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EFFICIENCY OF THERMAL AND NON-THERMAL METHODS IN REDUCING THE MICROBIAL LOAD OF STREET VENDED FRESHLY SQUEEZED SWEET LIME JUICES

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Abstract – Consumption of fresh fruit juices increased dramatically in recent days. They contain essential nutrients which support the growth of microbes. This study was conducted to assess microbial quality and establish the risk factors for contamination of raw Sweet Lime Juices vended in Kolkata City, West Bengal, India. Determination of Most Probable Number (MPN) to count the coliforms in the juice samples determines whether it was safe for human consumption or not. A total of 10 juice samples showed Total viable count (TVC) from 0.075×10^4 - 398×10^4 CFU/mL. Coliform (1.82×10^4 - 40×10^4 CFU/mL) *Alicyclobacillus* (15.1×10^4 - 65.2×10^4 CFU/mL), *Vibrio* (0.1×10^4- 49×10^4 CFU/mL), *Salmonella – Shigella* (2.3×10^4 - 60×10^4 CFU/mL), yeast (9.5×10^4 - 76×10^4 CFU/mL) were detected. High Temperature and Short time (HTST), Low Temperature Long Time (LTLT) as thermal treatment and sonication, UV-C as non-thermal treatment showed great reduction in the microbial count of the fruit juices and render them for human consumption.

INTRODUCTION

People are now more conscious about their health. They prefer fruit juices more than drinks like cola, pepsi etc. because of their high nutritional value and low consumption of calorie. There is great demand of street vended fruit juices particularly in tropical countries because of the easy availability, low price and for their high nutritional value. But consumption of contaminated fruit juice may cause food borne diseases. Food borne diseases are harmful illness mainly affecting the gastrointestinal tract and are transmitted through consumption of contaminated juices. Such juices have shown to be potential sources of bacterial pathogens notably *E.coli*, species of *Salmonella*, *Shigella*, and *S.aureus* (Buchmann *et al*., 1999; Sandeep *et al*., 2001; Barro *et al*., 2006). As they contain many nutrients, vitamins, antioxidants and phytochemicals they support growth of many micro-organisms. Street vended juices normally get contaminated during their preparation due to use of contaminated water, unclear utensils, contaminated ice for dressing, preservation without refrigeration and any other kind of preservatives.

In Kolkata (West Bengal, India) there is always a great demand for fresh fruit juices as the climate remains hot and humid for most part of the year and street vendors serve a great portion of this demand, but their hygiene practices and microbial quality are questionable (Mukhopadhyay *et al*., 2011). Particularly in Kolkata most of the street vended shops are beside the waste disposal system and roads. Such unhygienic conditions contribute to the contamination. Unclear utensils, contaminated water and ice, Swarming sand flies, fruit flies present in such open environment may also contaminate juices.

A rapid review of the street vended fruit juices was undertaken during summer due to high demand of fruit juices (June–August), at that time of year due to very hot climate. An attempt was taken to lower down the microbial load by using thermal and non thermal techniques. As thermal treatment LTLT and HTST were performed. Both of these techniques are forms of pasteurization process. In case of low temperature long time (LTLT) fruit juice has been traditionally pasteurized by batch heating

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at 63-65°C for relatively longtime (D'Amico *et al.* 2006). As non-thermal technique UV-C radiation at 260 nm and sonication (70 Hz.) were used. Non-thermal techniques are more useful because they do not change the organoleptic properties during the treatment than thermal techniques. That is why the non-thermal treatments are more preferable than the thermal ones.

MATERIALS AND METHODS

Collection of Freshly Prepared Street Vended Sweet Lime Juice Samples

The samples were collected from 10 different places of Kolkata in a sterile container from street venders. The sites are as follows: National medical college (NMC), College Street, Park circus, Cossipore, Sealdah, Chiriamore, Shyambazar, Howrah, Esplanade and Wellington square.

MPN Test

MPN test of the collected samples were performed by standard microbiological methods.

Isolation and Enumeration of Microorganisms From Samples

Isolation of microbes was done by serial dilutions, 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 sweet lime juice samples, the samples were treated with 2 different types of non -thermal and thermal treatments. Sonication 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 48 hours. In case of sonication the samples were sonicated for 5 minutes with 30 second 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°C temperature for 48 hours.

For low temperature long time pasteurization treatment (LTLT) 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 pasteurization treatment (HTST) juices were treated at 90-95°C temperature for 10-15 seconds, after treatment dilutions were prepared, plated and incubated.

