

Original Research Article**Assessment of microbial load of milk shakes available in various educational institutes of Lahore****ABSTRACT:**

A milkshake is a delicious and non-carbonated refreshment produced using dairy, frozen flavor related item. The unhygienic condition grow microbes in milk shake and produce toxins in food which is very harmful and cause food poisoning. This study was established to calculate total plate count (*Staphylococcus aureus*, *Total coliform* and *Salmonella*) present in Apple and Banana milk shakes. 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. Total plate count in August was significantly higher than in November. In August TPC range of banana shake 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 positive 18 (72%) in the month of August and 16 (64%) were also positive in the month of November. The *Total coliform* specie in apple milk shake were positive 16 (64%) in the month of August and 14 (56%) were also positive in the month of November. The total positive *coliforms* were 15 (64%) in the month of August and 14 (56%) were also positive in the month of November. 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

25 much higher than recommended by the Gulf standard. So these drinks are not fit for
26 consumption.

27 INTRODUCTION:

28 A milkshake is a delicious and non-carbonated refreshment produced using dairy,
29 frozen yoghurt and flavor related item being sold in streets and mostly sailed in educational
30 setups. It is served in disposable glass with a straw or in various serving styles. The
31 milkshake is made blending the apple and banana pulp with milk, sugar in a blender or drinks
32 blender and by including ice at last (Petridou et al. 1997). Natural product juices contains
33 vitamins, and minerals that are necessary for individual nourishment and they play a critical
34 part in the antipathy of heart problems, tumor and diabetes. Natural product juices are
35 essential and good sources of supplements and contain a few vital properties that may lessen
36 the danger of different illness. They contain a lot of cancer prevention agents, vitamins C and
37 E, and have charming taste and fragrance (Aghajanzadeh and Ziaifar 2018).

38 In developing countries, the 916 cases were reported for each 100,000 populace.
39 Considering WHO reports could be assessed at one billion dollars, considering therapeutic
40 expenses and profitability (Jackson et al. 1991). Poor cleanliness practices have been
41 connected with ingenious pathogenic organisms like *Staphylococcus aureus* (Djalma Chaves
42 et al. 2018).

43 Various types of liquid shakes are consumed day by day by a vast member of the
44 populations. The majority of these shakes are accessible in shops or canteens. It is
45 additionally noticed that most of the shopkeeper utilizes tap water for making juices, which
46 can be the fundamental source of bacterial contamination (Babu et al. 2006).

47

48 MATERIAL AND METHODS:**49 Study design:**

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

55 Sample collection:

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

58 Microbial Analysis:

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

62 Sample preparation:

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

69 Laboratory analysis:

70 From 6th and 7th dilution, 1 ml of diluted sample was taken and poured into two
71 separate sterile petri. After 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 Manito 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 were carried out on MacConkey Agar counting distinct pinkish colored colonies.

Colony counting:

After 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.

$CFU / ml = (no. \text{ of colonies } \times \text{ dilution factor}) / \text{volume of culture plate}$ (Rutala et al. 2006).

. Statistical Analysis:

Only descriptive statistics was used for the variation of milk shake samples.

RESULTS:

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 $2.3-7.2 \times 10^7$ and $2.2-7.5 \times 10^7$ respectively Banana and Apple range of TPC in November $2.1-6.7 \times 10^7$ and

2.08-6.5 $\times 10^7$ respectively. (Table 1) were shown mean of banana milk shake all samples in month of August which was (5.96×10^7) and relatively (4.25×10^7) in November.

Table 1: Institute wise comparative analysis of total plate count of banana milkshakes

Month	No. of Samples	Mean all samples cfu/ml of TPC
August	25	5.96×10^7
November	25	4.25×10^7

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

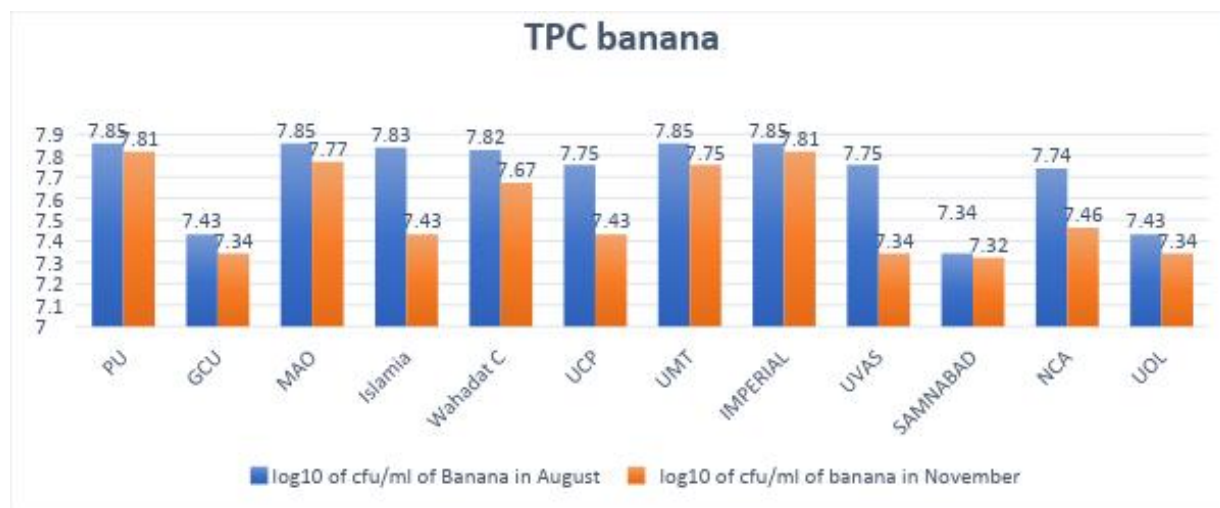


Figure 1(a): Institute wise comparative analysis of total plate count of banana milk shake

