*ORIGINAL ARTICLE*

# Fate of *Salmonella* species and *E. coli* in Fresh-Prepared Orange, Avocado, Papaya and Pine Apple Juices.

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**ABSTRACT**

**Background:** ***Fruits are consumed for their various nutritional qualities. They contain organic acids in quantities that are generally sufficient to produce pH values of 4.6 or lower and the large majority of fruits are considered high acid products. Some fruits, such as avocado, are rich in fat.***
***The aim of this study was to evaluate the microbiological safety of fruit juices by determining the growth potential of some food-borne pathogens in the juices at different holding temperatures.***
**Methods:** ***Juices were prepared from fresh ripened papaya, pine apple, avocado and orange fruits in Addis Ababa between February and May, 1999. They were separately inoculated with young cultures of*** **Salmonella typhimurium, Salmonella choleraesuis,** ***and*** **E. coli** ***(strains 9637 and 25922). Counts of test strains were made at intervals for 48 hours at ambient and refrigeration temperatures.***
**Results:** ***Papaya and avocado juices had initial pH values of >5.7 and allowed all test strains to reach numbers >$10^7$ cfu/ml at ambient temperature holding. At refrigeration temperatures, at least no elimination was observed. In pine apple juice (pH 3.8), the E. coli test strains were eliminated at both holding temperatures within 16 h whereas slight increase in counts of Salmonella test strains was observed at ambient temperature holding. Orange juice (pH 3.1) did not allow the survival or growth of the test organisms at both holding temperatures.***
**Conclusion:** ***Although pasteurization may not be practical in the Ethiopian fruit juice market, it may be useful to require fruit juice vendors to have procedures in place to reduce the number of disease-causing microorganisms to the same level achievable by pasteurization. Microbial reduction methods other than pasteurization, including washing, scrubbing, antimicrobial solutions, alternative technologies or a combination of techniques are suggested. In addition, it would be appropriate to determine the microbiological quality and safety of other fruit juices sold in various vending houses as made available for consumption in order to establish their role in the epidemiology of food- borne diseases in the country.***

**Key words:** ***Fruits juice, Salmonella, E. coli.***

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## INTRODUCTION

Fruits are consumed for their various nutritional qualities. They contain organic acids in quantities that are generally sufficient to produce pH values of 4.6 or lower and the large majority of fruits are considered high acid products (1). Some fruits, such as the avocado, are rich in fat.

Fruits are usually recommended in the daily menu to acquire a good nutritional status. In addition, some workers have demonstrated the anti-cancer properties of orange juice and indicated that citrus flavonoids are effective inhibitors of human breast cancer cell proliferation *in-vitro* (2). Similarly, grape and apple juices have been reported to be inhibitory particularly to polio virus type 1 (3).

Apart from the natural state, fruits can be obtained as frozen, fermented, pasteurized, canned, salted or pickled forms and also as components of different products like cosmetics, beverages, soft drinks, jams and candies. One of the natural forms with a little mechanical modification is the juice form, consumption of which is a relatively recent practice worldwide (2).

In whatever form they exist, fruits are never totally free of microorganisms even after being preserved. Contamination may arise from poorly handled containers, mechanical damage, and some environmental sources as soil, water, air and animal or human bodies (4).

Acidic fruit juices are generally believed to be unusual vehicles of transmission for human pathogens. However, various acidic fruit juices have been identified as vehicles of transmission in outbreaks of typhoid fever, hepatitis A virus, viral gastroenteritis, enterotoxigenic *E. coli, Salmonella typhimurium* infections, post diarrhoeal uremic syndrome and cryptosporodiosis (5,6). Illness also caused by other *Salmonella, Bacillus cereus* and *Clostridium* species from various fruit juices have also been reported (7).

In order to prevent such fruits from the action of pathogenic microorganisms, several preservation methods are practiced in many developed countries (8). In general, most preservation methods include actions like asepsis (removal of microbes), use of heat or low temperature, drying, use of preservatives, irradiation and ionization, fermentation, pasteurization, and canning of fruits.

