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 **t**otal 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 $2.3x10^7$-$7.2x10^7$ cfu/ml and respectively in November range between 2.1 $x10^7$-6.7 $x10^7$cfu/ml. In august TPC range of apple shake between $2.2x10^7$-$7.5x10^7$ cfu/ml and respectively in November range between 2.08 $x10^7$-6.5 $x10^7$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

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

INTRODUCTION:

A milkshake is a delicious and non-carbonated refreshment produced using dairy, frozen yoghurt and flavor 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 (Petridou et al. 1997). Natural product juices contains 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 (Aghajanzadeh and Ziaiifar 2018).

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 (Jackson et al. 1991). Poor cleanliness practices have been connected with ingenious pathogenic organisms like *Staphylococcus aureus* (Djalma Chaves et al. 2018).

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

MATERIAL AND METHODS:

Study design:

This present research work was designed to calculate TPC (*Staphylococcus aurous, 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 University of Veterinary and Animal Sciences (UVAS), Lahore-Pakistan for further processing.

Sample collection:

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

Microbial Analysis:

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

Sample preparation:

Before culturing of samples, all the sample of milkshake were stored at 4 . After thawing, 1 ml sample was taken by using of sterile pipette and transferred to sterilized test tube which comprises normal saline (9 ml) for make 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 9^{th} test tube, discarded 1ml from the 10^{th} test tube for obtaining desired dilution.

Laboratory analysis:

From 6^{th} and 7^{th} dilution, 1 ml of diluted sample was taken and poured into two 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 aurous and Salmonella* count (CFU/ml). After the process of incubation, all bacteria colonies with distinction with yellow color for *Staphylococcus aurous* 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. of colonies x dilution factor) / 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 $x10^7$ 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. (Table 1) were shown 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.

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 x10^7
November	25	4.25 x10^7

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.

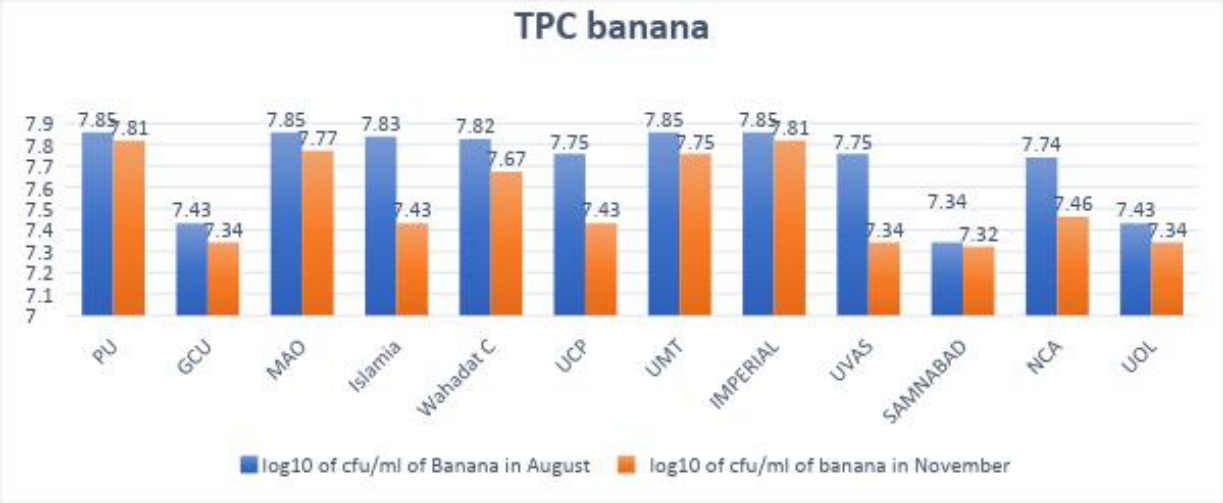

Figure 1(a): Institute wise comparative analysis of total plate count of banana milk shake