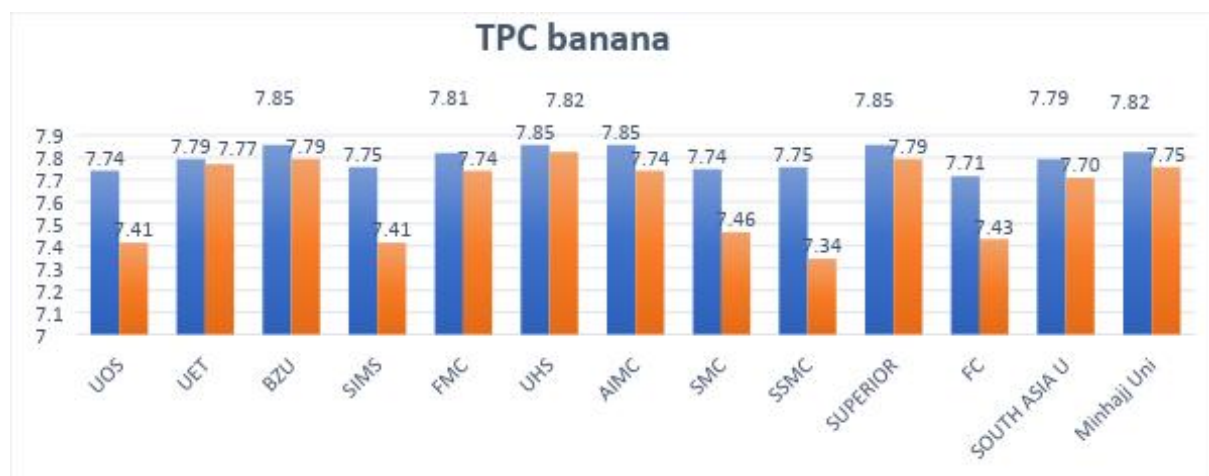


Figure 1(b): Institute wise comparative analysis of total plate count of banana milk shake

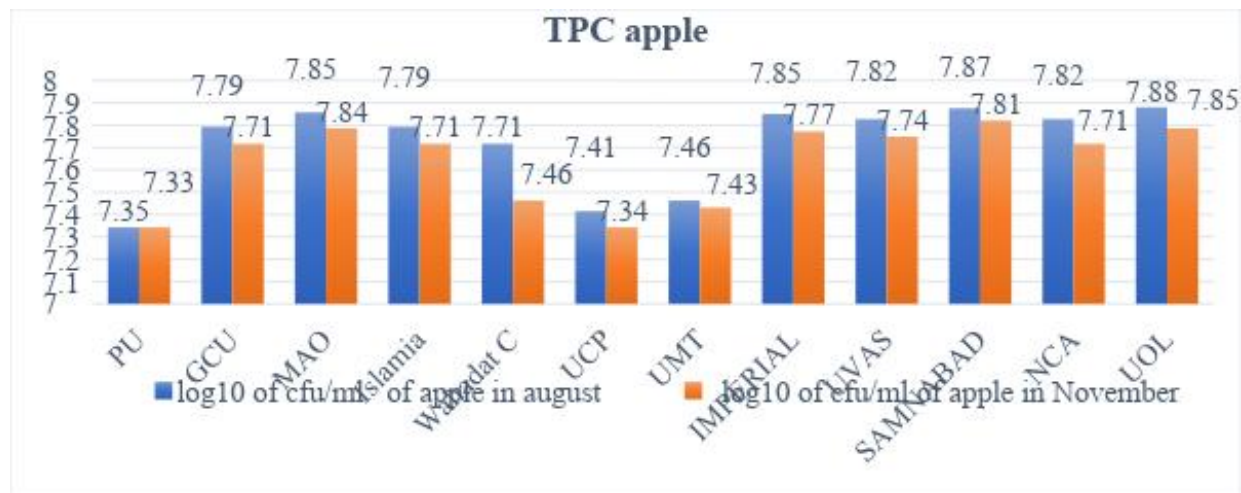
(Table 2) were shown mean of apple milk shake all samples in month of August which was (5.38×10^7) and relatively (4.26×10^7) in November.

Table 2: Institute wise comparative analysis of total plate count of apple milk shake in cfu/ml

Month	No. of samples	Mean of all samples cfu/ml of TPC
August	25	5.38×10^7
November	25	4.26×10^7

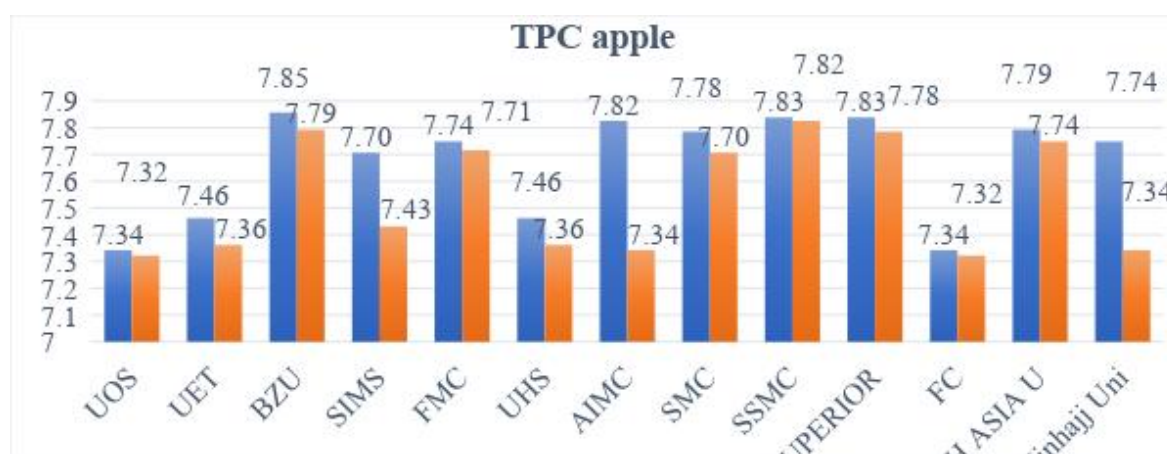
Figure 2 (a) (b) were shown all logs value for comparative analysis of apple shake in August and November which range was between 7.88-7.32.