In Ethiopia, particularly in large urban areas, fruit juices are available in supermarkets in canned or bottled forms. In addition, fruit juice vending houses, which serve different types of juices in their fresh forms are proliferating. In addition, customers of different social and age groups are served in such vending houses. Although there is a report on the microbial level of some fruits in Ethiopia (9), there is no scientific information on the safety of fruit juices prepared and consumed in Ethiopia. The aim of this study was, therefore, to evaluate the microbiological safety of orange, papaya, avocado and pine apple juices by determining the growth potential of some food-borne pathogens in the juices at different holding temperatures.

## MATERIALS AND METHODS

*Sample collection and preparation*: Fresh ripened papaya, pine apple, avocado and orange fruits were purchased from grocers in Addis Ababa during the months of February and May, 1999. The fruits were thoroughly washed in tap water separately and rinsed in sterile tap water. The skin of the fruits was aseptically removed and the flesh was separately blended into juice in a thoroughly washed and rinsed blender. Pine apple and orange juices were further filtered to remove fibrous material.

To obtain a consistency similar to that prepared in fruit juice vending houses,

different volumes of water were added to the fruit juice and further blended to make a final juice volume of 500 ml (100 ml of water to papaya, 150 ml to pine apple, and 250 ml to avocado). These juices were separately poured into screw cap bottles in duplicate and steamed at 100$^{o}$C for 10 min. The bottles were then left to cool to ambient temperature before inoculation with test strains.

*Bacterial cultures*: *E. coli* (9637), *E. coli* (25922), *Salmonella typhimurium* and *Salmonella choleraesuis* were kindly donated by Dr. Aberra Geyid of Bacteriology laboratory, Ethiopian Health and Nutrition Research Institute.

*Determination of growth potential of test strains:* The different juices were separately inoculated with an overnight broth culture of each of the test strains and thoroughly mixed to give an initial inoculum level of $10^2 - 10^3$ cfu/ml. Then 0.1 ml of an appropriate dilution was surface plated in duplicate on MacConkey agar and XLD agar (both from Oxoid) plates and incubated at 32$^{o}$C overnight to determine the initial inoculum level of *E. coli* and *Salmonella* test strains, respectively. One group of juice bottles was held at ambient temperatures and the duplicate were stored under refrigeration temperature (4$^{o}$C). For those maintained at ambient temperature enumeration continued until 48 hour at 8 hours intervals. For refrigerated juices, counting was done at 24 hours intervals.

The pH of stored juices was recorded using digital pH meter at time of initial inoculation and during sampling for counting thereafter.

## RESULTS

The initial pH of papaya juice was 5.7 and it decreased to 5.0 and 4.7 after 24 and 48 hour holding, respectively at ambient temperatures. Both *E. coli* test strains could reach counts as high as $10^9$ cfu/ml within 16 h and the count was maintained until 48 h. At 4$^{o}$C holding temperature, counts were maintained at a level not higher than the initial inoculum level (Table 1). *S. typhimurium* and *S. choleraesuis* could also proliferate in papaya juice luxuriously when held at ambient temperatures. *S. typhimurium* reached counts as high as $10^9$ cfu/ml in 24 h and the count kept on increasing slightly until 48 h. *S. choleraesuis* reached its maximum count ($10^8$ cfu/ml) at 24 h and slight decrease in count was noted *thereafter* (Table 1*).* Counts of both *Salmonella* test strains increased by one log unit in 24 h under refrigeration holding (4$^{o}$C), but did not increase any higher than $10^5$ cfu/ml throughout the holding period.

Pine apple juice had an initial pH of 3.8 and this was maintained throughout the 48 h holding period. Both *E. coli* test strains were not detected at and after 16 h of holding at ambient temperatures or at 4$^{o}$C (Table 2). *S. typhimurium* and *S. choleraesuis*, however, not only survived during ambient temperature holding but their count almost doubled in 24 h. There was a limited but steady increase in count until 48 h (Table 2). Pine apple juice stored at 4$^{o}$C did not allow the growth of either *Salmonella* test strains.

