

Microbiological Quality of Fresh Cut Fruit Salad Sold in Thanjavur City

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The aim of this study was to evaluate the microbiological quality of fruits salad commercially sold in Thanjavur city to reveal the microbial safety and possible health hazard linked with consuming this product. A total of 100 samples, were collected from major fruit stalls and local markets are analyzed for aerobic mesophilic and psychrotropic bacterial counts. 85 samples were analyzed for the presence of yeast and moulds, enumeration of coliforms and the presence of *E.coli* O157:H7 and *Salmonella* or *Shigella* species. Presence of aerobic mesophiles ranged from 2.5 to 5.8 log cfu/g, with the lowest and highest counts observed for pineapple (2.0 log cfu/g) and watermelon (6.8 log cfu/g) respectively. The highest coliforms were observed from grapes and freshcut salads with 70% of the samples contain 5 log cfu/g. Similarly, the maximum counts of yeasts and moulds were detected in pomegranates and grapes. There is no psychrotrophic microorganisms were isolated in this study. Biochemical characterization of bacteria was done using Biolog Microplate™ culture method and pathogens such as *Salmonella* were present in a few sample and *E.coli* O157: H7 or *Shigella* was not found in any of the samples used for the study. However, the presence of bacteria and yeast in higher range shows that the microbiological quality of fresh cut fruit salad sold in Thanjavur city were not recommended level and vendors should practice Good Manufacturing Practices before selling the products to the consumers.

Keywords: Microbiological quality, fruit salads, coliforms, *E.coli* O157:H7.

Consumption of raw fruits and vegetables such as fresh cut fruit salads or ready to eat fruits have been increased in many parts of the world. In India, vending of fresh cut salads, vegetables and sprouts becoming popular and as well as common practice among urban population (Viswanathan and Kaur, 2001). In particular, fresh-cut fruits attract consumers because they are fresh, nutritious, low priced, and ready-to-eat. As a consequence, a wide assortment of minimally processed fruits has been developed to meet consumer's needs for "quick" and convenient products, and to benefit from fruit's

healthy image (Ahvenainen 1996). Apart from these benefits, the readily available fruits and vegetables became a carrier for the infections and diseases. The sliced/peeled fruits are processed and sold by unlicensed vendors with poor education levels and untrained in food hygiene (Muinde *et.al.*, 2005), Barro *et.al.*, 2007). The consumption of sliced/peeled fruits may thus potentially increase the risk of food-borne diseases caused by a wide variety of pathogen (Mensah *et.al.*, 1999). There are also several reports about the human gastroenteritis have been associated with the consumption of fresh cut fruit salads and vegetables (Brackett and Splittstoesser, 1992; Larry 1995). Consumption of lettuce and spinach has resulted in the outbreaks of gastroenteritis and *E.coli* O157:H7 infection in

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various states of US (Ackers *et.al.*, 1998; Fan *et al.*, 2009). Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetable borne outbreak, occurred in Japan in 1996 in which 11,000 people affected and about 6,000 cultures were confirmed. The outbreak involved the death of three children and was carried by *Escherichia coli*. The most common bacterial enteropathogens associated with fruits and vegetables are *Salmonella spp.* (Thunberg *et.al.*, 2002). Beauchat, 1996, related the risk of consuming the contaminated watermelons due to the risk of infection with salmonellosis. There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere (Bhaskar *et.al.*, 2004; Chumber *et.al.*, 2007; Ghosh *et.al.*, 2007). Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* O157:H7, species of *Salmonella*, *Shigella*, and *Staphylococcus aureus* (Buchmann *et.al.*, 1999). In India the presence of coliforms and *Staphylococci Spp.* in kinnow and mandarin juices in Patiala city was reported by Ganguli *et.al.*, in 2004. Similarly coliforms were observed, in fresh fruit and vegetable juices sold by the street vendors of Nagpur city (Titarmare *et.al.*, 2009). Although, The Food Safety Standards Authority of India is well established and regulating norms for the safety of the consumers, the food safety is still lacking in the most parts of India for the fresh cut fruit salads sold by the local vendors. Therefore, a preliminary study has been conducted to evaluate the microbiological quality of fresh cut fruit salad commercially sold in Thanjavur city of Tamil Nadu in the food safety aspect.

