

## Microbial safety of street vended and laboratory prepared dragon-fruit (pitaya) juices in Penang, Malaysia

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**Abstract:** This study reports on the microbiological quality of fresh and laboratory prepared pitaya fruit juice compared this with those collected from street vendors in the Penang island of Malaysia. Additionally, the microbial growth patterns were monitored during extended storage for up to 7 days at 2 different temperature ranges (at 10°C and 25±1°C). Results revealed the presence and increase in the incidence of bacteria, yeasts and moulds in all the juice samples (fresh and stored). Except for *Klebsiella pneumoniae* all the other foodborne pathogens screened in this study such as: *Staphylococcus aureus*, *Salmonella* sp. and generic *Escherichia coli* were absent. Our results highlights and stresses the need to employ proper hygienic conditions during harvesting and processing stages of pitaya fruit or their juices to improve the overall microbial quality to ensure adequate safety for consumers.

**Keywords:** Dragon-fruit, pathogens, *Klebsiella pneumonia*, safety, consumers, street vended fruit juice

### Introduction

Consumption of fresh fruits and their juice provides potential health benefits to the general population (Allothman *et al.*, 2009; Bhat *et al.*, 2011). Dragon fruit (*Hylocereus undatus* Haw; Cactaceae) or the pitaya fruit is a native of Mexico, Central and South America. This exotic tropical fruit is also extensively grown and is consumed in Malaysia, Vietnam, Taiwan, Philippines and Indonesia (Haber, 1983; Mizrahi *et al.*, 1997). The fruits are eaten directly or as a part of desserts, while the prepared juice is consumed as a healthy refreshing drink. Traditional medicine or Aurvedic or Unani medicine in parts of Asia indicate that consumption of pitaya fruit juice can lower the blood cholesterol levels, improve blood circulation and neutralize the toxic elements in the blood (Chen Wu *et al.*, 2006).

Pitaya is refereed to as 'dragon fruit' due to its bright red to pink colour with green overlapping scales on the surface. Generally, three types of pitaya fruits are available, including pink skin with pink pulp, pink skin with white pulp (flesh) and yellow skin with yellow or white pulp. The flesh (or pulp) portion of the fruit consists of infinite numbers of minute black colored seeds that are also eaten along with the fruit or with juice (Barbeau, 1990). The red or pink flesh varieties are rich in betalains and meet the demand for the presence of natural antioxidants and natural food colorants (Le Bellec *et al.*, 2006). The fruits are also a rich source of beta-carotene,

lycopene and vitamin E (Charoensiri *et al.*, 2009).

Fruit juices sold by the street vendors (as thirst quenching aid) are consumed regularly by the local population in most of the tropical countries. Above all, in these countries, often consumer preference is for fresh-cut fruits and juices rather than their processed counterparts. The main reason for this is a general belief that fresh fruit juice retains the original nutritional and sensory attributes. However, available reports on the outbreak of foodborne diseases (attributed to cross-contamination) have raised serious concerns regarding the safety of consuming unpasteurized or unprocessed fruit juices. Reports are available on the presence of pathogenic or toxin-producing microorganisms such as *E. coli* 0157:H7, *Salmonella* sp., *Penicillium expansum*, *Aspergillus* sp., *Byssochlamys* sp., and *Fusarium* sp., in the unprocessed apple, orange and grapes fruit juices (Tournasa *et al.*, 2006; Bae *et al.*, 2009; Tribst *et al.*, 2009; Sant'Ana *et al.*, 2010).

Even though some reports are available on the overall quality (nutritional and antioxidant properties) of pitaya fruit, to the best of our knowledge, no scientific reports are available on the microbial safety or hygiene of this fruit or their juices. Considering the ever increasing demand for safer food products and implementation of HACCP (Hazard Analysis and Critical Control Point), GAP and GMP (Good Agricultural and Manufacturing Practices) in juice manufacturing, the present study was designed to screen for the possible presence of

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microbial contaminants (foodborne pathogens) and enumerate their status during storage conditions at 10°C and at room temperature ( $25 \pm 1^\circ\text{C}$ ) up to 7 days. It is envisaged that the results generated in the present study will be useful for both health conscious consumers and to juice manufacturers to improve microbial safety and hygiene quality.

## Material and Methods

### Samples

The dragon fruit juices used in this study were either prepared in the laboratory or was obtained from the local street vendors in the Penang state of Malaysia. The current work is mainly aimed to study the prevalence of the microorganisms, especially those of foodborne pathogens.