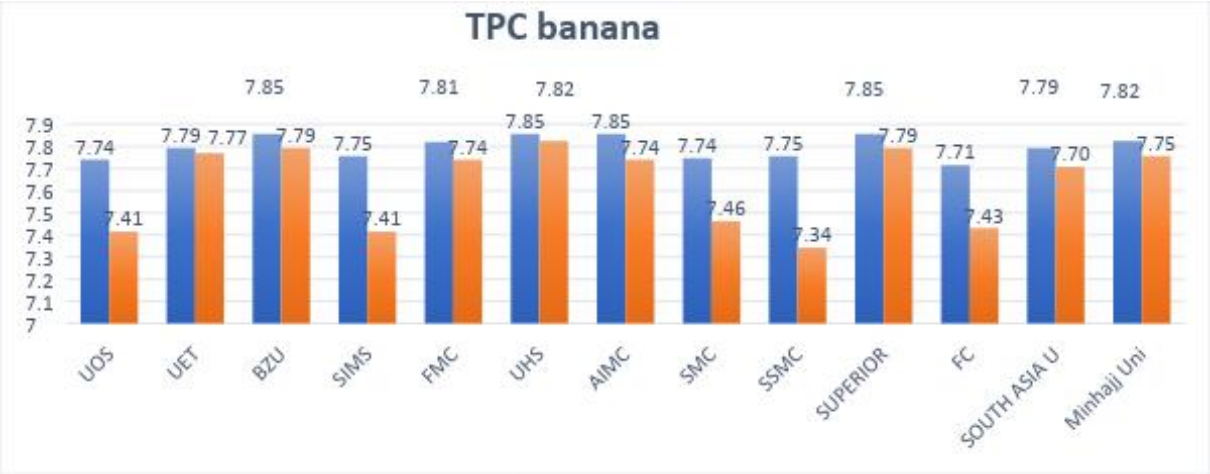

Figure 1(b): Institute wise comparative analysis of total plate count of banana milk shake

(Table 2) were shown mean of apple milk shake all samples in month of August which was (5.38 x10^7) and relatively (4.26 x10^7) 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 x10^7
November	25	4.26 x10^7

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.

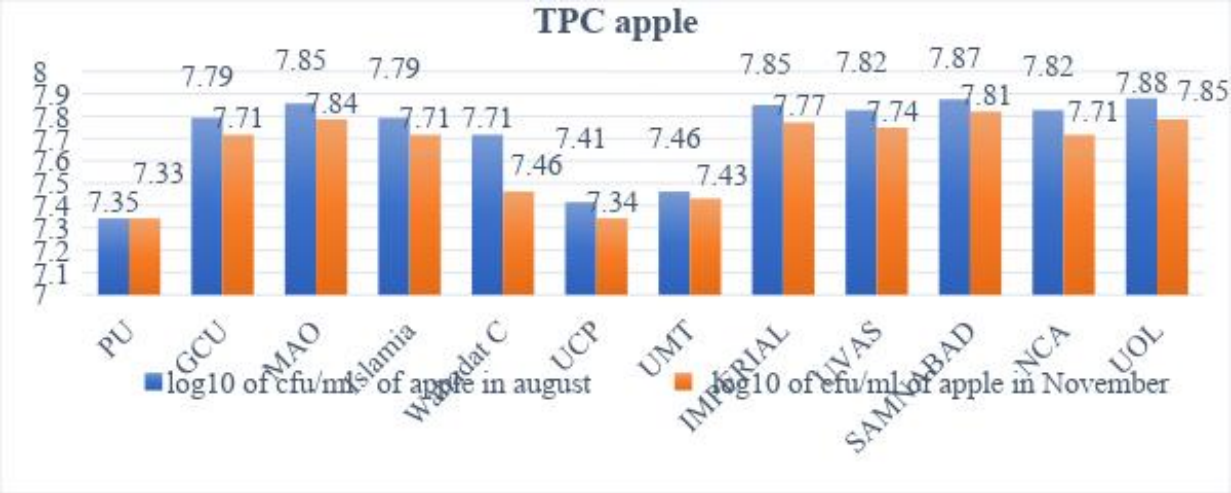

Figure 2 (a): Institute wise comparative analysis of total plate count of apple milk shake

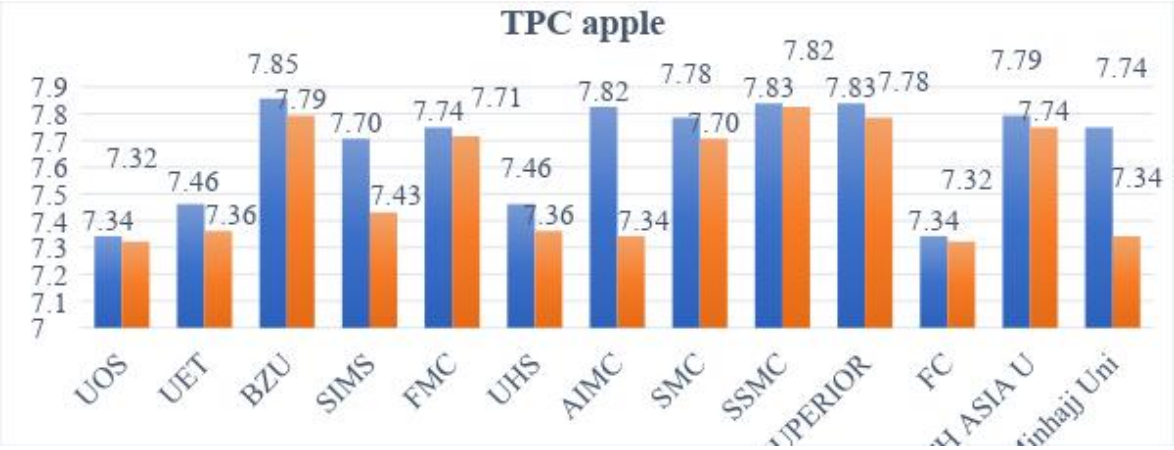

Figure 2 (b): Institute wise comparative analysis of total plate count of apple milk shake

Total *Staph 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 3.3-3.7x10^3 and 2.2 to 3.9 x10^3 in November respectively.