MATERIALS AND METHODS

Collection of samples

A total of 100 samples of fruit salad packets were purchased from 10 fruit stalls and also collected from local vendors across Thanjavur city. Commonly, each packet of fruit salad had a piece of apple, pineapple, papaya, watermelon and a small proportion of pomegranate and grapes. These fruits are imported from other states whereas, a few like papaya and pomegranate grown locally. Most of these samples were kept in refrigerated conditions in most of the fruit stalls.

Transportation of the sample

The fruit salad packets along with their original packaging, were transferred to a sterile zip lock covers and kept in icebox immediately after purchasing, and transported to the laboratory at Indian institute of Crop Processing Technology, Thanjavur, Tamil Nadu. The sample details such as brand name, packed date and expiry date were recorded thoroughly and kept in refrigerated (4 °C) conditions until analyses to maintain their original conditions. All the samples were analyzed within 24h after the time of purchasing and as well as before their expiry date. Also the packets get damaged during transportation were discarded.

Total Plate Count and psychrotrophic count

Approximately 25g of each fruit sample were homogenized in a laboratory blender (Waring Laboratory Blender, Sigma Aldrich, India) with 225 ml of 0.1% sterile peptone water (Himedia, India) and serial dilution technique (Rahman, *et.al.*, 2011) was performed to prepare 1:10 dilutions. Plate count agar (PCA) (Himedia) plates were inoculated with 1ml of selected dilutions and have incubated for 24 h at 37 °C (FDA, 1998; BAM) and Another set of plates were incubated at 10 °C for 7 days (Hantsis-Zacharov *et.al.*, 2007) for the detection of psychrotropic plate count. The plates with at least 20 colonies were counted using a digital model plate counter. The results were observed after incubation period and reported in colony forming units (Cfu/g).

Coliform count

The aliquots prepared from the fruit salad samples for total plate count were used for the enumeration of coliforms. Appropriate dilutions were chosen and 0.1ml of the samples was spread plated on MacConkey agar (Hi media, India) and Eosine Methylene Blue (EMB) agar (Himedia, India). After inoculation, the plates were incubated at 37 °C for 24 h and observed for pink and purple colonies for the presence of coliforms.

Isolation of yeasts and moulds

Samples were prepared as described above. 0.1ml of sample was inoculated using spread plate technique on Sabouraud Dextrose (SDA) (HiMedia, India) agar containing 0.1% chloramphenicol (HiMedia, India). The inoculated plates were incubated at 25 °C for 5-7 days and after incubation, the yeast and mould colonies were

counted (FDA, 1998; BAM) and expressed as yeast and mould/g.

Detection of *E.coli* O157:H7

An enrichment broth was prepared by the methods described by Shin Sata *et.al.*, 2003 for the isolation of *Escherichia coli* O157. Buffered peptone water (BPW) with 0.5% sodium thioglycolate (STG; Sigma Aldrich, India) was used for the enrichment which is specific for the growth of *E.coli* O157:H7 bacteria if any. For the enrichment, the whole homogenized sample was incubated with the BPW-STG broth for 24 h at 37 °C. Following incubation, the sample is streaked on chromogenic agar (HiCrome EC O157: H7 Agar, HiMedia, India) and incubated at 37 °C for 24 h. The colonies cultivated on the chromogenic plates were further identified by performing biochemical analysis.

Isolation of *Salmonella* and *Shigella* species

Approximately 25g of each fruit sample were homogenized in a laboratory blender (Waring Laboratory Blender, Sigma Aldrich, India) and enriched with 225 ml of Rappaport Vassiliadis *Salmonella* Enrichment Broth (Himedia, India) for the detection of *Salmonella* from the fruit salad samples. After incubation, a loopful of sample was streaked on XLD agar (HiMedia) and SS (*Salmonella* *Shigella* agar), incubated for 24 h at 37 °C. The black colored colonies observed on XLD agar the colonies grown on SS agar were used subsequently for biochemical tests.