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115

116 **Figure 2 (a): Institute wise comparative analysis of total plate count of apple milk shake**



117

118 **Figure 2 (b): Institute wise comparative analysis of total plate count of apple milk shake**

119 **Total *Staph aureus* count of banana milk shake:**

120 Total 25 sample of banana milk shake collected from different educational institute in
 121 the month of August and November. In banana shake *Staphylococcus aureus* range in August
 122 was 3.3-3.7x10³ and 2.2 to 3.9 x10³ in November respectively.

123 (Table 3) were shown mean of banana milk shake all samples in month of August
 124 which was (3.54) and relatively (3.49) in November.

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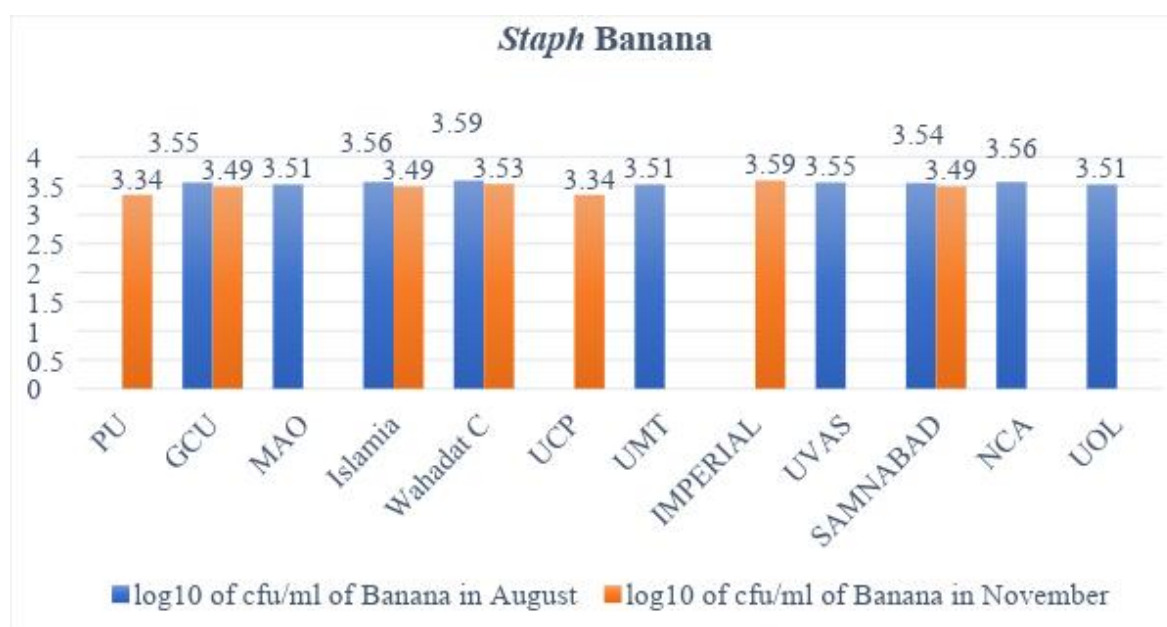
126 **Table 3: Comparative analysis of banana milk shake in log form**

Month	No. of samples	Positive samples	Negative Sample	Mean of Log 10 <i>S. aureus</i> count
August	25	19	6	3.54
November	25	15	10	3.49

127

128 Figure 3 (a) (b) were shown all logs value for comparative analysis of banana shake in

129 August and November which range was between 3.34-3.59.



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131 **Figure 3 (a): Institute wise comparative analysis of total *Staphylococcus aureus* count of**
 132 **banana milk shake**

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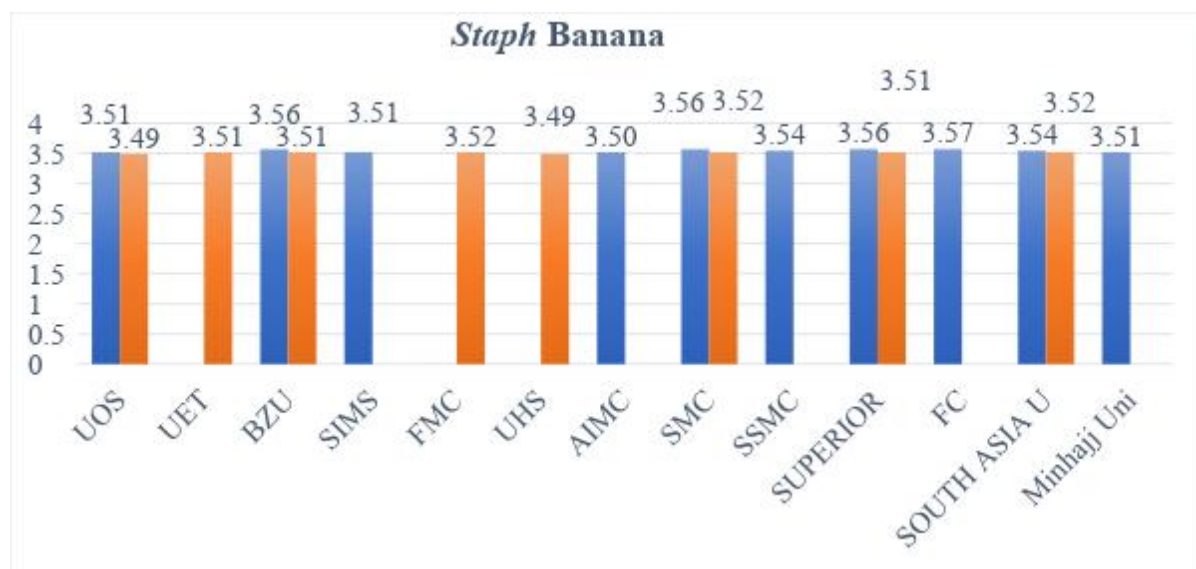


Figure 3 (b): Institute wise comparative analysis of total *Staphylococcus aureus* count of banana milk shake

Figure 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.

