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FUNGAL AND BACTERIAL CONTAMINATION OF FRESH FRUITS AND VEGETABLES SOLD IN HAWASSA TOWN OF SOUTHERN ETHIOPIA

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Abstract

For good health, including fresh fruits and vegetables in our daily diet is very important. However, fresh fruits and vegetables are highly perishable and affected by different microbial contaminants from production up to consumption. Therefore, the objective of this study was to evaluate the important microbial spectrum of selected fruits and vegetables and their management from Hawassa (Ethiopia) town markets. A total of 27 fruit and 9 vegetable samples were analyzed for pathogens from their surface wash. The mean aerobic mesophillic count (AMC) ranged from $2.04x10^{-7}$cfu/ml to $3.2x10^{-6}$cfu/ml from the surface wash and the total coliform count (TC) ranged from 2.50^{LTBc}cfu/ml $x10^{-7}$ to $1.5x10^{-6}$cfu/ml. Fecal coliform ranged from $3.2x10^{-6}$ to 0 cfu/ml and the range for Salmonella spp. and *Staphylococcus aureus* were $1.8x10^{-6}$ to 0 cfu/ml and $2.50^{LTBc}x10^{-7}$ to 0 cfu/ml respectively. Mold and yeast count ranged from $7.6x10^{-6}$ to $0.2x10^{-4}$ from the fruit surface wash. There was statistically significant difference regarding to AMC and TC between markets in between banana and orange samples and they were detected in all fruit samples. From the 36 samples *E. coli* (except M2 in cabbage) and molds and yeast were found in all fruit samples, and Salmonella and *Staphylococcus aureus* were detected in 89% fruit samples in each. Activities during harvesting, transportation, storage and marketing conditions favored contamination of most commonly used fruits banana and orange. Besides, poor hygiene of the venders, using microbially unsafe containers, poor handling practice and poor environmental conditions such as sanitarily unsafe marketing environment were identified to be another sources of

contamination. Hence, for safe and clean supply of fruits, community members working with the fruits must be trained on the ways by which fruits can be contaminated and the safe methods of harvesting, transportation, storage and vending the fruits.

Keywords: Total coliform, Fecal coliform, Fresh fruits, Hygienic condition, Contamination

1. INTRODUCTION

Fruit is part of flowering plants that is derived from specific tissues of the flower, one or more ovaries, and in some cases accessory tissues. The importance of fresh fruits in nutrition and healthy diet are well known and nowadays many countries have undertaken various initiatives to encourage consumers to eat more of these products. Fruits supply much needed vitamins and minerals. They play an important role in health through the prevention of different diseases (Asmaru Gultie *et al*. 2013).

Besides, it is obvious that humans and many animals have become dependent on fruits as a source of food (Asmaru Gultie *et al*. 2013). Fruits are low in calories and naturally sweet. Fruit and their juices are good sources of water. Different fruits contain different vitamins, so it is important to eat a variety of fruits. Mangoes, papayas, melons and citrus fruits, like oranges and grape fruits are high in vitamin c, whole fruits like apples and gapes contain more fiber than juices and sauces, like apples sauce and grape juice.

The consumption of fruits and vegetables helps in protecting human body from number of diseases by providing nutrients, minerals, vitamins, proteins and fibers. Fruit also contain various phytochemicals which could have a positive impact on body weight regulation and related condition including diabetes, hypertension, as well as reduce the risk of cancer, cardiovascular diseases, cataracts, and some of the functional weakness associated with aging (Mauseth, 2003).

Diets that include a sufficient amount of potassium from fruits and vegetables also help reduce the chance of developing kidney stones and may help reduce the effects of bone-loss. Fruits are also low in calories which would help lower one's calorie intake as part of a weight-loss diet. Fruits are essential for good health and they form major components of human diet in every family. Thiamine, riboflavin, vitaminB6, niacin, folate, vitamin and phytochemicals in fruits and vegetables, such as polyphenolics, carotenoids, and glucosinolates, may also have nutritional value. While many fruits and vegetables are

consumed primarily in their fresh state, some produce such as tomatoes, snap beans, corn, peaches, nectarines, and pineapples are also consumed to a significant degree in their processed state. They are vital energy contributors which are depended upon levels of human as food supplement for nutrient (Manso *et al*., 2009).