RESULTS AND DISCUSSIONS

Freshly prepared sweet lime juice samples were collected from 10 different places in Kolkata in a sterile container and checked for their pH values. Such p^H of the juice samples play an important role in the survival of the micro-organisms.

MPN test of the collected juice samples showed the presence of Coliform ranging from 210-2400 CFU / 100 mL and therefore not potable for human consumption.

In spite of freshness, the freshly squeezed sweet lime juice may contain potential pathogens. Al-Jedah and Robinson (2002) had reported that a number of factors may be responsible for contamination of fruit juices. Prior to processing, most fruit contains bacterial counts of 1×10^5 CFU/mL on their surface. These microorganisms may be reflected in the final fruit juice products. To determine the microbial status of the collected street vended sweet lime juice samples the serial dilution and spread plate method was performed. From the result (Table 1) it was observed that Total viable count (TVC) was high ranging from 0.075×10^4 - 398×10^4 CFU/mL. Coliform count ranges from 1.82×10^4 - 40×10^4 CFU/mL. The huge contamination of *E.coli* was also recorded by Subbannayya *et al*., (2007) in street vended juices and indicating possible risk of infection involved with drinking of such juices. *Alicyclobacillus* count varied between 15.1×10^4 - 65.2×10^4 CFU/mL. *Vibrio* (0.1×10^4- 49×10^4 CFU/mL), *Salmonella-Shigella* (2.3×10^4 - 60×10^4 CFU/mL), Yeast

(9.5×10^4 - 76×10^4 CFU/mL) were also detected. The presence of *Candida* sp. (in 58% of juice samples), *Rhodotorula*, *Pichia*, *Candida*, and *Saccharomyces* (Ghenghesh *et al*., 2005) were also reported in Tripoli-Libya. Only one sample showed the presence of Moulds (0.1×10^4 CFU/mL). In another study some species of *Rhizopus* and *Penicillium* have been reported from fruits and vegetable juices and produce potent mycotoxins responsible for various mycotoxicosis in humans (Efiuvuwevwere, 2000).

The antibiotic sensitivity test for bacteria with 8 different antibiotics reveal that Coliforms were highly sensitive towards Chloramphenicol and Ciprofloxacin and resistant to Ampicilin. The *Vibrio* and *Alicyclobacillus* strains were resistant to most of the tested antibiotics and therefore highly pathogenic. Such multidrug resistant strains pose a real threat to the mankind.

Thermal treatment was performed to reduce the microbial load and to make them safe for human consumption. Using HTST and LTLT maximum 100% reduction occur in *Vibrio*, Coliform and *Salmonella – Shigella* count in 4 most contaminated samples. Maximum 97.88% reduction occurs in Yeast count applying HTST and maximum 97.45% reduced using LTLT. TVC was reduced to 98.74% by HTST and 90.54 % by LTLT. After treating the juice with HTST and LTLT the % reduction of *Alicyclobacillus* count was respectively 99.50% and 97.55% (Table 2). In a similar study apple juice were treated by HTST at 77 to 88°C for 25 to 30 seconds and reduction in microbial count was observed (Moyer & Aitken., 1980).

Similarly for non- thermal treatment 4 most contaminated juice samples were taken, sonication and UV-C treatment were applied to reduce the microbial load (Table 3). The application of high power ultrasound in the food industry has been widely investigated. By applying sonication and maximum 100% reduction occur in *Vibrio* count. In case of *Salmonella–Shigella* using sonication and UV-C maximum 60.41% and 100% was reduced. Coliforms are also reduced to maximum 94.92% by UV-C and 56.70% by sonication. After UV-C treatment yeast count was reduced to 76.87% and in case of sonication 69.73% was observed. Maximum

Table 1. Microbial load (CFU/ml) of collected freshly squeezed sweet lime juice sample