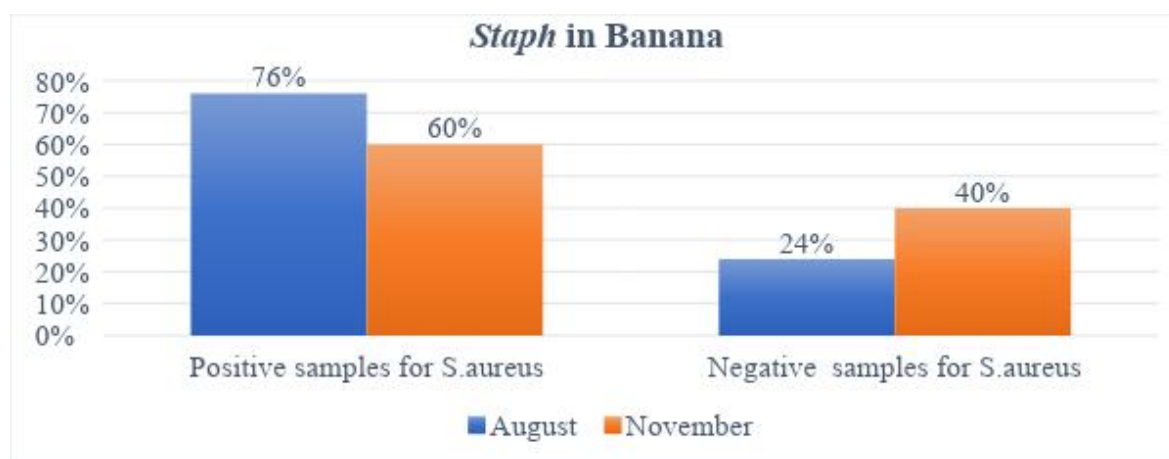


Figure 4: Comparative analysis of total *Staphylococcus aureus* count of banana milk shake

Total *staph 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.

(Table 4) were shown mean of apple milk shake all samples in month of August which was (3.55) and relatively (3.52) in November.

Table 4: Institute wise comparative analysis of apple milk shake in log form

Month	No. of samples	Positive	Negative	Mean of log 10 of <i>S. aureus</i>
August	25	18	7	3.55
November	25	16	9	3.52

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

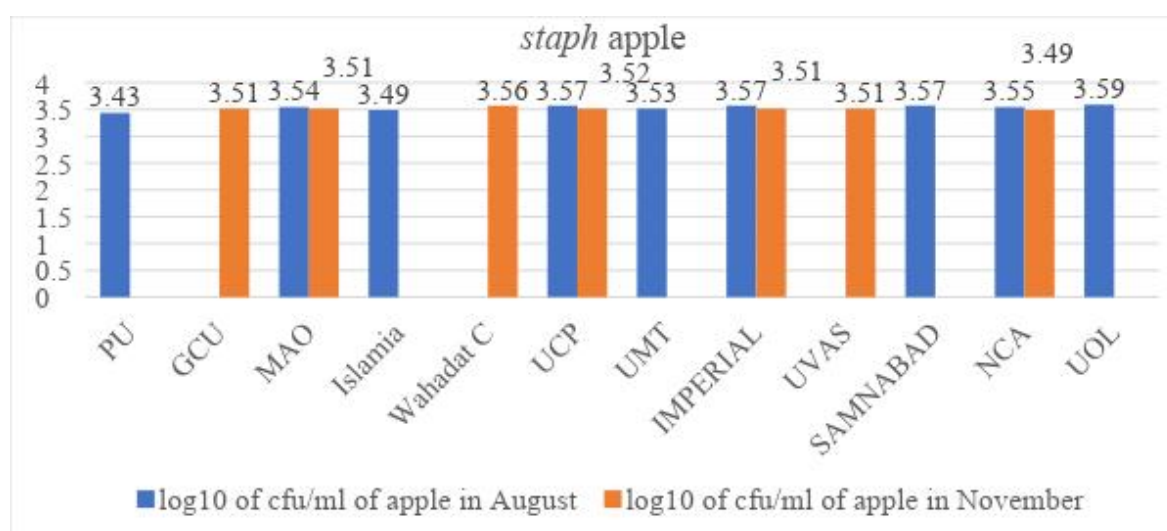


Figure 5 (a): Institute wise comparative analysis of total *Staphylococcus aureus* count of apple milk shake