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

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

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

Figure 3 (a) (b) were shown all logs value for comparative analysis of banana shake in August and November which range was between 3.34-3.59.

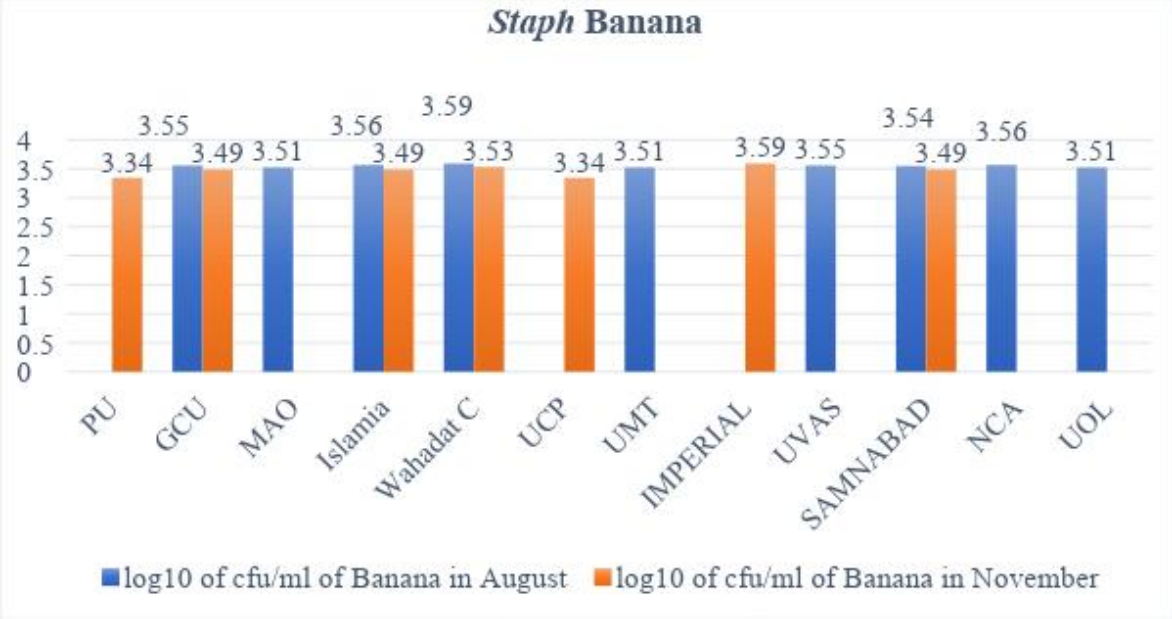

Figure 3 (a): Institute wise comparative analysis of total *Staphylococcus aureus* count of banana milk shake

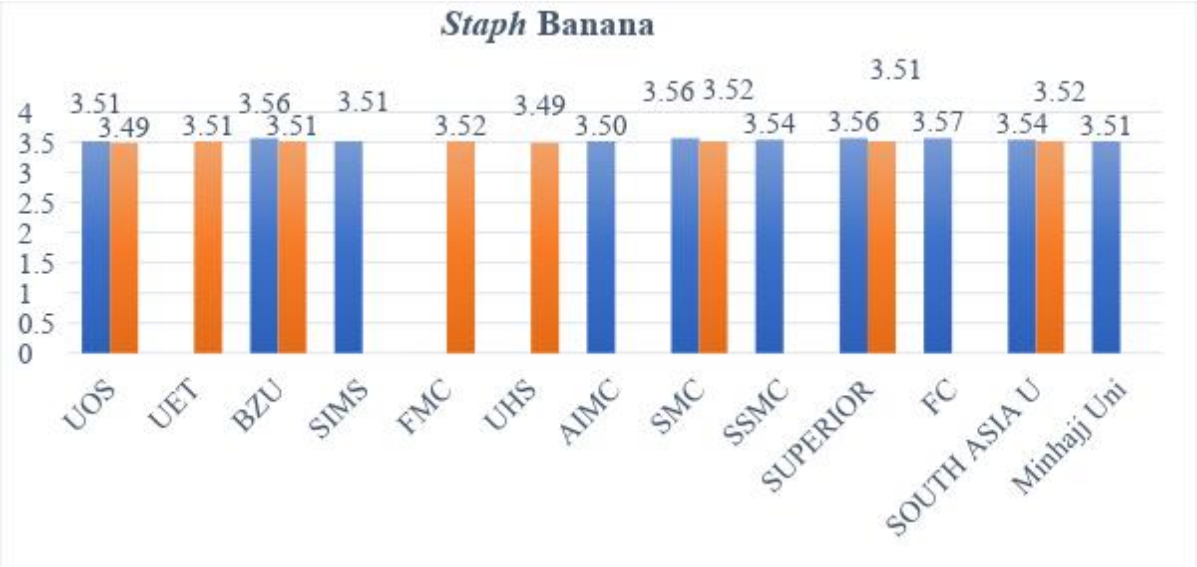

Figure 3 (b): Institute wise comparative analysis of total *Staphylococcus aureus* count of banana milk shake

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.