**Table 1.** Growth and Inhibition (Log Cfu/Ml) of *E. Coli* and Salmonella Test Strains in Papaya Juice Stored at Different Temperatures, Addis Ababa, February -May 1999.

| Time (h) | pH | *E. coli* (9637) | | *E. coli* (25922) | | *S. typhimurium* | | *S. choleraesuis* | |
|---|---|---|---|---|---|---|---|---|---|
| | | $37^0$c | $4^0$c | $37^0$c | $4^0$c | $37^0$c | $4^0$c | $37^0$c | $4^0$c |
| 0 | 5.74 | 2.78 | 2.78 | 2.70 | 3.52 | 3.99 | 3.99 | 3.52 | 3.52 |
| 16 | 5.02 | 9.1 | ND | 9.41 | ND | 8.46 | ND | 8.56 | ND |
| 24 | 5.02 | 9.1 | 2.54 | 9.36 | 4.45 | 9.15 | 5.2 | 8.66 | 4.45 |
| 40 | 4.70 | 9.6 | ND | 9.38 | ND | 9.20 | ND | 8.48 | ND |
| 48 | 4.69 | 9.4 | 2.53 | 9.40 | 4.08 | 9.23 | 4.43 | 8.18 | 4.08 |

Table 2. Growth and Inhibition (Log Cfu/Ml) of E. Coli and Salmonella Test Strains in Pine Apple Juice Stored At Different Temperatures, Addis Ababa, February -May 1999.

| Time (h) | pH | *E. coli* (9637) | | *E. coli* (25922) | | *S. typhimurium* | | *S. choleraesuis* | |
|---|---|---|---|---|---|---|---|---|---|
| | | $37^0$c | $4^0$c | $37^0$c | $4^0$c | $37^0$c | $4^0$c | $37^0$c | $4^0$c |
| 0 | 3.80 | 2.78 | 2.78 | 2.78 | 2.78 | 2.30 | 2.3 | 2.95 | 2.30 |
| 16 | 3.76 | <1 | ND | <1 | ND | 3.52 | ND | 3.77 | ND |
| 24 | 3.76 | <1 | <1 | <1 | <1 | 4.20 | <1 | 5.22 | <1 |
| 40 | 3.78 | <1 | ND | <1 | ND | 4.74 | ND | 4.71 | ND |

The initial pH of orange juice was 3.1, which fell to 2.9 after 16 h of holding at ambient temperature. None of the four test strains were detected in orange juice at and after 16 h (Table 3) at ambient temperature or refrigeration holding.

Avocado juice had an initial pH of 6.0 and this fell by one unit within 24 h. Both *E. coli* test strains increased to counts $>10^7$ cfu/ml between 16 and 24 h and the counts dropped to $\leq 10^4$ cfu/ml in 48 h at ambient temperature holding. At $4^oC$, there was a limited but steady increase until end of holding time (48 h). A similar pattern of growth was also noted for *S. typhimurium* at ambient temperature holding, where highest count was noted at 16 h (Table 4).

Table 3. Growth and Inhibition (Log Cfu/Ml) of E. Coli And Salmonella Test Strains in Orange Juice Stored at Different Temperatures, Addis Ababa, February -May 1999.

| Time (h) | pH | *E. coli* (9637) | | *E. coli* (25922) | | *S. typhimurium* | | *S. choleraesuis* | |
|---|---|---|---|---|---|---|---|---|---|
| | | $37^0$C | $4^0$C | $37^0$C | $4^0$C | $37^0$C | $4^0$C | $37^0$C | $4^0$C |
| 0 | 3.1 | 2.78 | 2.78 | 1.98 | 1.98 | 2.75 | 2.75 | 2.70 | 2.70 |
| 16 | 2.9 | <1 | ND | <1 | ND | <1 | ND | <1 | ND |
| 24 | 2.9 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |
| 40 | 2.9 | <1 | ND | <1 | ND | <1 | ND | <1 | ND |
| 48 | 2.9 | <1 | <1 | <1 | <1 | <1 | <1 | <1 | <1 |

**Table 4.** Growth and Inhibition (Log Cfu/Ml) of E. Coli and Salmonella Test Strains in Avocado Stored at Different Temperatures, Addis Ababa, February -May 1999.