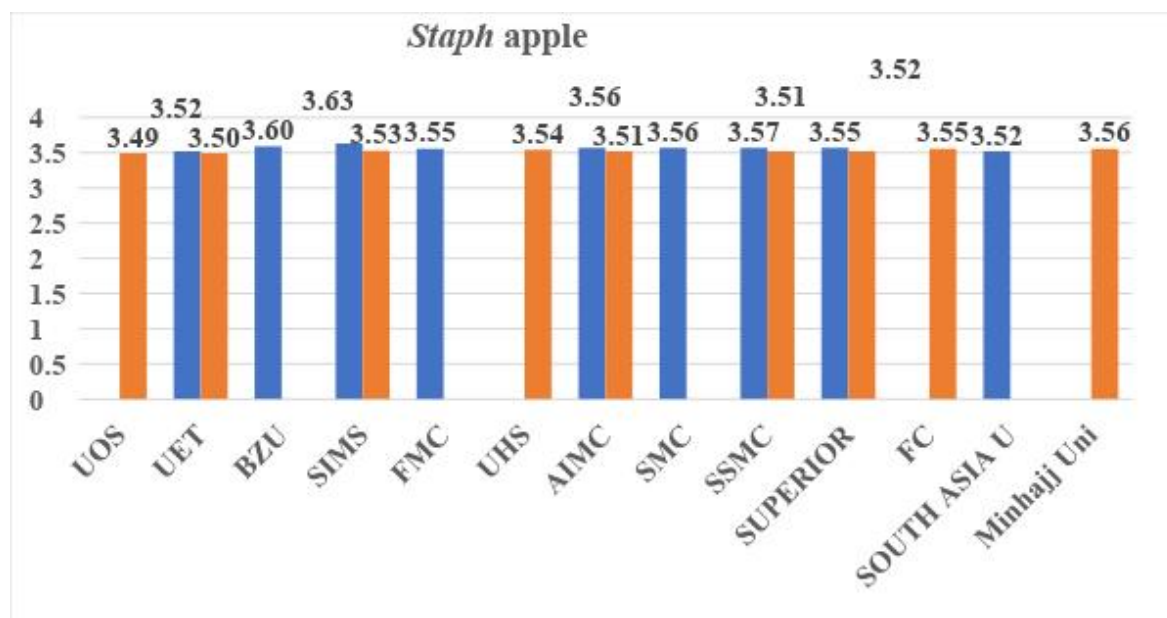


Figure 5 (b): Institute wise comparative analysis of total *Staphylococcus aureus* count of apple milk shake

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

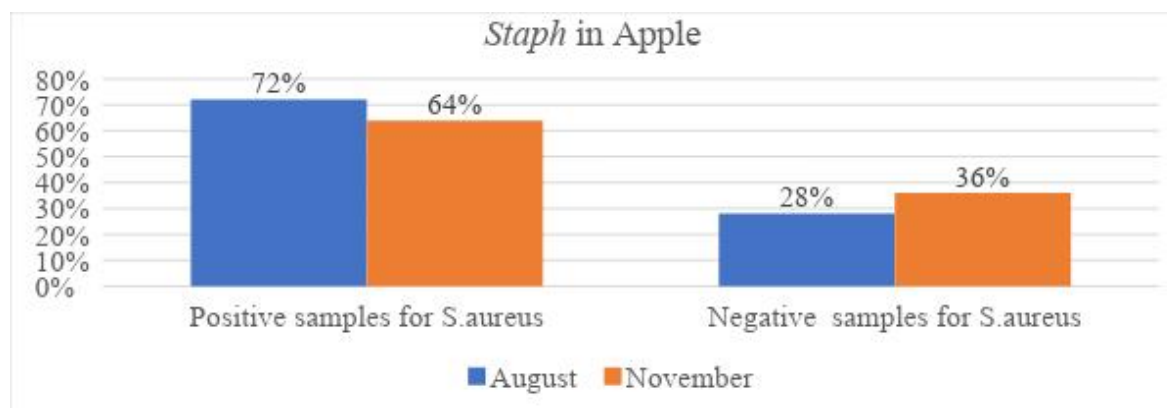


Figure 6: Comparative analysis of total *Staphylococcus aureus* count of apple milk shake

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 x10² and 1.1-3.3 x10² in November respectively. (Table 5) were shown mean of

169 banana milk shake all samples in month of August which was (2.39) and relatively (2.28) in
 170 November.

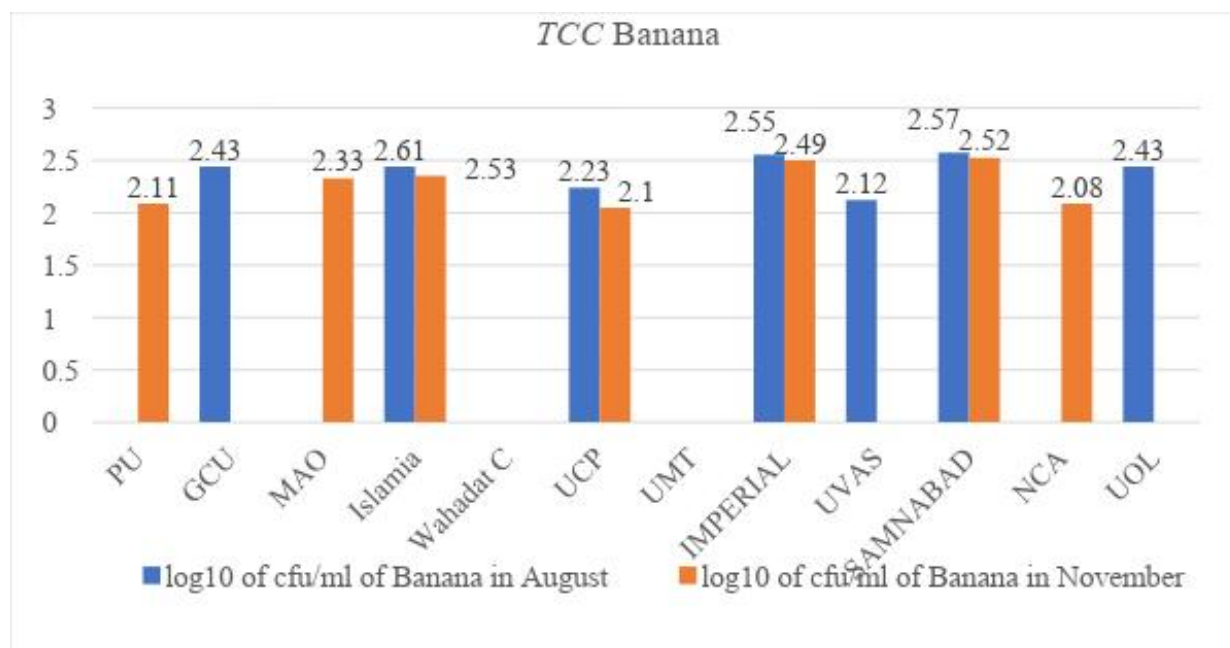
171 **Table 5: Comparative analysis of banana milk shake in log form**

Month	No. of samples	Positive	Negative	Mean log 10 of <i>Total coliform</i>
August	25	16	9	2.39
November	25	14	11	2.28

172

173 Figure 7 (a) (b) were shown all logs value for comparative analysis of banana shake in
 174 August and November which range was between 2.08-2.61.