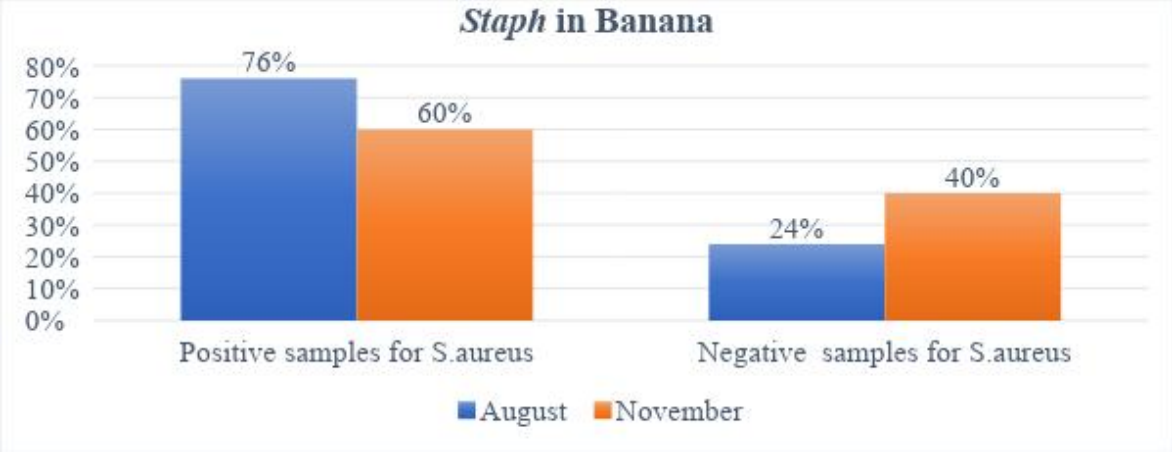

Figure 4: Comparative analysis of total *Staphylococcus aureus* count of banana milk shake

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 $x10^3$ and 3.1 to 3.7 $x10^3$ in November respectively.

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

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

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

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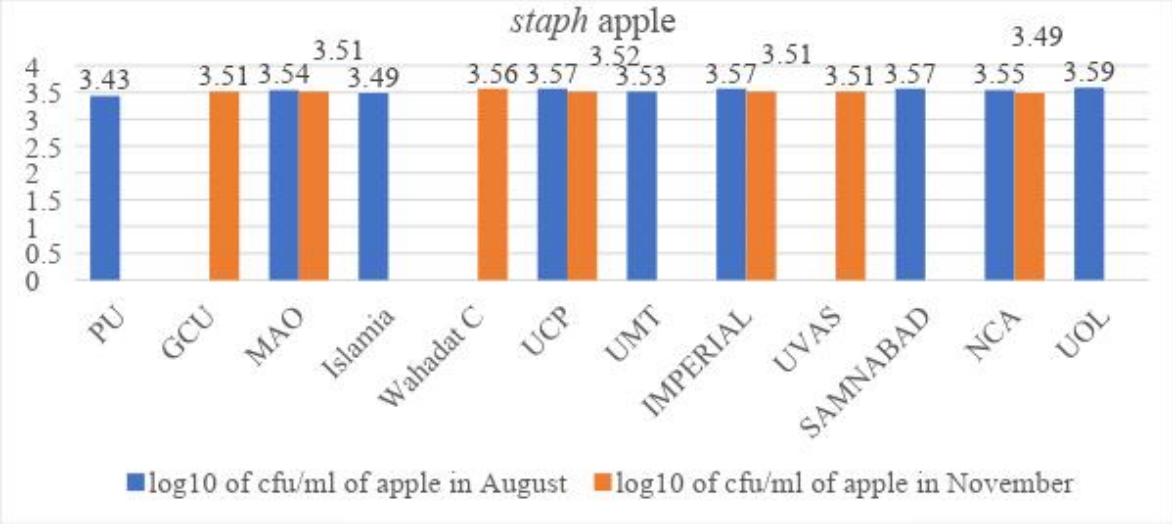
151 Figure 5 (a) (b) were shown all logs value for comparative analysis of apple shake in
152 August and November which range was between 3.43-3.63.



