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A brief profiling of microbes of freshly squeezed pineapple juices in Kolkata city, India and reduction of microbial load following thermal and non-thermal methods

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Fruit juices are popular drinks as they contain various nutrients like vitamins, minerals, antioxidants which are very helpful for healthy life especially to combat several diseases. For being so nutritious they support a lot of microorganisms to grow in it. In this study, an effort has been made to know the microbial profile of the most demanding freshly squeezed Pineapple Juices collected from street vendors of different areas and branded pineapple juice was also monitored. All samples were found to be acidic in nature. A total of 9 juice samples (8 fresh and 1 branded/packaged) were examined for their microbiological analysis, after being collected in sterile containers. Total Viable Count (TVC) was high ranging from 2.45×10^5 - 28×10^5 CFU/ml, and hence not safe for human consumption. Coliform count ranged from 0.8×10^4 - 9.8×10^4 CFU/ml. *Alicyclobacillus* count varied from 0.20×10^4 - 9.6×10^4 CFU/ml with few being numerous in count. *Vibrio* (0.3×10^4- 5.2×10^4 CFU/ml), *Salmonella-Shigella* (0.1×10^4 – 12.5×10^5 CFU/ml), Yeast (0.10×10^4 -2.5×10^4 CFU/ml) with few being numerous in count, molds (1.1×10^4-6.8×10^4) with 1 being numerous were also detected. Sensitivity of the isolated colonies were checked for 8 antibiotics. One species of *Alicyclobacillus* and *Salmonella* were found to be resistant in Ampicillin which may pose health challenge whereas others being sensitive towards most or all antibiotics.The attempt was taken to reduce the microbial load in the juice samples using non thermal (UC-C radiation and Ultrasonication) as well as thermal methods (Low temperature long time and high temperature short time treatment). Both thermal and non thermal methods were effective to reduce the microbial load to a great extent. Though thermal methods were most effective but they may compromise the nutritional value and organoleptic properties of the fruit juice samples. So the non thermal methods are recommended for healthy processing of the fruit juice samples.

Key words: Fruit juice, coliform, total viable count, thermal treatment, non thermal treatment, UV-C, Ultrsonication, LTLT, HTST

INTRODUCTION

Consumption of fruit juice increased in recent years due to high nutritional value and low calorie (Mukhopadhyay and Biswas, 2018). Fruit juices are normally extracted from the pulp of the fresh fruits (Pilo *et al.* 2009). They are mostly available in packged condition, but the demand of freshly squeezed juices are also high, mainly in tropical country during summer months.

Though the actual components varies from fruits to fruits, in general they contain flavonoid glycosides, dietary fiber, calcium, vitamin C, carotenoids, lutein, lycopene, ²-carotene, phenolic acids, stilbenes, ellagic acid, amino acids, aroma compounds, anthocyanin, flavonols, polyphenols, potassium, vitamin D, low amount of sodium, cholesterol, fat etc (Ahmed *et al*. 2018). The high potassium and low sodium characteristic of most juices help in maintaining a healthy blood pressure. Vitamin C is naturally present in juices which are essential for the body to form collagen, cartilage, muscle, and blood vessels. It also helps in the absorption of iron (Aneja *et al*. 2014). But consumption of contaminated fruit juices may cause different types of diseases mostly affecting the gastro intestinal tracts (Mukhopadhyay and Biswas, 2018). Such juices have shown to be potential sources of bacterial pathogens notably

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E.coli, species of *Salmonella, Shigella*, and *S.aureus* (Baro *et al.* 2006). The juice borne other bacterial genera include *Acetobacter*, *Alicyclobacillus*, *Bacillus*, *Gluconobacter*, *Lactobacillus*, *Leuconostoc*, *Zymomonas*, and *Zymobacter*. Among yeasts *Pichia*, *Candida*, *Saccha-romyces*, and *Rhodotorula* are commonly encountered genera responsible for spoilage of juices (Bevilacqua *et al.* 2011). Though lower pH serves as the main barrier against different food borne pathogens, but some may survive in such low pH level (Aneja *et al.* 2014). The main source of microorganisms in fruit juice include the microbial population present on skin during harvest or post harvest treatments or during handling of fruits and also from contaminated utensils (Tournas *et al.* 2006).