Fresh fruits without any apparent physical damage were purchased from the local supermarket (Tesco, Penang, Malaysia) and were used for preparing the juice under laboratory conditions. All the fruits were of eating quality and were carefully selected to be identical in terms of shape, size, color and physical maturity of the fruits. Further, the fruits were surface cleaned with a fine muslin cloth, washed with chlorinated water followed by rinsing in sterile distilled water (5-10 min). After cleaning and peeling of the skin, fruits were thinly sliced (5-6 mm thickness) using a sterile stainless steel kitchen slicer and the juice was prepared by blending at high speed in a kitchen blender (Panasonic, MX 898M, Malaysia). This juice sample served as control and was used for comparison with the street vended samples.

All the samples were collected during the fasting month of the local Malay population in Malaysia, as the selling and consumption of dragon fruit or their juice is high during this period. Also, as this is a preliminary work that is being reported, we have tried to restrict the samplings and tried to evaluate the prevalence of pathogens only. For the purchased dragon fruit juices, the samples (total 2 from 2 different locations in Penang island) were collected in a pre-sterilized plastic bag and were brought to the Food Microbiology Laboratory (School of Industrial Technology, Universiti Sains Malaysia, Malaysia) within 30 min under aseptic conditions, placed in an icebox (0 to 4°C). All the microbial analysis was conducted at room temperature ( $25 \pm 1^\circ\text{C}$ ) within 2 h on arrival to the laboratory. Aseptic techniques were employed wherein all the equipments were pre-sterilized prior to experimentation.

### Sample preparation and serial dilution

Replicates ( $n = 3$ ) of each juice sample (25 g, pH 5.0) were vortex mixed (3-4 sec) before placing it in a stomacher bag containing 225 ml of sterile peptone water. Further, the samples were homogenized (2-3 sec) using a stomacher and serial dilutions were prepared (up to  $10^{-7}$ ). Spread plate technique was used with appropriate selective media for enumeration of microorganisms.

### Microbial analysis

The microbiological analysis included enumeration of total microbial counts along with the enumeration and identification of potential foodborne pathogens based on the standard procedures for coliforms, *E. coli*, *Staphylococcus aureus* and *Salmonella* species.

### Total and aerobic plate counts

The total plate counts (TPC) and Aerobic Plate Counts (APC) were determined by spread plate method on plate count agar (PCA). The plates were incubated at 37°C and counted after 24 h (BAM, 2001; Sheth *et al.*, 2005). The results were expressed as colony forming units (cfu's) per ml.

### Total yeast and mould counts

For total yeast and mould counts, spread plate method using potato dextrose agar (PDA) was employed and the colonies were counted after 5-7 days of incubation at room temperature ( $25 \pm 1^\circ\text{C}$ ). The results were expressed as colony forming units (cfu's) per ml.

### Total coliform and *E. coli* counts

The total coliforms and *E. coli* were determined using the multiple tube fermentation technique based on the available standard procedures (Refai, 1979; Hirokazu *et al.*, 2002; Sheth *et al.*, 2005).

### Determination of *Staphylococcus aureus* and *Salmonella* sp.

To detect the presence of *Staphylococcus aureus* and *Salmonella* sp., in the dragon fruit juice samples, standard methods were used (BAM, 2001). The presence of *Klebsiella pneumoniae*, an enterobacter was also confirmed by adopting the same method.

### Storage studies

Storage (shelflife) studies were conducted for periods ranging from 1 to 7 days to access the status of microbial quality of dragon fruit juices. The storage temperatures employed were 10°C and  $25 \pm 1^\circ\text{C}$ . Samples were bottled individually in air tight glass

bottles which were filled under aseptic conditions and placed on a table at room temperature ( $25 \pm 1^\circ\text{C}$ ), while the second set prepared similarly was stored in an incubator (at  $10^\circ\text{C}$ ; SANYO, Japan; Model: MK 254). For all the storage studies conducted, the values are reported as means of triplicate measurements  $\pm$  standard deviation. On each day, the individual samples were taken from storage and microbial analysis was conducted for TPC, APC, TYC and TMC and for certain pathogenic microorganisms.

### Statistical analysis

Analysis of variance followed by Duncan test was performed using SPSS version 15.0, and comparisons of means were made using Tukey's test at the 95% confidence level (significance level at  $P \leq 0.05$ ).

## Results and Discussion

Even though fresh fruits or their unpasteurized juices offer potential health benefits, they can be a potential substrate for microbial contamination (Parish, 1997; Tribst *et al.*, 2009). Results on the microbial quality of laboratory prepared (under sterilized conditions) and street vendor collected dragon fruits juice samples, and their status after extended storage are shown in Table 1. From the results (Table 1), all the juice samples were found to be contaminated by bacteria (TPC and APC), yeasts and molds (TYC and TMC). The presence of microorganisms in the juice samples might be attributed to the pre- and post-harvest storage conditions as well as to improper handling during transportation of the fruit. Also, the pulp portion of the dragon fruit contains high amount of gelatinous carbohydrates (Ariffin *et al.*, 2009) that might provide suitable base for spoilage microorganisms

to grow and proliferate. Overall comparison, higher microbial load was recorded in the juice samples collected from street vendors compared to those prepared in the laboratory. However, except for *Klebsiella pneumoniae* all the juice samples analyzed (street vendors and laboratory prepared) were devoid of any foodborne pathogens screened in the present study (*Staphylococcus aureus*, *Salmonella* sp. and generic *E. coli*).