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

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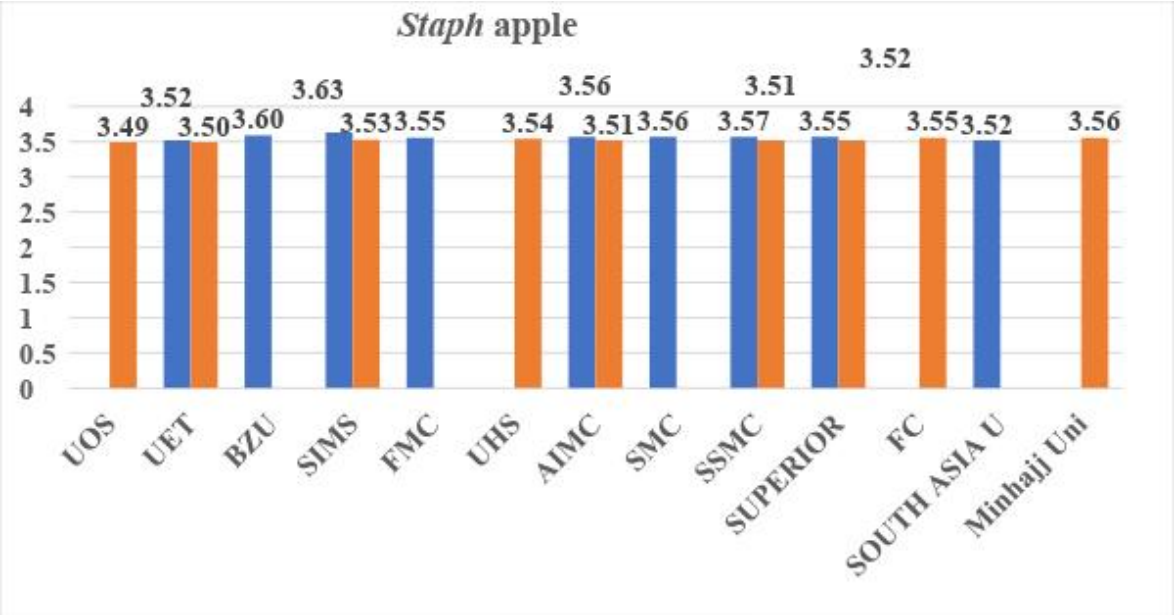

Figure 5 (b): Institute wise comparative analysis of total *Staphylococcus aureus* count of apple milk shake

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.

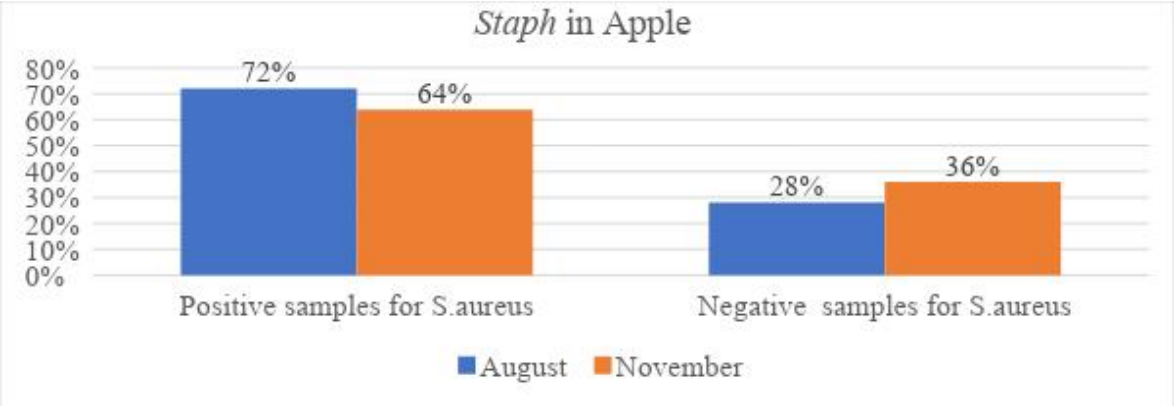

Figure 6: Comparative analysis of total *Staphylococcus aureus* count of apple milk shake

***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 $x10^2$and 1.1-3.3 $x10^2$ in November respectively. (Table 5) were shown mean of

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

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

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

Figure 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.