Fruits require water for their growth and those eaten raw and without peeling have been demonstrated to be a way for transmission of a wide range of parasites (Gharavi *et al*., 2002). Range of microorganisms associated with outbreaks linked to fresh fruits encompasses bacteria, viruses and parasites. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* O157 in sprouted seeds and fruit juices are of particular concern. The viruses involved in outbreaks have a human reservoir (example. Norwalk-like and Hepatitis A) and can be associated with intact products grown in contact with the soil and/or water. Outbreaks linked to protozoa (e.g. *Cryptosporidium, Cyclospora, Giardia)* have been associated more with fruits than with vegetables.

Food borne illness caused by microbial contamination is still a public health problem in developing countries like Ethiopia. Raw fruits and vegetables are one of the main sources of illness because they have been consumed without proper washing. According to Ethiopian Public Health Institute (EPHI) food microbiology guideline, no person shall sell or consume fruits that; is assumed to have any poisonous or harmful substance, unfit for human consumption, consists in whole or in part of any filthy, putrid, disgusting, rotten, decomposed or diseased fruits, spoiled, manufactured, prepared, preserved, packaged or stored under unsanitary conditions.

Further no person shall manufacture, prepare, preserve, package or store for sale any fruit under unsanitary conditions (Kiiyukia, 2003). Personal observation evidence shows that, different market areas in Hawassa city are not properly hygienic area for fruit vending. Additionally, it seems that the distributors, vendors and consumers have no significant awareness about the possibility of getting infection through these contaminated fruits. Most of the consumers eat these fruits directly after purchasing them without proper washing. Therefore, the objective of this study is to assess the microbial contamination rate, frequency, and prevalence on raw fruits sold in Hawassa markets.

3. MATERIALS AND METHODS

3.1 Study area description

The study was conducted in Hawassa, a capital city of SNNPR located at 275km from Addis Ababa. The geographical coordinates of the city is 7°3′N 38°28′E/7.050°N 38.467°E and an elevation of 1708 meters above sea level.

3.2 Sample collection

Market places from where to collect samples were selected. Three different fruits; orange, banana and tomato and one vegetable, cabbage were randomly collected from three market sites selected and were packed in polyethylene bags separately and were transported to the laboratory of department of Biology Hawassa University for examination.

3.3 Sample preparation

Samples were prepared for Microbiological Analysis on the Fruit Surface (Wash)**.** The fruit and vegetables surface wash was examined for aerobic plate count, total coliform, fecal coliform, enumeration of Salmonella, *Staphylococcus aureus and* mold and yeast count. 25 g of the sample was aseptically weighed and rinsed thoroughly in 250 ml of distilled water for five minutes. The washes were analyzed according to standard procedures.

3.3.1 Aerobic mesophilic count (AMC)

5.8 g of the powdered plat count agar (PCA) medium was suspended in a 250ml of distilled water, mixed and left on the bench to stand until the mixture is uniform. The mixed solution was heated with gentle agitation and allowed to boil until completely dissolved and sterilized in the autoclave at 121 ^{0}C for 15 minutes. Allowed to cool to 45^{0}C and powered onto sterile petri-dishes. The plates then, allowed to cool at room temperature for two hours for the media to solidify and stored at suitable temperature in the refrigerator.

Aerobic mesophilic count of all the fruits at surface was determined by standard plate count method as described by APHA on prepared plat count agar medium (Don whitely eqp. Pvt.ltd-India). One mL of the sample was serially 10 fold diluted in 0.1 % of buffer peptone water (Don whitely eqp. Pvt.ltd-India). 0.1 mL from each serially diluted sample (10^{-1} to 10^{-6}) was pour plated on standard plate count agar medium. The samples were incubated at 37 ^{0}C

for 24 hours. After incubation plates with colonies in between 25-250 were counted according to Downes *et al*. (2001).

3.3.2 Enumeration of total coliform

Eosin Methyl Blue agar (EMB) was prepared according to manufacturer's instructions. 10.4 g of the powdered EMBA medium was suspended into 250ml of distilled water. The medium was boiled for 1 minute with frequent agitation to dissolve completely. Then, the medium solution was sterilized in the autoclave at 121 ^{0}C for 15 minutes and then allowed to cool to 45 ^{0}C and powered to sterile petri dishes. The peti dishes were left at room temperature for two hours for the medium to solidify and then stored at appropriate temperature in the refrigerator.