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177 **Figure 7 (a): Institute wise comparative analysis of *Total coliform* count of banana milk**
 178 **shake**

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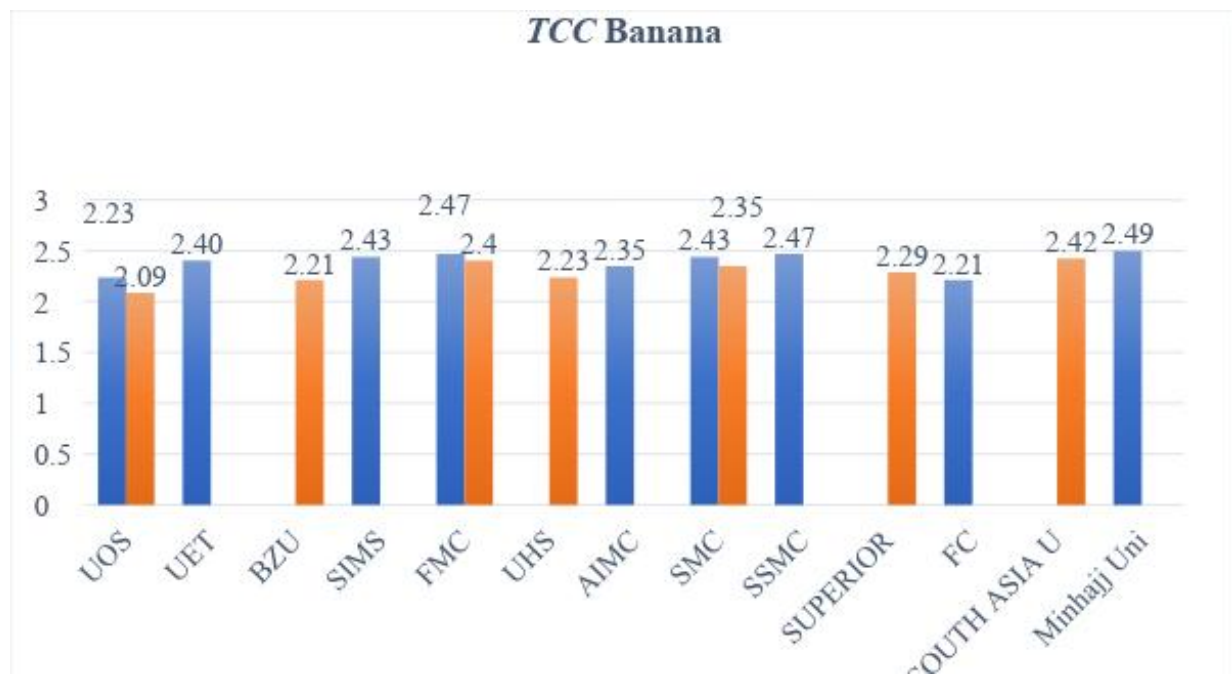


Figure 7 (b): Institute wise comparative analysis of *Total coliform* count of banana milk shake

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

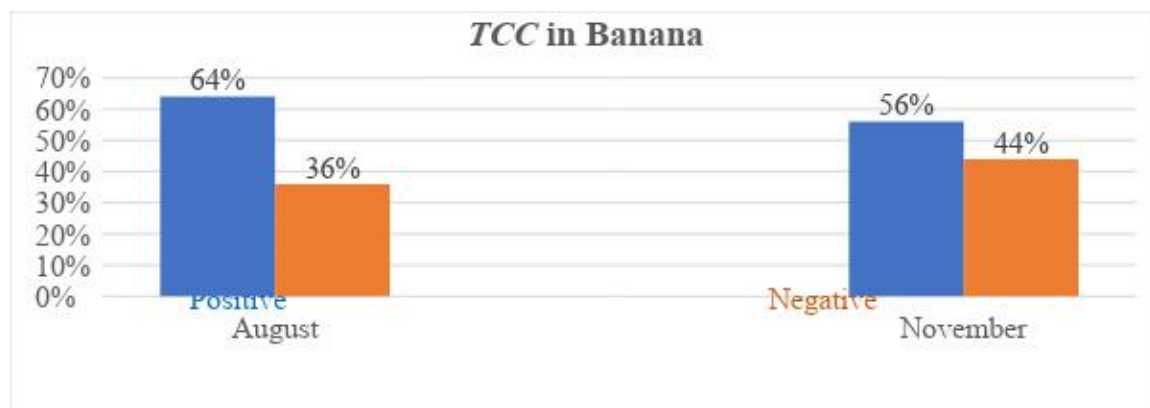


Figure 8: Comparative analysis of *Total coliform* count of banana milk shake
***Total coliform* count of apple milk shake:**

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. (Table 6) were shown mean of apple milk shake all samples in month of August which was (2.32) and relatively (2.22) in November.