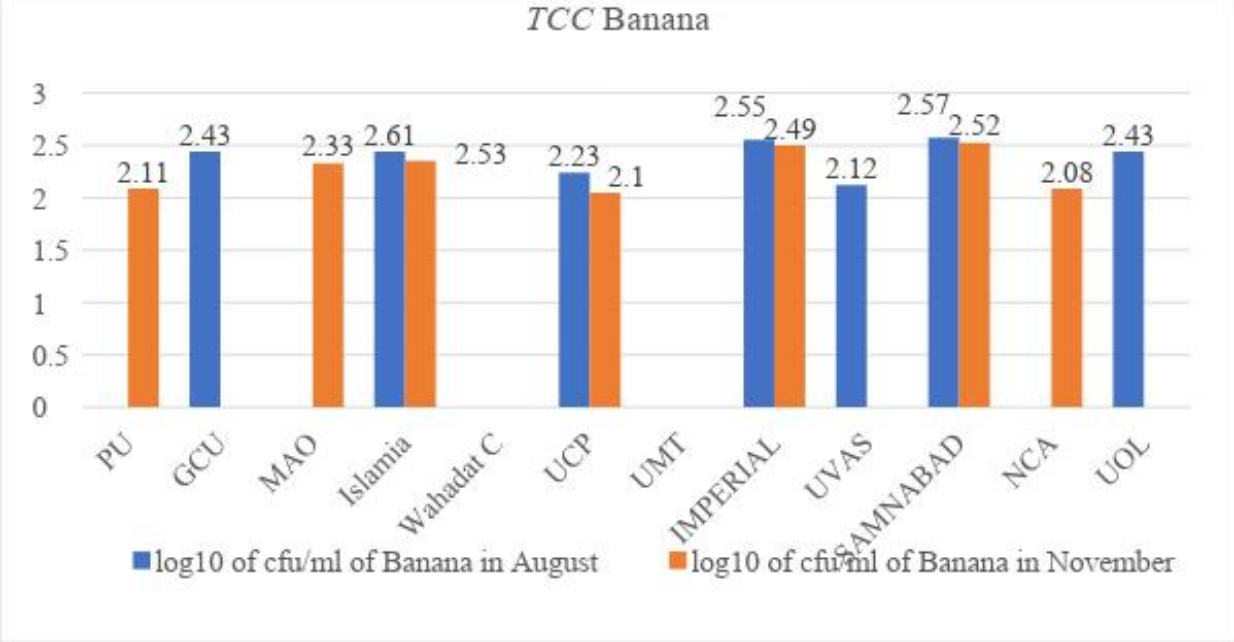

Figure 7 (a): Institute wise comparative analysis of *Total coliform* count of banana milk shake

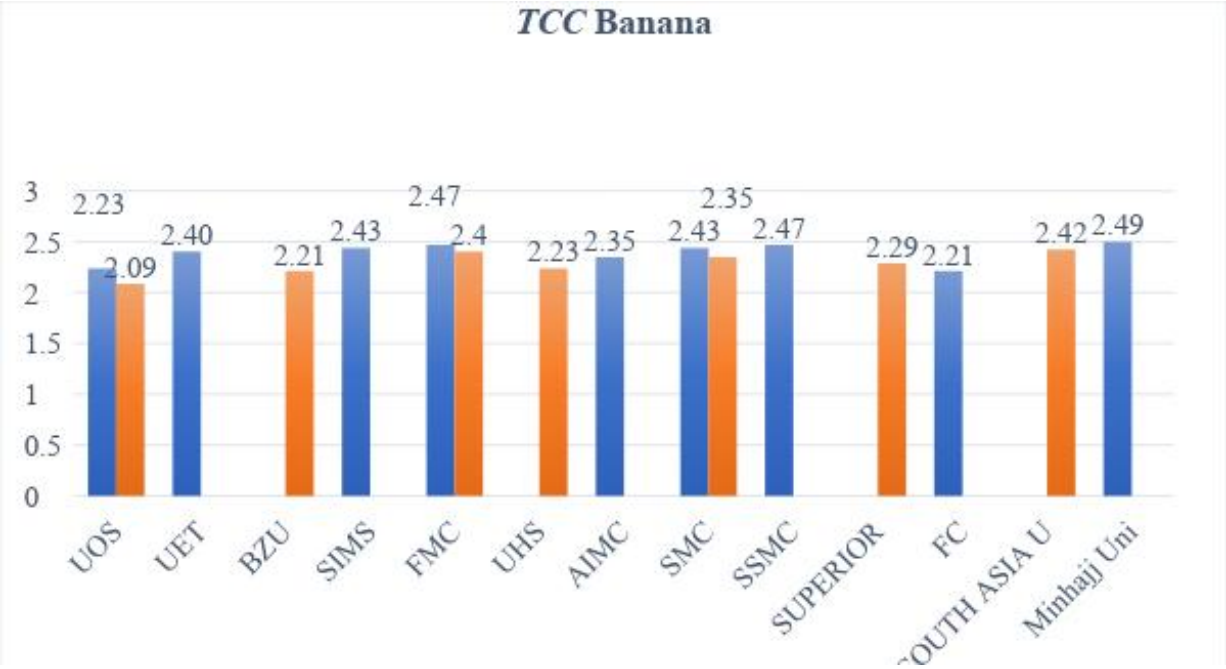

Figure 7 (b): Institute wise comparative analysis of *Total coliform* count of banana milk shake

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 x$10^2$and 1.1 to 3.3 x10^2 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 *total coliform*
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.