Total coliform count (TCC) was determined using EMB as recommended by APHA. For the enumeration of total coliform using Eosin Methyl Blue agar 0.1 ml from each serially diluted sample (10^{-1}- 10^{-6}) were pour plated on Eosin Methyl Blue agar (EMB). Then the plates were incubated at 37℃ for 24-48 hours. After incubation colonies between 25- 250 were counted.

3.3.3 Isolation and identification of *E. coli*

The presence of *E. coli* was confirmed by using the following standard method. One mL of the sample was diluted in nine mL of lactose broth and incubated at 37^{0}C for 24 hours. The sample was examined for gas formation. After shaking the lactose positive broth one loop full sample from the tube was transferred in to 10 mL of *E. coli* (EC) (DIFCO 0314-01-0) broth. The sample was incubated for 24 hours at 45 ^{0}C. From EC broth cultures which were gas positive one loop of culture was streaked on EMB agar (Himedia, M022S India). The culture was incubated at 35 ^{0}C for 18-28 hours. Dark center colonies with metallic sheen were considered as indicative of *E. coli* (FDA, 2002). After incubation colonies between 25- 250 were counted.

3.3.4 Detection of *Salmonella spp.*

For the isolation of *Salmonella spp.*, *Salmonella-shigella*(SS) agar was used (Don Whitley eqp. pvt.ltd.-India). After preparing the medium following manufacturer's instructions, 0.1 mL of the sample was spread plated on the *Salmonella-shigella* agar and incubated at 37^{0}C for 24 hours. After incubation colorless colonies between 25- 250 were counted.

3.3.5 Enumeration of *Staphylococcus aureus*

Staphylococcus aureus was counted using Manitol salt agar (MSA). 0.1mL of the appropriate sample dilution was inoculated by spread plating on to manitol salt agar (MSA) plates and incubated at 37 ℃ for 24-48 hours. After incubation colonies between 25- 250 were counted.

3.3.6 Mold and yeast count

9.75gm of Potatoes Dextrose Agar (PDA) was suspended in 250 ml of distilled water. The content was boiled to dissolve the medium completely. Then the medium was sterilized by autoclaving at 121^0C under 15Lb pressure for 15 minutes and allowed to cool to 45^0C. Then, the medium was well mixed before dispensing and poured to Petri plates.

One ml of the sample was serially 10 fold diluted in 0.1 % buffer peptone water. 0.1 ml from each dilution (10^{-1} to 10^{-6}) of the serially diluted sample was spread plated on PDA in duplicates. To suppress growth of bacterial colonies broad spectrum antibiotic was added to the medium before incubation. The plates were incubated at 21^0C for 5-7 days. After incubation colonies between 10 -150 were counted.

3.4 Data Analysis

The data collected from all the experiments was subjected to the analysis of appropriate statistical packages and SPSS version 20 computer software. Average values were used for duplicates; the data and all the countable dilution were used to calculate the average number of colonies in terms of colony forming unit per milliliter cfu/ml. $P<0.05$ was used as statistically significant association.

Besides, quantitative data collected in the laboratory, qualitative data collected through social survey were analyzed by one way ANOVA. The analyzed data was presented in the form of Mean±SEM. The counted bacterial colonies were calculated using the following formula:

cfu/ml= no. colonies x dilution factor/volume of culture plate

4. RESULTS AND DISCUSSION

4.1 Results

4.1.1 Microbiological Analysis on the surface Wash of Fruits and vegetables

The fruit and vegetable types, number of samples, count in cfu/mL of Aerobic Mesophilic Count, Total Coliform, Faecal coliform, *Salmonella* sp., *Staphylococcus aureus* and Yeast and Mold Count are provided in table 1.