MATERIALS AND METHODS

Collection of freshly prepared street vended pineapple juice samples

The samples were collected from 8 most populated sites of Kolkata in a sterile container from street venders. The sites are as follows: Gariahat, Kidderpore, Sealdah, Behala, Howrah, Rasbehari, Dhakuria and Esplanade.

Isolation and enumeration of microorganisms

From freshly collected fruit juice samples isolation of microbes was done by serial dilutions and spread plate method. Nutrient agar was used for total viable count. Thiosulfate Citrate Bile Salt sucrose agar (TCBS), Eosine Methylene Blue agar (EMB), Salmonella-Shigella agar (SS Agar), Yeast extract Potato Dextrose Agar (YPDA), Sabouraud agar, Yeast Extract Starch Glucose agar (YSG) were used to isolate various groups of micro-organisms. After inoculation, plates were incubated at 37°C for 48 hours. Enumeration was determined as number of colony forming unit (CFU/ml).

Antibiotic sensitivity test of selected bacteria

The antibiotic sensitivity test of selected bacteria was done by paper disc diffusion method using 8 different antibiotics.

Non-thermal and thermal treatments

To reduce the microbial content of collected freshly squeezed Pineapple juice samples, the samples were treated with 2 different types of non –thermal and thermal treatments. Ultrasonication and UV-C treatments were used as non -thermal treatment and LTLT and HTST were used as a thermal treatment.

For UV- C treatment the samples were taken on a sterile Petri plate and exposed at wavelength 260 nm to UV-C light for 30 minutes under continuous stirring with the help of magnetic stirrer. Then dilution of UV-C treated juices were prepared and plated on selected media, incubated at 37°C temperature for 24-48 hours. In case of ultrasonication the samples were sonicated for 5 minutes with 30second gaps. The intensity of ultrasonic wave was 70 kHz. After treatment dilutions of the sonicated samples were prepared and plated on selected media, incubated at 37° temperature for 48 hours.

For low temperature long time (LTLT) pasteurization treatment juices were treated at 60-65°C temperature for 30 minutes, dilutions were prepared and plated on 7 different media and incubated. For high temperature short time (HTST) pasteurization treatment juices were treated at 90-95°C temperature for 10-15 seconds, after treatment dilutions were prepared, plated and incubated.

RESULTS AND DISCUSSION

Freshly in such prepared Pineapple juice, one of the best choice of consumers, are collected from 8 different places of Kolkata and packaged pineapple juice of renowned brand is also selected for microbiological analysis. The pH of the juice samples are ranging from 3.0 to 4.0. Such pH values play a very important role in survival of different microorganisms in juice samples. Aneja *et al*. (2014) reported that pH less than 4.5 in fruit juices is the main barrier for most of the microorganisms and only acid tolerant microorganisms can grow in such pH condition. Such low pH condition is due to organic acid content of the fruit itself (Hariyadi, 2013)

Though its freshness, the fruit juice is contaminated by several ways like unhygienic water, unclean utensils and hands, preparation process and often swarming of flies on place of preparation promote the contamination of juices (Oranusi and Wesley, 2012; Asghar *et al.* 2018) . It is reported that fresh

fruits contain 1×10^5 CFU/ml on their surface and that may be reflected in the juice (Al-Jeddah and Robinson, 2002). The 9 samples collected from 8 different places of Kolkata showed Total Viable Count of organisms ranging from $2.45x10^5$ to $7x10^5$ CFU/mL (Table 1).

A study in Dar es Salaam city, Tanzania showed that the total plate counts (TPC) ranged between 2.32 and 8.54 (Log cfu/ml) and about 72.2% of tested juice samples had TPC above Codex recommended maximum levels (3.7–4.7 Log cfu/ml) (Simforian *et al*. 2015). In another study in Lahore City showed about 80% of fruit juice