Storage temperature is one of the crucial factors that play a significant role in determining the microbial growth and their survival in fruit juices. Storage temperature of fruit juice is also vital to ensure the wholesomeness of the product. In the present study, storage of dragon fruit juice samples at varying temperature range up to 7 days exhibited a significant and gradual increase in the microbial load. Juice samples stored at  $10^\circ\text{C}$  had lower microbial load compared to the samples stored at room temperature ( $25 \pm 1^\circ\text{C}$ ). After 3 days of storage, the microbial load increased substantially in all the samples stored at room temperature and could not be counted (too numerous to count; TNTC) (Table 1).

Interestingly, in control samples, initially on first day a decrease in the TPC counts were recorded, which were significantly enhanced on second and third day onwards. Overall, no significant differences were observed in control samples, as these were prepared aseptically in the laboratory. However, significant differences were observed for street vended juice samples (juice 1 and 2), which might be attributed to pre-contamination that might have occurred during or prior to purchase or preparation of juice. Additionally, this might be due to the fact that the bacteria might have tried to adapt to the low temperature initially for their growth, thus going for a bacteriostatic stage. The occurrence of high microbial load in the street

**Table 1.** Microbiological quality of laboratory prepared and street vendor collected dragon fruits juice samples, and their status after extended storage ( $n = 3 \pm \text{S.D.}$ ; Results expressed as  $\log_{10}$  CFU/ml; dilution factor  $10^{-3}$ )

Microbial analysis	Sample	Fresh juice	10 °C (incubated temperature)			25 ± 1 °C (room temperature)		
			Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Total Plate Count	Control	5.7±0.1 <sup>b</sup>	<20 <sup>a</sup>	6.0±0.1 <sup>a</sup>	TNTC <sup>b</sup>	6.0±0.1 <sup>a</sup>	6.4±0.1 <sup>a</sup>	TNTC
	Juice 1	<20 <sup>a</sup>	<20 <sup>a</sup>	5.5±0.1 <sup>a</sup>	TNTC <sup>b</sup>	5.9±0.2 <sup>a</sup>	7.0±0.2 <sup>b</sup>	TNTC
	Juice 2	6.5±0.0 <sup>c</sup>	6.3±0.1 <sup>c</sup>	6.4±0.1 <sup>b</sup>	TNTC <sup>b</sup>	6.3±0.1 <sup>b</sup>	9.3±0.0 <sup>c</sup>	TNTC
Aerobic plate count	Control	<20 <sup>a</sup>	<20 <sup>a</sup>	6.3±0.1 <sup>b</sup>	6.4±0.0 <sup>a</sup>	5.9±0.0 <sup>a</sup>	TNTC <sup>d</sup>	TNTC
	Juice 1	<20 <sup>a</sup>	<20 <sup>a</sup>	6.3±0.1 <sup>b</sup>	6.3±0.0 <sup>a</sup>	6.0±0.0 <sup>a</sup>	TNTC <sup>d</sup>	TNTC
	Juice 2	6.2±0.1 <sup>c</sup>	4.9±0.1 <sup>b</sup>	6.0±0.0 <sup>b</sup>	6.5±0.0 <sup>a</sup>	6.4±0.1 <sup>b</sup>	7.4±0.1 <sup>b</sup>	TNTC
Total Yeast and Mould Count	Control	<20 <sup>a</sup>	<20 <sup>a</sup>	6.2±0.1 <sup>b</sup>	6.4±0.1 <sup>a</sup>	6.3±0.1 <sup>b</sup>	6.4±0.0 <sup>a</sup>	TNTC
	Juice 1	<20 <sup>a</sup>	<20 <sup>a</sup>	6.4±0.0 <sup>b</sup>	6.5±0.1 <sup>a</sup>	6.1±0.2 <sup>a</sup>	6.4±0.1 <sup>a</sup>	TNTC
	Juice 2	6.0±0.0 <sup>b</sup>	5.9±0.1 <sup>c</sup>	6.4±0.1 <sup>b</sup>	6.4±0.1 <sup>a</sup>	6.3±0.1 <sup>b</sup>	6.5±0.1 <sup>a</sup>	TNTC
<i>Staphylococcus aureus</i>	Control	NP	NP	NP	NP	NP	NP	NP
	Juice 1	NP	NP	NP	NP	NP	NP	NP
	Juice 2	NP	NP	NP	NP	NP	NP	NP
<i>Salmonella</i> sp.	Control	NP	NP	NP	NP	NP	NP	NP
	Juice 1	NP	NP	NP	NP	NP	NP	NP
	Juice 2	NP	NP	NP	NP	NP	NP	NP
<i>Escherichia coli</i>	Control	NP	NP	NP	NP	NP	NP	NP
	Juice 1	NP	NP	NP	NP	NP	NP	NP
	Juice 2	NP	NP	NP	NP	NP	NP	NP
<i>Klebsiella pneumoniae</i>	Control	6.1±0.1 <sup>b</sup>	5.9±0.0 <sup>c</sup>	TNTC <sup>c</sup>	TNTC <sup>b</sup>	6.4±0.1 <sup>b</sup>	TNTC <sup>d</sup>	TNTC
	Juice 1	6.0±0.0 <sup>b</sup>	5.9±0.0 <sup>c</sup>	TNTC <sup>c</sup>	TNTC <sup>b</sup>	6.4±0.0 <sup>b</sup>	TNTC <sup>d</sup>	TNTC
	Juice 2	6.3±0.1 <sup>c</sup>	6.2±0.1 <sup>c</sup>	TNTC <sup>c</sup>	TNTC <sup>b</sup>	6.5±0.0 <sup>b</sup>	TNTC <sup>d</sup>	TNTC