Table 1. Number of cfu/ml sample for each market and the respective fruits and vegetables (n=3)

Types of Fruits and vegetables	No. of Samples	AMC cfu/ml	TC cfu/ml	FC cfu/ml	Sal cfu/ml	Sta cfu/ml	MY cfu/ml
Cabbage							
M1	3	$9.2x10^{-6}$	$6.5x10^{-6}$	$5x10^{-5}$	$1.2x10^{-6}$	$5x10^{-5}$	$2.2x10^{-6}$
M2	3	$7.5x10^{-6}$	$2.2x10^{-6}$	0	$0.1x10^{-4}$	$3.6x10^{-6}$	$1.01x10^{-7}$
M3	3	$3.2x10^{-6}$	$1.5x10^{-6}$	$0.2x10^{-4}$	$0.1x10^{-4}$	$0.4x10^{-4}$	$2x10^{-5}$
Tomato							
M1	3	$4.2x10^{-6}$	$1.11x10^{-7}$	$2.6x10^{-6}$	$0.7x10^{-4}$	$0.4x10^{-4}$	$1.45x10^{-7}$
M2	3	$9.5x10^{-6}$	$3.5x10^{-6}$	$3.2x10^{-6}$	$0.6x10^{-4}$	$2.2x10^{-4}$	$7.6x10^{-6}$
M3	3	$7.6x10^{-6}$	$6.6x10^{-6}$	$0.1x10^{-4}$	$0.2x10^{-4}$	$4.6x10^{-4}$	$7.5x10^{-6}$
Orange							
M1	3	$2.24x10^{-7}$	$2.50x10^{-7}$	$2.7x10^{-6}$	$1.5x10^{-6}$	$1.95x10^{-7}$	$1.36x10^{-7}$
M2	3	$2.04x10^{-7}$	$1.91x10^{-7}$	$0.7x10^{-4}$	$2x10^{-5}$	$2.50x10^{-7}$	$5.1x10^{-6}$
M3	3	$1.19x10^{-7}$	$1.15x10^{-7}$	$0.4x10^{-4}$	0	$1.01x10^{-7}$	$1.8x10^{-6}$
Banana							
M1	3	$1.82x10^{-7}$	$1.17x10^{-7}$	$0.4x10^{-}$	$6x10^{-5}$	$1.62x10^{-7}$	$2.8x10^{-6}$
M2	3	$1.81x10^{-7}$	$1.79x10^{-7}$	$1.7x10^{-6}$	$0.4x10^{-4}$	$4x10^{-5}$	$0.2x10^{-4}$
M3	3	$1.12x10^{-7}$	$1.09x10^{-7}$	0	$1.8x10^{-6}$	0	$0.4x10^{-4}$

Key: *AMC-aerobic mesophilic bacterial count; TC-total coliform; FC- faecal coliform; Sal-Salmonella sp.; Sta- staphylococcus aureus; MY- Mold and Yeast; M1-market 1; M2-market 2; M3-market 3*

Comparative study of all markets with respect to bacterial load indicates the maximum mean AMC from market 1 (2.24 x 10^{-7}cfu/mL) in orange to the least (3.2 x 10-6cfu/mL) from market 3 in cabbage. The highest total coliform load was recorded on banana samples from

all markets and ranged from 1.79 x 10^{-7}cfu/mL in market 2 to 1.5 x 10-6 in market 3 on cabbage samples. Fecal coliform load ranged from 0cfu/mL on cabbage on M2 and banana M3 to 3.2 x 10-6 on tomato samples from market 2(Table 1).

Load of Salmonella species, *Staphylococcus aureus* and Mold and Yeast load was ranged from 0.1 x10-4 in markets 2 and 3 on cabbage samples to 1.8 x 10-6 in market 3 on banana samples, from 1.95 x 10^{-7} cfu/mL in market 1 on orange to 0 cfu/mL from market 3 on banana samples, and from 0.2 x 10-4 on banana samples from market 2 to 7.6 x 10-6 on tomato samples from market 2 respectively (Table 1).

Figure 1 below demonstrates that, in all cases AMC is larger than all other values. The least load of *Staphylococcus aureus* was also recorded for tomato samples and the least mold and yeast load was recorded for banana samples.