Confirmation of presumptive colonies

The isolated colonies from the presumptive tests were suspended in the IF (Inoculation fluid, Biolog, Hayward, USA) till it reaches 65% transmittance with Biolog turbidity meter. 100µl of the fluid is then transferred to 99 well Biolog Microplate (Biolog, Hayward, USA). The plates were incubated for 24 h at 37 °C. After incubation, the plates were inserted in the Biolog automatic system and identified using GENIII Biolog software™.

Statistical analysis

All the experiments were carried out in triplicates and the data were represented as mean (Table1; Table 5). MS Excel was used to plot the graphs. SPSS18.0 was used to conduct analysis of variance (ANOVA) followed by Duncan's multiple range test to investigate the significant difference among treatments.

RESULTS AND DISCUSSION

Microbiological safety of fresh cut fruit salad

Tables 1-4 results of fresh cut fruit salads analyzed for total plate count, psychotropic colonies, enumeration of coliforms and yeast and mould growth. There was no visible mark of any spoilage or any defect in the samples analyzed but surprisingly, highest microbial diversity was observed among the commodities. The total plate count mean value for the freshcut fruit salad analysed is about 5.5 log cfu/g (Table.1) with a range of 2.5 to 5.8 log cfu/g, which is unacceptable for consumption. According to HACCP, food containing <4 log cfu/g is unfit for human consumption and those containing approximately >8 log cfu/g is considered spoiled. The mean total plate count results are similar to the mean aerobic plate count ranges from 2.0×10^6 to 8.2×10^8 on pineapple and watermelon obtained from the Nigerian local market reported in 2011 by Oranusi and Olorunfemi. In a similar study, 52 bagged salads were analysed and reported in US upto mean aerobic count of 7.0 log cfu/g (Hagenmaier and Baker, 1998).

Among the seven fresh cut fruits and salads, all watermelon samples has 6.8 log cfu/g (Table 1) of total plate count which also having a highest range. This must be due to the usage of contaminated water and unclean utensils and storage conditions. In earlier work done by Brooks in 2014, microbial quality of street vended fruit salad resulted in the high microbial load upto 90% of the samples examined. Most of the fresh cut fruit products were resulted in the high total plate count. The lowest range was observed in pineapple with 2.0 log cfu/g and the highest was observed in grapes and watermelon as 6.5 and 6.8 log cfu/g respectively. Only samples such as apple, pine apple and pomegranate were resulted in the lower microbial load when compared to other fruits. This may be attributed with the thick skin of pine apple and pomegranate which acts as a surface barrier for the bacterial infection. Also the edible wax coated on apple's skin for increasing the shelf life will be the reason behind the lower microbial count. Comparatively, a study conducted by Viswanathan and Kaur (2001) had a higher aerobic plate count range between 6 log cfu and 8 log cfu/g of fruits

collected from the street vendors of Mumbai. This can be correlated with the type of fruits, storage conditions, utensils etc.

In this present study, there is no psychrotrophic microorganisms were isolated when incubated at 10°C (Table 2). The psychrotrophs are a real problem for the vendors who stored the fruit products under refrigerated conditions; usually 7-10 °C. But, the recommended temperature for storing fruits are around 1-5°C while none of the samples collected for the present study were stored in such conditions during collection.

In the present study, fresh cut fruit salad had a mean coliform count between 1.0 to 5.5 log cfu/g (Table 3). The highest mean coliform counts were observed in grapes, fresh cut fruit salad, papaya followed by watermelon and with 5.5, 5.4, 4.5 and 4.1 log cfu/g respectively. These four samples were not safe for consumption due to the

high prevalence of coliforms. Other samples such as apple, pineapple and pomegranate exhibit very less coliform count compared to the above mentioned samples and assuming safe for consumption. Chukwu *et al.*, 2010, conducted a study to determine the microbial safety of pre-cut fruits from 150 samples composed of pineapples, paw-paw and watermelon and out of these, 70% of the resultant microbial population was coliforms which includes *E.coli*, *Salmonella*, *Proteus*, *Enterobacter* and *Klebsiella* species. The possible explanation behind this high coliform count is exposure of fruits to microbial contamination through contact with soil, dust, water and handling thus harbouring a diverse range of pathogenic microbes including coliforms. During prolonged storage of cut fruits, nutrients may leach from cut fruits (Viswanathan and Kaur, 2000) could have caused an increase in the coliform growth.

