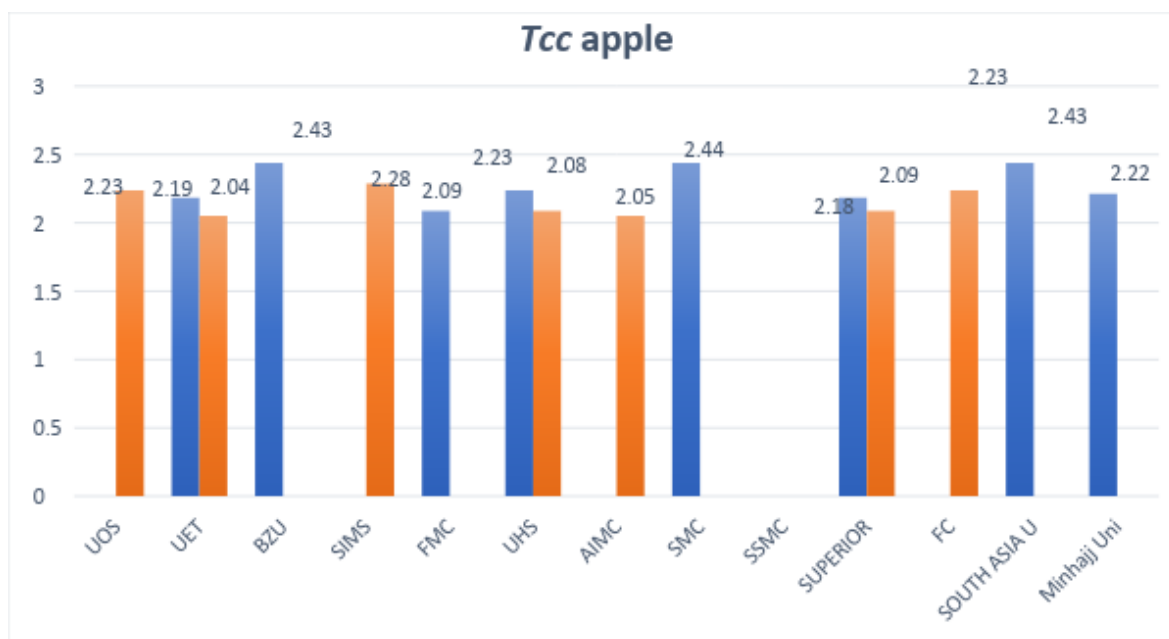


Fig. 9(b): Institute wise comparative analysis of Total coliform count of apple milk shake

Total coliform count of Apple milkshake in the month of November showed the highest ranges of 3.3×10^2 cfu/ml and also showed the mean of total samples as 1.7×10^2 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).

Figure 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.

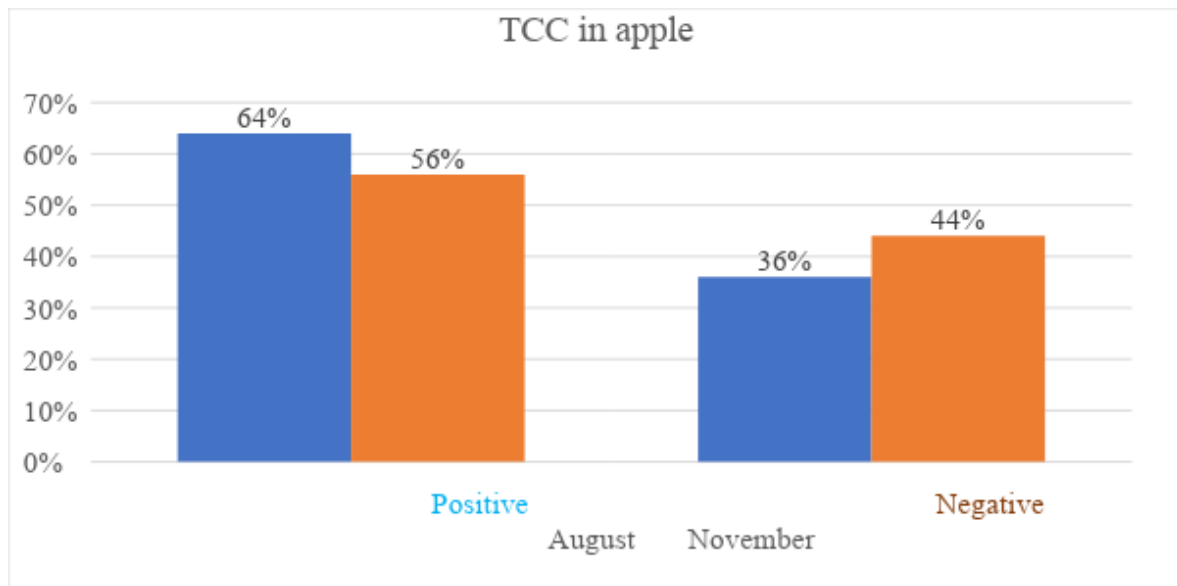


Fig. 10: Comparative analysis of Total coliform count of apple milk shake

DISCUSSION:

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 9.3×10^8 and for Apple, it was 7.3×10^9 .

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 6.6×10^6 cfu/ml and *Total coliform* was 1.4×10^3 cfu/ml. Whereas banana had TPC of 2.2×10^6 cfu/ml and *Total coliform* were 3.2×10^3 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 (7.7×10^3 - 9×10^8 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.

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