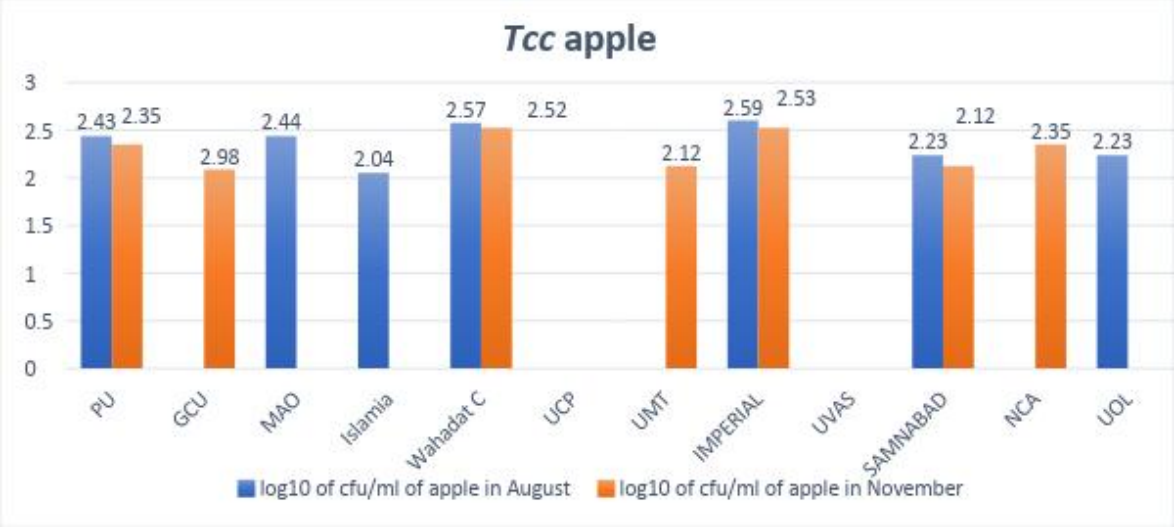

Figure 9(a): Institute wise comparative analysis of *Total coliform* count of apple milk shake

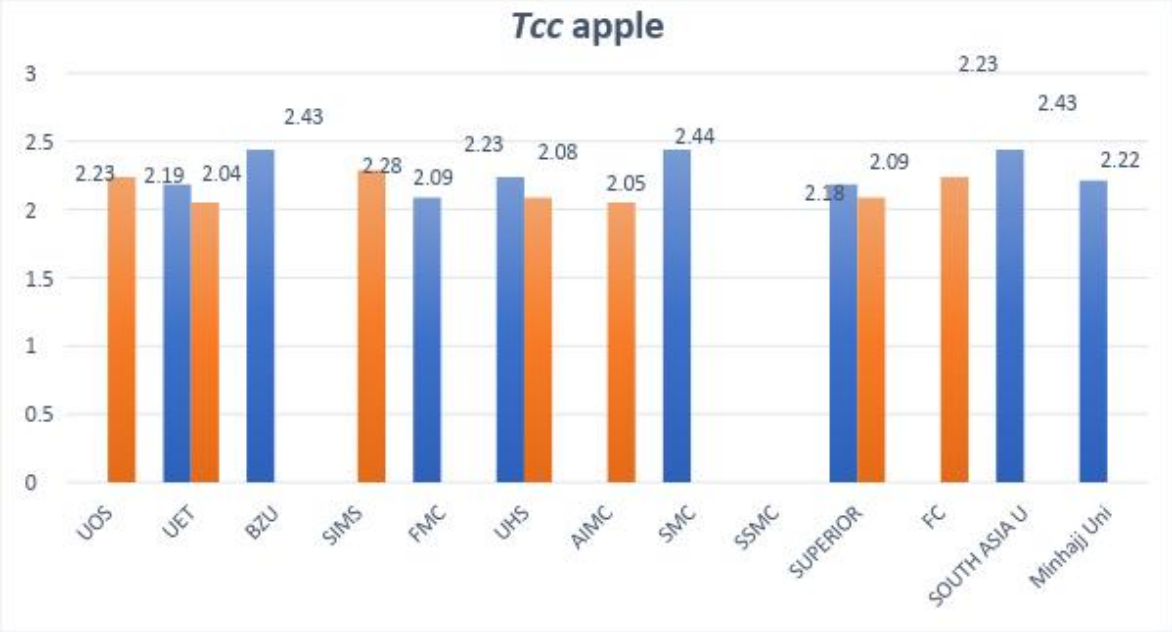

Figure 9(b): Institute wise comparative analysis of *Total coliform* count of apple milk shake

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.