Table 1: Microbial load (CFU/mL) in Freshly Squeezed Pineapple juice samples

COLLECTION SITE	TVC ($X 10^5$)	*Salmonella-Shigella* ($X 10^4$)	Coliforms ($X 10^4$)	*Alicyclobacillus* ($X10^4$)	*Vibrio* ($X10^4$)	*Yeast* ($X10^4$)	*Molds* ($X10^4$)
Gariahat	7	8.9	5.4	0.63	5.2	0.10	1.1
Kidderpore	4	12.5	0.8	6.5	0.4	Numerous	3.5
Sealdah	28	4.5	1.1	0.20	0.3	2.5	4.9
Behala	4.7	0.6	9.8	2.1	0.6	Numerous	Numerous
Howrah	4.3	0.1	5.2	9.6	2.9	1.2	1.4
Rashbihari	4.8	-	8	7.2	-	1.0	6
Dhakuria	2.89	1.6	8.5	Numerous	-	Numerous	2.5
Esplanade	2.45	7.5	7.8	Numerous	-	Numerous	6.8
Packaged Pineapple juice (Branded)	0	0	0	0	0	0	0

Table 2: Percent reduction of microbial load with non thermal treatment of freshly squeezed Pineapple juice

Non thermal treatment	Microorganisms	Control (CFU/mL)($\times10^4$)	After treatment (CFU/mL)($\times10^4$)	Percentage of reduction
UV C	TVC	35	7.7	78
	Vibrio	0.5	0	100
	Coliforms	3	0	100
	Salmonella- Shigella	64	0.1	99.8
	Alicyclobacillus	15.2	8.8	42.2
	Yeasts	25	7	72
	Moulds	15.9	2.6	83.7
Ultrasonication	TVC	3.9	2.04	47.7
	Vibrio	8.4	0.04	99.5
	Coliforms	9.2	1	89.1
	Salmonella- Shigella	7.7	1	87
	Alicyclobacillus	5.35	0.4	92.5
	Yeasts	3.2	1.5	53.1
	Moulds	4.15	0.14	96.7

samples showed higher value and 20% samples showed less total plate count of maximum bacterial load according to gulf standard (Asghar *et al.* 2018). In addition, higher value of TPC (Total Plate Count), might indicated the preparation of fresh juice under unhygienic conditions (Gulf Standards, 2000). In a study with fresh lime juice in Kolkata city Mukhopadhyay and Biswas (2018) reported that the number of total viable count ranges from 0.075×10^4 to 398×10^4 CFU/ ml may be due to the operational hygiene. In the present study another important result is the absence of any microbial contamination in a branded packaged Pineapple juice. Though in a study with 21 different types of packaged juice samples from either single or mixed fruit type showed a however huge microbial contamination in Nigeria City (Onuoha *et al.* 2018), which reflects safe production of packaged fruit juice in our country. Coliform count ranges from 0.8×10^4 to 9.8×10^4 CFU/ml. (Eva *et al.* 2017) also reported the presence of high coliform content in fruit juice samples established by MPN test in Dhaka, Bangladesh. *Alicyclobacillus* ranged from $0.20\text{x}10^4$ to $9.6\text{x}10^4$ CFU/mL , *Salmonella- Shigella* were ranged from $0.1\text{x}10^4$ to $8.9\text{x}10^4$ CFU/mL, *Vibrio* were ranged from $0.3\text{x}10^4$ to $5.2\text{x}\ 10^4$ CFU/mL,

Table 3: Percent reduction of microbial load with non thermal treatment of freshly squeezed Pineapple juice

Thermal treatment	Microorganisms	Control (CFU/mL)($\times10^4$)	After treatment (CFU/mL)($\times10^4$)	% of reduction
LTLT	TVC	2.4	0.06	97.5
	Vibrio	3.92	0	100
	Coliforms	7.6	0	100
	Salmonella- Shigella	2.2	0	100
	Alicyclobacillus	5.38	0.39	92.7
	Yeasts	2.6	0.17	93.5
	Moulds	2.1	0.36	82.9
HTST	TVC	2.4	0.99	58.8
	Vibrio	3.92	0.1	97.5
	Coliforms	7.6	2.0	73.7
	Salmonella- Shigella	2.2	0.4	81.8
	Alicyclobacillus	5.38	1.9	64.7
	Yeasts	2.6	0.8	69.3
	Moulds	2.1	0.56	73.4

Table 4: Comparative studies among various techniques used for reduction of microbial load in freshly squeezed Pineapple juice