Table 1. Results of Total Plate Counts

Name of sample (Each 25g)	No. of sample units	Percentage (%) of samples in the indicated interval					Range ^a	Mean ^a	
		<4 ^a	4-5	5-6	6-7	7-8			
Apple	15	30.2	28.4	18.6	15.4	7.4	0.0	2.5-5.1	2.8 ^D
Pineapple	18	35.3	32.8	20.1	8.4	3.4	0.0	2.0-5.6	3.8 ^{CD}
Papaya	12	28.4	48.6	19.1	4.0	0.0	0.0	2.2-5.4	5.2 ^D
Watermelon	15	38.0	22.1	28.8	11.1	0.0	0.0	3.0-6.8	5.4 ^C
Pomegranate	13	10.1	1.8	44.4	6.2	32.4	5.1	2.1-3.4	2.8 ^D
Grapes	14	10.5	22.2	34.2	18.6	14.5	0.0	2.4-6.5	4.5 ^B
Fresh cut Salad pack	13	31.2	21.5	24.2	23.4	0.0	0.0	2.5-5.8	5.5 ^A

Means having different letters (A-D) are significantly different (P <0.05).

^a The unit of number is log cfu/g.

Table 2. Results of psychrotrophic count in the sample

Name of sample (Each 25g)	No. of sample units	Percentage (%) of samples in the indicated interval					Range ^a	Mean ^a	
		<3 ^a	3-4	4-5	5-6	6-7			
Apple	15	41.2	18.6	25.1	15.2	0.0	0.0	ND ^b	ND
Pineapple	18	35.6	30.2	15.8	18.4	0.0	0.0	ND	ND
Papaya	12	37.4	24.3	28.1	10.2	0.0	0.0	ND	ND
Watermelon	15	5.8	22.8	41.2	28.2	2.0	0.0	ND	ND
Pomegranate	13	84.2	6.5	9.3	0.0	0.0	0.0	ND	ND
Grapes	14	64.4	10.1	25.5	0.0	0.0	0.0	ND	ND
Fresh cut Salad pack	13	24.4	22.4	14.5	24.2	14.5	0.0	ND	ND

^a The unit of number is log cfu/g.

^b Not detected.

Samples of the fresh cut fruit salads had significantly higher microbial load (Tables 1 and 3), and yeasts and moulds counts (Table 4) but relatively lower when compared with the previous studies reported. This must be due to the difference between the processing steps like peeling, slicing and handling which initiate the nutritional availability for the growth of bacteria and yeast and mould colonies. The mean yeast and mould count in pomegranate was 1.0 log cfu/g which is a lowest among the fruits samples, whereas grapes accounts for a high yeast and mould growth of 4.1 log cfu/g followed by fresh cut fruit salad with 3.9 log cfu/g colonies. Even though the presence of yeast and mould count compared to bacterial count, the presence of mould poses health risks – moulds may produce mycotoxins and allergens (Tournas, 2005) which are serious threat to the world.

Occurrence of Food borne pathogens in the fresh cut fruit salad

No samples analysed in this study harbors either *E.coli* O157:H7 strains or *Shigella* species in it but *Salmonella typhi* was found in three different samples viz. watermelon, grapes and fresh cut fruit salad packs (Table 5). This indicates that there is still food safety risk in consuming the fresh cut fruits salads. *Salmonella* species were responsible for two different outbreaks resulted upon consuming pre cut wrapped watermelons (Gayer *et al.*, 1955). This may be attributed to the formation of broth from the pre cut watermelon due to handling and storage influences the microbial growth.