<sup>a</sup> Control, lab prepared fresh juice; <sup>b</sup> Juice 1 and Juice 2, collected from street vendors; <sup>c</sup> NP, not present; <sup>d</sup> TNTC, too numerous to count.

<sup>e</sup> Values in the same column with different superscript letters are significantly different from each other at  $P < 0.05$ .

\*\* Result from day 4 to 7 is not reported as the microbial (bacteria) load were too numerous to count in all the samples.

vended samples might be attributed directly to the improper washing of fruits, use of crude stands and carts, preservation without adequate refrigeration and to the unhygienic surrounding environment as opined earlier by Lewis *et al.* (2006). The TPC count shows the estimation of aerobic and facultative anaerobic bacterial populations in the juice sample, while APC count shows the estimation of only aerobic bacterial population in the juice sample. Results on the aerobic plate count showed presence of spoilage bacteria after day 3 at 10°C, while it was as early as day 2 in juice 1 and day 3 in juice 2 at 25 ± 1°C, respectively. The presence of yeast and moulds were high on day 4 onwards at 10°C and on day 3 at 25 ± 1°C. The absence of *E. coli* is an indication on the presence of *Klebsiella pneumoniae*, which is also fecal coliform. *Klebsiella pneumoniae* were found at 10°C and 25 ± 1°C at day 1, but their growth was slow at low temperature compared to room temperature. However, gradually their number increased in both temperatures on extended storage. Furthermore, the absence of *E. coli* might be attributed to the quality of potable water used for preparing the juice. The absence of *Salmonella* sp., might be due to the minimal use of contaminated animal manure during fruit growth stages. However, the presence of *Klebsiella pneumoniae* is alarming as this pathogen has been previously isolated from some of the street foods served in Malaysia (Haryani *et al.*, 2007). It has been reported that the presence of *Klebsiella pneumoniae* in the street food samples might pose serious health risks and can lead to rapid cross-contamination with other 'ready- to-eat foods' sold by the street vendors (Haryani *et al.*, 2007). Our results on the presence of *Klebsiella pneumoniae* are in agreement with some of the earlier reports on their presence in fruit juices (Fuentes *et al.*, 1985; Ghenghesh *et al.*, 2004). In order to avoid contamination from pathogenic bacteria and other foodborne pathogens, vital factors such as juice processing conditions, storage environment, processing equipments, cleansing water of the fruits and hygiene of the handler needs to be taken care (Tsige *et al.*, 2008).

The present study being a preliminary investigation, only a few of the selected foodborne pathogens were screened. However, still there are high possibilities that acidothermophilic spoilage bacteria like *Alicyclobacillus acidoterrestris* might be present in the pitaya juice samples. Additionally, there are high chances that spoilage microorganisms like *Lactobacillus*, *Leuconostoc* and thermophilic *Bacillus* species might be present, which needs to be investigated in the near future. Application of physical, non-thermal preservation techniques like

ultraviolet radiation, ozone or sonication treatments might prove to be useful for commercial exploitation of pitaya fruit juice. It is envisaged that the results obtained in the present study might be useful for monitoring the microbial quality of pitaya fruit juices for human consumption to avoid any future foodborne disease outbreaks and also be useful to implement a proper HACCP approach with good GMP practices.

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