Organisms	% Reduction			
	Non- thermal treatment	Ultrasonication	Thermal treatment	
	UV C		LTLT	HTST
TVC	78	47.7	97.5	58.8
Vibrio	100	99.5	100	97.5
Coliforms	100	89.1	100	73.7
Salmonella- Shigella	99.8	87	100	81.8
Alicyclobacillus	42.2	92.5	92.7	64.7
Yeasts	72	53.1	93.5	69.3
Moulds	83.7	96.7	82.9	73.4

Yeast were ranged from $0.10x10^4$ to $2.5x10^4$ CFU/mL, Molds were ranged from $1.1x10^4$ to $6.8x10^4$ CFU/mL. Low pH of the juice samples supports the fungal growth. A total of 34 bacterial, 12 yeast, and 25 mould isolates were isolated from juices classified by grouping them into 9 bacterial species, 5 yeast species, and 11 mould species on the basis of phenotypic characteristics in freshly prepared Citrus and Carrot juice (Aneja *et al*. 2014). Previously Molds were reported to be rare in freshly squeezed Fresh lime juice though yeasts were reported in 100% of the samples (Mukhopadhyay and Biswas, 2018).

The antibiotic sensitivity test for bacteria with 8 different antibiotics revealed that only *Alicyclobacillus* and *Salmonella-Shigella* showed resistance to Ampicillin but rest of the organisms showed sensitivty towards all the antibiotics (Table 2).

In a separate study some MDR strains were reported from fruit juice samples, posing a real health threat (Mukhopadhyay and Biswas, 2018). Some MDR strains isolated from orange juice samples also identified as a health hazard (Jain and Yadav 2015).

To reduce the load of microorganisms by non thermal methods 2 different techniques were adopted like UV C treatment and ultrasonication using most contaminated fruit juice samples. 100% reduction in *Vibrio* and coliform population was established (Table 3), other bacterial and fungal population also showed a marked reduction in number by the UV C treatment. 78% reduction in TVC was achived with UV C treatment where as nearly 47.7% reduction found with sonication. To reduce *Vibrio* and coliforms UV-C treatment was found to be better (100% reduction) than ultrasonication99.5% and 89.1% reduction respectively. Among the fungal population moulds were found to be more sensitive to ultrasonication. *Salmonella* and *Shigella* population also reduced to a higher extent with UV-C treatment. Torkamani and Niakousari (2011) proved that bacteria cells were more sensitive to UV-C treatment than fungi by conducting an experiment on orange juice. Jayawardena *et al*. (2019) ultrasonication as one of the non-thermal pasteurization techniques used for liquid food processing, especially in juices, purees and smoothies. The researches proved that Ultrasonication improves the flavors, stabilizes and preserves the quality of juices/purees as well (Zoran *et al*. 2016).

Thermal treatment was also applied to reduce the microbial load of the fresh pineapple juice. Both Low temperature long time (LTLT) and high temperature short time (HTST) were found to be effective to reduce the microbial count (Table 3).

LTLT was very much effective against *Vibrio*, coliforms and *Salmonella Shigella* with 100% reduction. About 97.5% reduction in TVC was also evident with LTLT. In other bacterial and fungal genera also LTLT was superior over HTST. Such heat sensitivity of *Vibrio, Salmonella, Shigella* and coliforms were also represented in another study with Sweet lime juice sample from Kolkata city (Mukhopadhyay and Biswas, 2018).

From the above discussion we can conclude that UVC treatment is better than Ultra sonication treatment and LTLT is better than HTST to reduce microbial load in freshly squeezed Pineapple juice (Table 4). Thermal treatment may reduce Microbial load and enzymes and extend the shelf life of food but exert negative effects on nutritional and organoleptic properties (Roobab *et al.* 2018). Non thermal methods either single or in combination are suitable alternatives for achieving the same purpose without the adverse effect of heat on the quality of juices and meet the consumer demand. The reduction in load may be optimized by changing the exposure time or change in wavelength of UV light or intensity of ultrasound or combining the two methods and may be implied for hygienic manufacture of fruit juices.

CONCLUSION

Fruit juice samples are in high demand for its nutritional values, though they support a high population of microorganisms. Thermal treatment and nonthermal treatments of the juice samples are found to be effective to reduce the microbial load in the juice samples. As thermal treatment may compromise the nutritional and organoleptic properties, nonthermal treatments may be recommended for healthy production of fruit juice samples and some measures may be adopted by the street vendors.

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