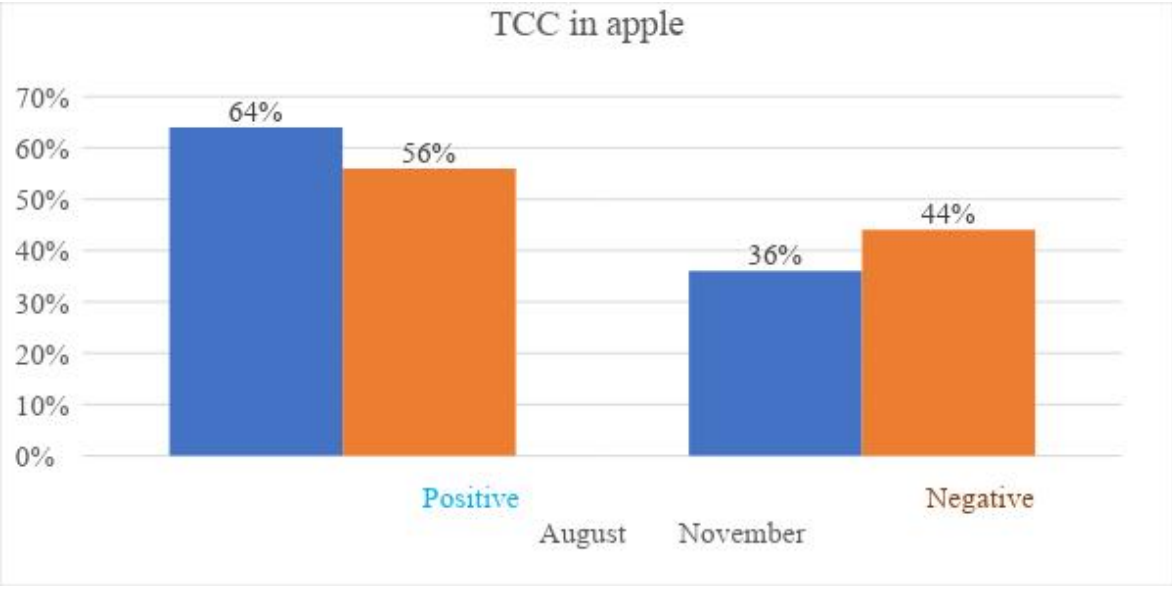

Figure 10: Comparative analysis of *Total coliform* count of apple milk shake

DISCUSSION:

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 10^7cfu/ml, whereas, average TPC was 5.96 x10^7cfu/ml (Table 1). TPC value of log 10 was between 7.85-7.34 (figure 1). Whereas in the month of November banana Shake, showed highest TPC of 6.7 x 10^7cfu/ml. average TPC was 4.25 x 10^7 cfu/ml (Table1). TPC value of log 10 was between 7.82-7.32 (figure 1).

In the month of August apple Shake, showed highest range of TPC is 7.5 x 10^7cfu/ml. whereas average TPC was 5.38 x10^7cfu/ml (Table 2). TPC value of log 10 was between 7.88-7.34 (figure 2). Whereas in the month of November Apple Shake, showed highest TPC of 6.5 x 10^7 cfu/ml, average TPC was 4.26 x 10^7 cfu/ml (Table 2). TPC value of log 10 was between 7.85-7.32 (figure 2)

According to our study was to calculate total *Staphylococcus aureus* count of banana milkshake in the month of August maximum value was 3.7x 10^3 cfu/ml and an average value of total *Staphylococcus aureus* count for the banana shake in the month of August was 3.52x 10^3 cfu/ml. Average log 10 of total *Staphylococcus aureus* count was 3.54 (Table 3). *TSC* value of log 10 was between 3.59-3.5 (figure 3). Total of 76% samples was positive for *Staphylococcus aureus* and 24% Negative (figure 4).

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 10^3 cfu/ml and Mean of different samples of banana shake in the month of November was 3.13 x 10^3 cfu/ml. (Table 3) 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 (figure 3). (Figure 4) was showed that sample of 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 4.3x 10^3 cfu/ml. The Mean of different samples of apple shake in the month of August was 3.57x 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 3.7x 10^3 cfu/ml. The Mean of different samples of apple shake in the month of November was 3.34x 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 3.7x 10^2cfu/ml and an average of 2.53x 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 3.3x 10^2 cfu/ml with highest Total *coliform* value. Average value was 2.1x 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 3.9x 10^2cfu/ml and mean of 2.2x 10^2 cfu/ml. Mean of log 10 of all the

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

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(Table 6). *TCC* value of log 10 was between 2.98-2.04 (figure 9). *Total coliform* count of 56% positive and 44% Negative samples (figure 10).

Our study showed a much higher prevalence of contamination as compared to Windratz and Arias (2000). 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 (2017) 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 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 × 108 and for Apple, it was 7.3 × 109.

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 X 10^6 cfu/ml and *Total coliform* was 1.4X 10^3 cfu/ml. Whereas banana had TPC of 2.2 X 10^6 cfu/ml and *Total coliform* were 3.2 X 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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