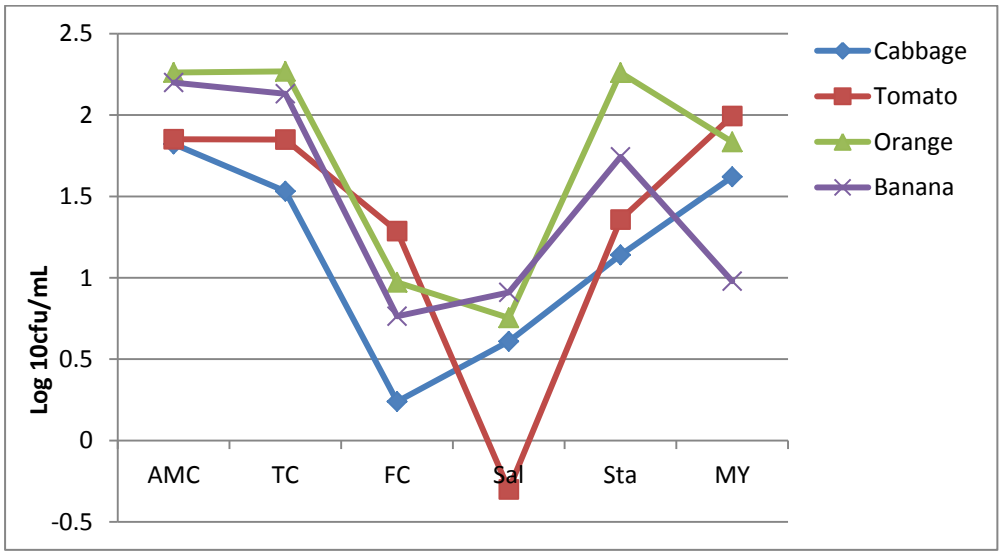

Figure1. For the presence of aerobic mesophilic count, total coliform, fecal coliform, salmonella sp., *Staphylococcus aureus* and mould and yeast count from the fruit surface wash samples analyzed (log10 cfu/mL). **Key:** *AMC-aerobic mesophilic bacterial count; TC-total coliform; FC- faecal coliform; Sal-Salmonella sp.; Sta- staphylococcus aureus; MY- Mold and Yeast.*

When the above values are log10 transformed, the grand mean aerobic mesophilic count (AMC) of orange and banana were 2.26 and 2.19 log10 cfu/ml, respectively (Table 2). The grand mean total coliform of banana and orange were 2.27 and 2.13 log10 cfu/ml respectively and the mean fecal coliform, S*almonella* sp. and *Staphylococcus aureus* were 1.29 & 0.97; 0.91 & 0.75 and 2.26 & 1.74 log10 cfu/ml respectively. The grand mean mold and yeast count of banana and orange were 1.99 & 1.83 log10 cfu/ml respectively. The AMC also indicated that all fruit surfaces contain large bacterial load in unacceptable level according to international guidelines.

Table2. (Mean±SEM) aerobic mesophilic count, total coliform, fecal coliform, Salmonella sp. *Staphylococcus aureus* and mold and yeast count from the fruit and vegetable surface wash samples log10(cfu/L).

Fruits and vegetables	AMC (logcfu/mL)	TC (logcfu/mL)	FC (logcfu/mL)	SAL (logcfu/mL)	STA (logcfu/mL)	MT (logcfu/mL)
Cabbage	1.82^{a}	1.53^{a}	0.24^{a}	0.61^{b}	1.14^{a}	1.62^{b}
Tomato	1.85^{a}	1.85^{a}	1.29^{d}	-0.30^{a}	1.36^{a}	1.99^{c}
Orange	2.26^{b}	2.27^{c}	0.97^{c}	0.75^{b}	2.26^{c}	1.83c
Banana	2.19^{b}	2.13^{b}	0.76^{b}	0.91^{c}	1.74^{b}	0.98^{a}

Key: *AMC-aerobic mesophilic bacterial count; TC-total coliform; FC- faecal coliform; Sal-Salmonella sp.; Sta- staphylococcus aureus; MY- Mold and Yeast. Similar letters in columns show not significant difference between samples at p<0.05.*

4.2 Discussion

This large bacterial load might be contributed from different sources such as from pre-harvest, harvesting and poor handling practices at the postharvest activities. The assessment on the management of fruit in these marketing areas indicated that there were different conditions such as poor handling of fruits, sanitary problem of some marketing areas. These conditions might have their own contribution on the microbial load for that area. Regarding to the market area the maximum mean aerobic plate count and total coliform was found at the three markets studied.