| Time (h) | pH | *E. coli* (9637) | | *E. coli* (25922) | | *S. typhimurium* | |
|---|---|---|---|---|---|---|---|
| | | $37^0$C | $4^0$C | $37^0$C | $4^0$C | $37^0$C | $4^0$C |
| 0 | 6.0 | 3.36 | 3.36 | 3.28 | 3.28 | 2.79 | 2.79 |
| 16 | 5.3 | 7.94 | ND | 8.26 | ND | 7.42 | ND |
| 24 | 5.0 | 8.13 | 3.51 | 7.73 | 4.72 | 5.30 | 3.68 |
| 40 | 4.8 | 4.23 | ND | 5.24 | ND | 3.61 | ND |
| 48 | 4.8 | 3.21 | 4.29 | 4.56 | 5.22 | 2.13 | 2.0 |

## DISCUSSION

The four test strains could grow luxuriously in papaya and avocado juice as the pH of these juice types was not inhibitory to the test strains. At the time when maximum count was reached, the pH of both juice types was $\geq$ 5.0. At this pH, most food borne pathogens can thrive well, thus making these juice types potentially hazardous to health. In a large outbreak of food poisoning caused by *Salmonella weltervreden* that occurred in 1996 in Singapore, papaya was among the fruits implicated in the outbreak (10). This is indicates that processing of such fruits for the preparation of the respective juices requires the following of strict hygienic procedures. This should include observing personal hygiene, thorough cleaning of fruits before blending, and thorough cleaning of the mixers or other blending equipment. It should also be noted that avocado and papaya juices are frequently used as major components of various juice cocktails. Thus, the fact that they are good media for the proliferation of pathogens should be considered during their preparation. In this study, the counts of the test pathogens reached infective doses in less than 16 h of storage. Thus the juices should be consumed within few hours of preparation and longer holding at ambient temperatures should be discouraged. As observed in this study, refrigeration markedly improves the safety of both juices. Counts after 24 h of refrigeration were either lower than the initial inoculum level or a slight increase was observed. In any case, final counts were much less than the infective doses for the respective strains.

The situation with orange and pine-apple juices was, however, different. They are generally considered as acidic juices and are not expected to support growth of pathogenic microorganisms. However, various acidic fruit juices have been implicated in outbreaks of gastroenteritis (7). Although orange juices were implicated in various *Salmonella* outbreaks (11), and although other laboratory studies on survival of *Salmonella* and other bacterial pathogens in orange juice support the hypothesis that acid juices could be vehicles of pathogen transmission (7), the orange juice in our study was much more acidic (pH $\leq$ 3.1) than the mean pH (3.7) reported elsewhere (7), and, therefore, eliminated the test strains at both holding temperatures. However, as pH of fruits may vary with season and within variety, the observation in this study may not be taken as conclusive.

The pH of pine apple in this study was around 3.8 and this was acidic enough to inhibit the *E. coli* test strains. This is particularly important as *E. coli* O:157 could jeopardize the health of young children and anyone with a challenged immune system. Both *Salmonella* test strains, however, could survive and even grow to a limited extent in pine apple juice

at ambient holding. Ooi et. al. (10) reported that pine apple juice was implicated in food poisoning outbreak caused by *Salmonella*.

Most of the well-publicized outbreaks of food borne illnesses have occurred in recent years due to the consumption of 'fresh' or unpasteurized fruit juices (6,7). Consequently, various recommendations are made to pasteurize fruit juices for the purpose of eliminating food-borne pathogens (6,12). Unfortunately, pasteurization also reduces the bioavailability of certain nutrients in the juice (13). In the developed countries, fruit juices are sold in the packed form and it is feasible to include pasteurization during the production process. In the case of the fruit juice market in Ethiopia, where preparation is done at juice vending house level, it may be useful to require fruit juice vendors to have procedures in place to reduce the number of disease-causing microorganisms to the same level achievable by pasteurization. They should be free to use microbial reduction methods other than pasteurization, including washing, scrubbing, antimicrobial solutions, alternative technologies or a combination of techniques as proposed by FDA (14).

This study indicated only the potential of some food-borne pathogens to grow in some fruit juices. It would be appropriate to determine the microbiological quality and safety of fruit juices sold in various vending houses as made available for consumption in order to establish their role in the epidemiology of food- borne diseases in the country.

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