Table 6: Comparative analysis of apple milk shake in log form

Month	No. of samples	Positive	Negative	Mean log 10 of <i>total coliform</i>
August	25	15	10	2.32
November	25	14	11	2.22

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

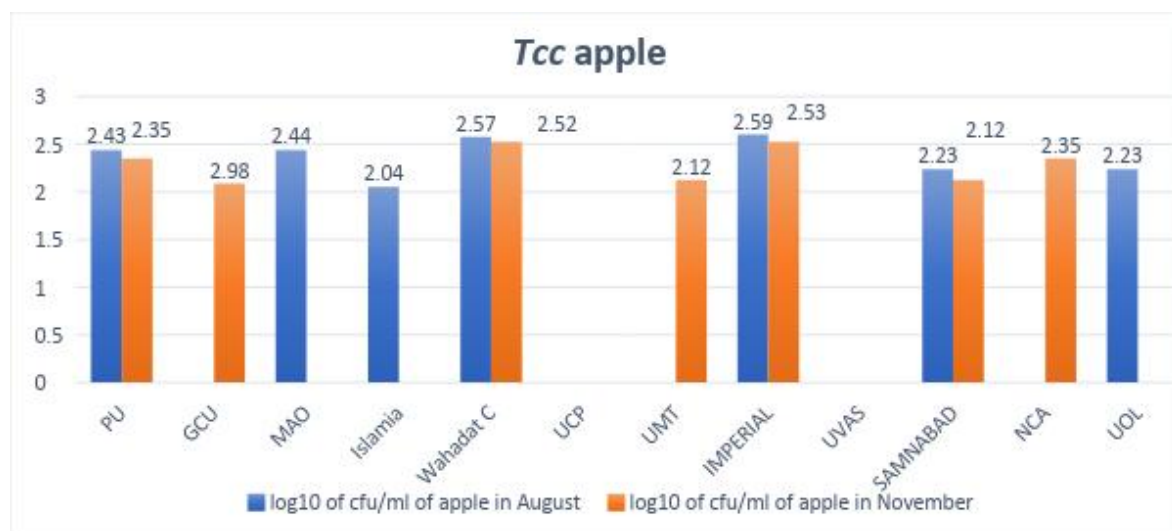


Figure 9(a): Institute wise comparative analysis of *Total coliform* count of apple milk shake

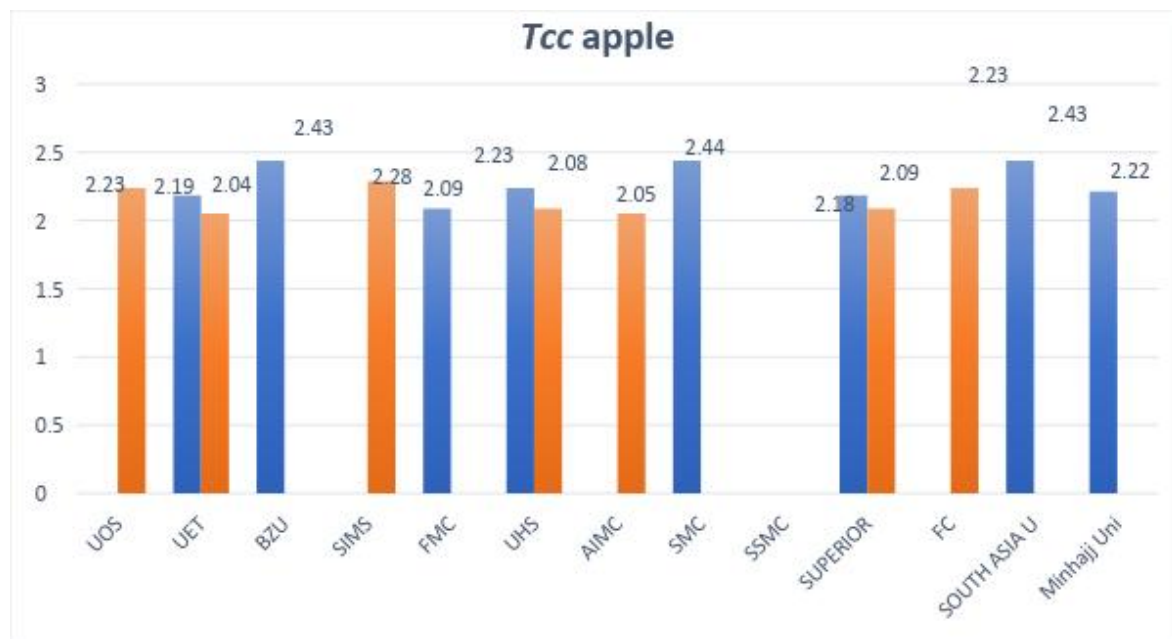


Figure 9(b): Institute wise comparative analysis of *Total coliform* count of apple milk shake

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.