The difference might be from the handling and sanitary differences in the market around the three markets. The markets are crowded by vehicles that emit dust particles; the fruits were transported from long distances, harvested un-hygienically and stored open exposed to several contaminants. When the fruits were collected (harvested) it may be exposed to feces from domestic animals which could be the source of different contaminants too.

More over the fruit handlers put fruits on ground without using covering material and used the measuring balance for different commodities such as onion, tomato, different vegetables and cereal crops. These factors could increase the microbial load of fruit. From statistical

analysis, there was no statistically significant difference between the mean AMC of the three markets ($p<0.05$). That is the markets or the areas in which fruit marketing taking place had significant impact on the value of AMC of fruits samples. This may be due to different variables such as the handling practice of fruits and sanitary condition of the area.

The presence of coliform bacteria especially *E. coli* indicated that fecal contamination of fruits which may be contributed from different sources such as the water used during pre-harvest and postharvest activities, exposure during transport facility, improper storage conditions and poor handling practice at any stage (NACMF, 1999). From statistical analysis $p<0.05$, there was significant difference between total coliform and fecal coliform counts among the markets. This shows that the market area had impact on the total and fecal coliform counts. This may be attributed from different variables such as the sanitary condition of the market area and handling practice difference in different markets. Based on the type of fruit, there were no significant differences between fruits that contained total coliforms.

The mean mold and yeast count ranged from 0.98 log10 cfu/ml to 1.99 log10 cfu/ml from both banana and orange. Since $p=0.003<0.05$ there was a significant difference between the mean of mold and yeast count between markets. As to date, in Ethiopia there is no standard guideline for comparing the microbiological safety of fresh fruits. However, different countries give more concern for the microbiological safety of fruits. For instance, South Africa has proposed a standard guideline for raw fruits and vegetables. According to the South African guideline the yeast and mold count greater than $1x10^5$cfu/ml in any raw fruits and vegetables has no acceptance (South Africa department of health, 1997). The average mold and yeast count from the Hawassa town markets was greater than $10^5$and therefore; they are unacceptable for consumption unless otherwise proper sanitary measures are taken.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

On the basis of the findings of the study it was concluded that the presence of *E. coli* in most fruit samples, banana and orange studied, harbored high microbial load most of which are dangerous to human health such as *Salmonella spp* caused by fecal contamination at any stage of fruit processing. High microbial load was found on the surface wash. However, there was no significant difference for aerobic mesophilic count and total coliform count from the

wash. Slight statistically significant difference in aerobic mesophilic count and total coliform count were identified among the marks which might be due to the presence of different variables that enhanced microbial load. All fruit types in the study markets harbored known postharvest fruit spoilage molds. The presences of such spoilage molds have an economical and health risk in which they are sources of different secondary metabolites. The presence of pathogens in these fruits can cause health risk on the consumers. From the assessment of fruit management, a number of deficiencies were identified in the study area. Fruit venders were unaware of the health risk of fruit and causes of fruit contamination. Poor handling practice during storage, measuring or weighting, loading and unloading from the vehicles, unable to use waste collection bin were also identified as deficiencies in most of the studded markets. Despite the high microbial counts obtained from the samples in this study it is important to note that the samples did not show any visible sign of spoilage. Thus out ward appearance may not be a good criterion for judging the microbial quality of fruits. So, all fruits should be adequately washed before consumption to reduce the microbial load.

5.2 Recommendation

The responsibility to safeguard fruits from contamination is shared by everyone involved from the grower to the consumer. Education and knowledge are tools to improve fruit safety controls. On the basis of these facts the following recommendations are forwarded:

- Farmers should be trained on the methods of fruit harvesting, storage and transportation to markets
- The vendors should also be trained on how to store, display, weight and sell the fruits
- training the fruit handlers such as fruit venders or sellers and workers engaged in loading and unloading fruits from the vehicles about fruit borne diseases, contamination of fruits, handling of fruits and the impact of hygienic problems on the safety of fruits is essential.
- Concerned bodies should introduce supervising and monitoring system on the sanitary condition of fruit marketing areas and the feasibility of transport system.
- Further studies should be conducted on the areas such as the necessary alternative decontamination methods of fruits and the economic impact of fruit loss.

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