Collection Site	TVC	*Salmonella Shigella*	*Alicyclobacillus*	Coliforms	Yeast	*Vibrio*	Molds
NMC	0.075×10^4	8.2×10^4	59×10^4	5.5×10^4	38×10^4	26.6×10^4	-
College street	100×10^4	3.9×10^4	52×10^4	4.8×10^4	12×10^4	13.5×10^4	-
Park circus	4.4×10^4	5.8×10^4	32×10^4	19.6×10^4	36×10^4	39.6×10^4	-
Cossipore	398×10^4	39.7×10^4	65.2×10^4	17×10^4	76×10^4	0.6×10^4	-
Sealdah	238×10^4	48×10^4	Numerous	Numerous	Numerous	49×10^4	-
Chiriamore	4×10^4	3×10^4	15.1×10^4	2.2×10^4	11×10^4	6.5×10^4	-
Shyambazar	Numerous	Numerous	56.4×10^4	Numerous	Numerous	40×10^4	-
Howrah	90×10^4	2.3×10^4	28×10^4	3×10^4	9.5×10^4	0.7×10^4	0.1×10^4
Esplanade	24×10^4	60×10^4	38.8×10^4	40×10^4	36×10^4	$O.1\times10^4$	-
Wellington	29.8×10^4	23.8×10^4	Numerous	1.82×10^4	34.5×10^4	1.6×10^4	-

Table 2. % Reduction of microbial load with Thermal Treatment of collected freshly squeezed fruit juice samples

Thermal Treatments	Sample Numbers	% Reduction					
		TVC	*E.coli*	*Alicyclobacillus*	*Salmonella Shigella*	*Vibrio*	Yeast
LTLT	1	60.30%	100%	63.75%	100%	100%	93.15%
	2	90.54%	100%	70.45%	100%	100%	97.5%
	3	9.90%	100%	67.81%	100%	100%	55.84%
	4	27.27%	100%	97.55%	100%	100%	97.45%
HTST	1	98.74%	100%	99.50%	100%	100%	43.15%
	2	91.89%	92.85%	63.63%	46.66%	100%	80.35%
	3	86.03%	100%	90.80%	100%	100%	90.64%
	4	77.65%	100%	73.07%	100%	100%	97.88%

Area from where samples are collected : 1-Sealdah, 2 – Cossipore, 3 – Shyambazar, 4 – Park Circus .

Table 3. % Reduction of microbial load with Non-Thermal Treatment of collected freshly squeezed sweet lime juice samples

Non-Thermal Treatments	Sample Numbers	% Reduction					
		TVC	*E.coli*	*Alicyclobacillus*	*Salmonella Shigella*	*Vibrio*	Yeast
UV	1	11.20%	77.41%	5.76 %	63.59%	99.77%	13.15 %
	2	67.64%	69.91%	10.50%	81.81%	79.68%	67.64%
	3	7.65 %	60 %	60.81 %	32.46%	83.33%	32.46%
	4	10.11%	94.92%	23.31%	100%	100%	74.36%
Sonication	1	22.19%	56.70%	3.36 %	9.70 %	5.28%	6.57%
	2	1.68%	33.33%	49.34%	27.27%	29.68%	6.86%
	3	1.02 %	48.23%	68.24 %	24.67 %	9.09 %	69.37%
	4	7.86%	55.07%	27.60%	60.41%	100 %	59.32%

Area from where samples are collected :1-Sealdah, 2 – Cossipore, 3 – Shyambazar, 4 – Park Circus

Table 4. Comparative studies among various techniques used for reduction of microbial load of collected freshly squeezed sweet lime juice samples

Organisms	*% Reduction*			
	Thermal Treatment		Non-Thermal Treatment	
	LTLT	HTST	Sonication	UV
TVC	47%	88.57%	8.18%	9.86%
Coliforms	100%	98.21%	48.33%	75.56%
Alicyclobacillus	74.89%	81.75%	37.13%	32.18%
Yeast	85.98%	78%	35.53%	58%%
Vibrio	100%	100%	36.01%	90.69%
Salmonella-Shigella	100%	86.66%	30.51%	69.46%

60.81 % and 68.24% reduction of *Alicyclobacillus* was observed for UV-C and sonication treatment respectively. TVC count was also reduced and maximum % of reduction was found to be 11.20% for UV-C and 22.19% for sonication. Short wave UV was also recommended for the inactivation of microorganisms in the food industry by Bintsis *et al.* (2000). In a study Ngadi *et al.* (2003) reported the remarkable inactivation of *E. coli* O157:H7 with UV light in fruit juice. In comparison to heat pasteurization, juices treated with UV-C did not change in taste and color profiles.

It is clear that the HTST is better than LTLT and UV-C treatment is better than sonication (Table 4). Thermal treatments may reduce the quality of juices therefore non thermal treatments are better for the disinfection purpose. If the intensity of ultrasound and time of treatment is increased, it is possible to get better result. Similarly the wavelength and time of exposure also can be changed of UV-C light to get better result.

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