Though the presence of non fecal coliforms such as *Klebsiella* spp., *Enterobacter* spp., and *Citrobacter* spp., were observed during the presumptive analyses from chromogenic agars,

Table 3. Results of coliform count in the fruit salad samples

Name of sample (Each 25g)	No. of sample units	Percentage (%) of samples in the indicated interval					Range ^a	Mean ^a
		<2 ^a	2-3	5-6	6-7	7-8		
Apple	16	95.2	4.8	0.0	0.0	0.0	ND-2.3	1.1 ^{CD}
Pineapple	14	64.4	14.2	21.4	0.0	0.0	ND ^b -1.1	0.1 ^D
Papaya	10	74.8	19.1	6.3	0.0	0.0	0.3-4.8	4.5 ^B
Watermelon	15	45.2	28.2	14.2	12.4	0.0	1.3-5.2	4.1 ^C
Pomegranate	18	68.6	0.0	31.4	0.0	0.0	ND-1.2	1.0 ^A
Grapes	14	24.2	12.2	28.5	35.2	0.0	1.2-6.2	5.5 ^B
Fresh cut Salad pack	16	65.2	24.4	10.4	0.0	0.0	1.5-5.6	5.4 ^A

Means having different letters (A-D) are significantly different (P <0.05).

^a The unit of number is log cfu/g.

^b Not detected.

Table 4. Results of yeast and mould count

Name of sample (Each 25g)	No. of sample units	Percentage (%) of samples in the indicated interval					Range ^a	Mean ^a	
		<4 ^a	4-5	5-6	6-7	7-8			
Apple	15	24.2	32.6	21.8	11.4	0.0	0.0	1.8-3.5	3.2 ^A
Pineapple	11	28.4	18.6	30.2	5.4	0.0	0.0	0.2-1.2	1.1 ^C
Papaya	10	5.2	8.4	58.2	28.2	0.0	0.0	0.1-2.1	2.1 ^D
Watermelon	13	0.0	55.8	15.6	28.6	0.0	0.0	1.2-3.4	3.1 ^A
Pomegranate	18	33.2	28.4	18.2	20.2	0.0	0.0	0.1-0.9	1.0 ^C
Grapes	16	43.4	31.5	12.6	12.5	0.0	0.0	1.8-4.6	4.1 ^B
Fresh cut Salad pack	12	0.0	27.2	33.1	20.4	19.4	0.0	1.5-4.1	3.9 ^A

Means having different letters (A-D) are significantly different (P <0.05).

^a The unit of number is log cfu/g.

Table 5. Food borne pathogens in the fresh cut fruit salad

Name of sample (Each 25g)	No. of sample units	Percentage (%) of samples with presumptive colonies			Percentage (%) of confirmed positive samples		
		<i>E.coli</i> O157:H7	<i>Salmonella</i> <i>spp.</i>	<i>Shigella</i> <i>spp.</i>	<i>E.coli</i> O157:H7	<i>Salmonella</i> <i>spp.</i>	<i>Shigella</i> <i>spp.</i>
Apple	13	ND ^a	ND	ND	ND	ND	ND
Pineapple	10	ND	ND	ND	ND	ND	ND
Guava	9	ND	ND	ND	ND	ND	ND
Watermelon	20	ND	10	ND	ND	3	ND
Pomegranate	15	ND	ND	ND	ND	ND	ND
Grapes	18	ND	10	ND	ND	4	ND
Fresh cut Salad pack	16	ND	12	ND	ND	3	ND

^a ND: not detected.

these were failed to develop their characterization in the confirmation tests. Still their presence is not affecting the public health as they are commonly present in water and other environments and thus common in fresh cut salads (Soriano *et al.*, 2000).

CONCLUSIONS

Present study exhibited the microbiological safety of locally available fresh cut fruit salads and their commodities to ensure food safety for a control on local public of Thanjavur city of Tamil Nadu. Though the total plate count was observed high, the coliform count was in the border line of food control, the risk of consuming the contaminated food products which having bacterial pathogens like *Salmonella typhi* is still at high risk. From the data presented in the current study, it can be concluded that the microbiological quality of most of the vendor and packaged fresh cut fruit samples collected from different areas of Thanjavur city were not satisfactory as the fecal coliform were detected in the samples. Considering the processing steps carried out by street vendors and by the fruit related super markets or outlets and the environment where the fruits were kept and practices they follow will not be ruled out unless any government agencies step in to educate them in groups. If a consumer should get the benefit of street vended fruits or fruit products, the government should ensure the food safety by strict practices and altering the local situation.

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