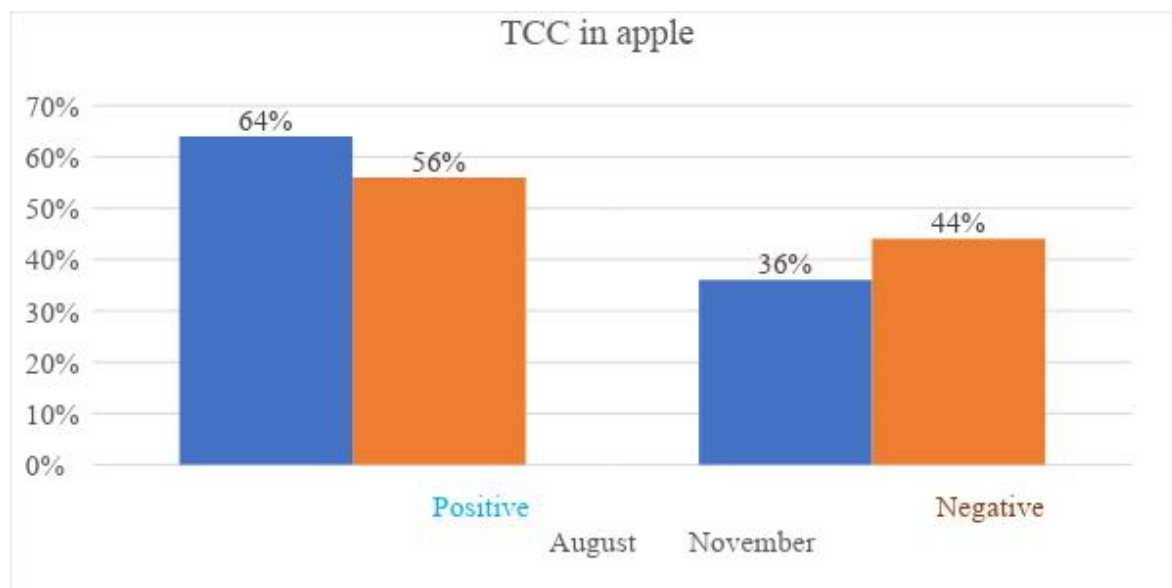


Figure 10: Comparative analysis of *Total coliform* count of apple milk shake

213 DISCUSSION:

214 Current study was aimed to evaluate pathogens in the milkshakes available in various
 215 public and private sector educational institutes. Our results showed that in the month of
 216 august banana Shake, showed highest TPC of 7.2×10^7 cfu/ml, whereas, average TPC was
 217 5.96×10^7 cfu/ml (Table 1). TPC value of log 10 was between 7.85-7.34 (figure 1). Whereas in
 218 the month of November banana Shake, showed highest TPC of 6.7×10^7 cfu/ml. average TPC
 219 was 4.25×10^7 cfu/ml (Table1). TPC value of log 10 was between 7.82-7.32 (figure 1).

220 In the month of August apple Shake, showed highest range of TPC is $7.5 \times$
 221 10^7 cfu/ml. whereas average TPC was 5.38×10^7 cfu/ml (Table 2). TPC value of log 10 was
 222 between 7.88-7.34 (figure 2). Whereas in the month of November Apple Shake, showed
 223 highest TPC of 6.5×10^7 cfu/ml, average TPC was 4.26×10^7 cfu/ml (Table 2). TPC value of
 224 log 10 was between 7.85-7.32 (figure 2)

225 According to our study, ^{calculation of} ~~was to calculate~~ total *Staphylococcus aureus* count of banana
 226 milkshake in the month of August ^Λ maximum value ~~was~~ ^Λ 3.7×10^3 cfu/ml and an average value
 227 of total *Staphylococcus aureus* count for the banana shake in the month of August was $3.52 \times$
 228 10^3 cfu/ml. Average log 10 of total *Staphylococcus aureus* count was 3.54 (Table 3). TSC
 229 value of log 10 was between 3.59-3.5 (figure 3). Total of 76% samples was positive for
 230 *Staphylococcus aureus* and 24% Negative (figure 4).

231 The Present study was to calculate total *Staphylococcus aureus* count of banana
 232 milkshake in the month of November, it was showed the maximum ranges of samples was
 233 3.9×10^3 cfu/ml and Mean of different samples of banana shake in the month of November
 234 was 3.13×10^3 cfu/ml. (Table 3) was showed that Mean of log 10 of all the samples was 3.49.
 235 TSC value of log 10 was between 3.59-3.34 (figure 3). (Figure 4) was showed that sample of
 236 total *Staphylococcus aureus* was positive 60% and 40% were Negative.

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. (Table 4) was showed that Mean of log 10 of all the samples was 3.55. TSC value of log 10 was between 3.63-3.43 (figure 5). (Figure 6) was 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. (Table 4) 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 (figure 5). (Figure 6) was showed that sample of total *Staphylococcus aureus* was positive 64% and 36% Negative.

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 (Table 5). TCC value of log 10 was between 2.61-2.21 (figure 7). 64% samples were positive and 36% samples were negative for the *Total coliform* count (figure 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 (Table 5). TCC value of log 10 was between 2.53-2.08 (figure 7). The *Total coliform* count was positive for 56% and Negative for 44% samples (figure 8).

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

260 samples was 2.32 (Table 6). *TCC* value of log 10 was between 2.59-2.04 (figure 9). In (figure
261 10) showed the *Total coliform* count was positive for 64% and Negative for 36%.

262 *Total coliform* count of Apple milkshake in the month of November showed the
263 highest ranges of 3.3×10^2 cfu/ml and also showed the mean of total samples as 1.7×10^2
264 cfu/ml. Mean of log 10 of all the samples was 2.22 (Table 6). *TCC* value of log 10 was
265 between 2.98-2.04 (figure 9). *Total coliform* count of 56% positive and 44% Negative
266 samples (figure 10).

267 Our study showed a much higher prevalence of contamination as compared to
268 Windratz and Arias (2000). Who documented that milkshake was contaminated with *E. coli*,
269 *Staphylococcus aureus* and *Salmonella*. The *E. coli* was a basic source of water, handler
270 hands, nose and clothes are the major source of contamination. Food poisoning occurred due
271 to *Staphylococcus aureus*, *salmonella* and *coliform*. The study was done in Costa Rica the
272 all samples of homemade milk shake were examined in this study we found the total fecal
273 coliforms, *E. coli*, and *Salmonella* was examined using pour plate culture method, in the
274 37.1% of samples of homemade milk shakes and 20% of commercial homemade milk shakes
275 did not meet international standards of *Total coliform* as designated of my research all of my samples
276 of banana and apples was free from *salmonella* but all the samples of banana and apples is to
277 improve the bacterial quality to meet the bacteria standard like TPC (*Staph* and *Total*
278 *coliform*).

279 According to Verma and Gaur (2017) the most probable number of samples (*Total*
280 *coliform*) the range of *Total coliform* s from 9.5 MPN/100ml to greater than 2400 MPN. It
281 was observed that all the juices were with *coliforms*.

282 Our findings showed a much higher prevalence of microbial load in banana and apple
283 as compared to Ahmed et al (2009). 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 (2013) findings were according to the set standards of ICMSF. Comparatively our findings had much higher prevalence. In another study by Tambekar et al. (2009) 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. (2015) 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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