A PRELIMINARY STUDY OF THE MICROFLORA LEVEL OF SOME FRUITS AND VEGETABLES:

Pre and Post - Preservation

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ABSTRACT: Samples of 15 different fruit and vegetable types were purchased from five small groceries around Addis Ababa. Enumeration, isolation and identification processes were performed for the microbial flora of each sample before and after 15-30 days of preservation. Both direct and enrichment culture media were used to distinguish these microflora as members of the groups of normal contaminants, indicators, spoilage and food-borne disease causing organisms. The overall result of this work indicates that a total of 25 different organisms, comprising 3 (12%) indicators, 15 (60%) spoilage, 5 (20%) food-borne pathogens and 2 (8%) normal contaminants, were isolated. The predominant isolates of these groups were *Escherishia coli* type I, among the indicators *Bacilli*, molds and *Enterobacter species* among the spoilage and *Staphylococcus aureus, Salmonella typhimurium* and *Bacillus cereus* among the pathogens.

The direct inoculation method revealed the isolation of more than one indicator organisms from each of 7 (46.7%) fresh or unpreserved fruit and vegetable samples, more than 3 spoilage organisms from each of 8 (53.3%) samples, and, at least, one pathogenic organism from each of another 8 (53.3%) samples. When enrichment culture method was applied, all these results were increased as: >1 indicators in 10 (67%), >3 spoilage organisms in 9 (60%), and pathogens in 9 (60%) of the unpreserved samples. The enumeration values of the indicator/organisms and the spoilage groups of mesophyllic aerobes/anaerobes, molds and/or yeasts were all above the accepted limits for such fruits and vegetable samples. This was shown to be true in 10 (66.7%), 4 (26.7%) and 6 (40%) samples for the indicators, spoilage mesopheles, and mold or yeasts respectively.

The preservation processes have proved to be effective in eliminating or reducing the numbers and types of the organisms isolated from each fresh sample. The effectivity of the preservation methods is discussed and its applicability in a simplified and comprehensive manual for a small-scale (household level) preservation of fruits and vegetables has been recommended.

INTRODUCTION

Fruits and vegetables can be obtained as fresh, frozen, fermented, pasteurized or canned, brined, salted and pickled forms. In whatever form they exist fruits and vegetables are never totally free of micro-organisms even after being preserved (1-3). The number and kinds of normal micro-organisms vary with the differences in the forms of the fruit

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and vegetables. They can also be contaminated with micro-organisms of three other categories, namely, indicator organisms, spoilage organisms and food-borne disease causing pathogens (4-8).

The indicator organisms are those which serve to indicate objectionable conditions of foods such as, recent or remote faecal contamination, presence of potential pathogens or potential spoilage of food, as well as the sanitary conditions of the food precessing, production and storage facilities. The most commonly encountered and widely accepted organisms include:

i) the coliforms which comprise *Escherishia coli, Citrobacter fruendi, Enterobacter aerogenes. Enterobacter cloacae* and *Klebsiella pneumoniae,*

ii) faecal coliforms which consist primarily of *E.coli* type I, a few *Enterobacter* and *Klebsiella* strains,

iii) faecal streptococci of the enterococci group which consist of *S. Faecalis,* and *S. faecium* along with two subspecies of *S. faecalis* (liquefaeciens and Zymogenss), and

iv) other organisms like *Clostridium perferingens* and *Pseudomonas aeruginosa* that act as remote faecal contamination indicators.

The spoilage organisms are those which result in the deterioration of food quality by the action of their enzymes altering certain food components and alterations in the appearance, texture, colour, odour, or flavour, or by slime formation. The organisms of this group vary with the type of food, condition of growth and action of their spoilage effect on the material. The third group of food micro-organisms consists of the food-borne disease causing pathogens which include all organisms that cause either food-borne infection or food intoxication (food poisoning) after the ingestion of the organisms themselves or their toxigenic products with the food. There are also very many specific types of pathogenic organisms which differ according to the kind of the food vehicle that allows selectively the growth of these organisms.

Freshly harvested fruits and vegetables usually contain micro-organisms whose sources include poorly handled containers, mechanical damage during transportation, agitation or spraying when washing or soaking in water and environmental sources like soil, air, water and animal or human bodies (9). Vegetables growing in close contact to soil have the highest number of aerobic spore formers, coryne forms and other soil organisms. The high acid and sugar content of fruit and the high carbohydrate content of vegetables favours the growth of yeasts or molds and lactic acid bacteria, respectively (10). Many reports (4,8,11,16-19) have indicated that the natural microflora of fresh fruits and vegetables include members of the following genera: *Achromobacter, Pseudomonas, Enterobacter, Alicaligenes, Bacillus, Chromobacter, Clostridium, Micrococcus, Serratia, Staphylococcus, Streptococcus* and many molds or yeasts.

Generally fruits and vegetables will have their qualities deteriorated by many intrinsic and extrinsic factors, primarily, the growth and action of the different categories of micro-organisms. Most of the spoilage organisms cause rots of different forms such as bacterial soft rots, mold rots, coloured rots and sliminess or souring effects. These can be acquired during storage, transit, marketing and processing periods. Because most fruits and vegetables are seasonal and perishable, their keeping qualities prevent their consumption throughout the whole year (12). In order to prevent such fruits and vegetables from the actions of spoilage or pathogenic organisms several preservative methods are practised in many developed countries (3). In general principle, most preservative methods include actions like asepsis (removal of microbes), use of heat or low temperature (chilling or freezing), drying, use of preservatives, irradiation and ionization (4,27-29). Fermentation, pasteurization and canning of fruit and vegetable juices and concentrates are also usual practices for preventing food hazards or spoilage factors.

In Ethiopia fruits and vegetables are among harvested products with their uses limited to only the season of abundance due to lack of facilities, skill and knowledge, as well as economic capability for food preservation activities. Food crisis and drought in the country has, however, forced all sectors of people to make use of home gardening. Interests on growing fruits, vegetables and root-crops and their use for human consumption have recently increased in the country. But their poor storage quality prevents fresh fruits and vegetables from

being consumed in the lean period. This then initiates high wastage and in turn affects the already shaky nutritional status of the people (10). The availability of home and cottage-level preservations of such perishable food stuffs is therefore, indispensable both in the urban and rural settlement.

Hence, the aim of this study is to assess the microbiological quality of some fruits and vegetables both pre and post-preservation using different methods. The ultimate objective is to prepare a simple and comprehensive manual that can serve for household, hotel and hospital level preservations of fruits and vegetables, thus contributing to the reduction in the existing wastage of large quantities of such products and make them sources of our essential nutrients throughout the whole year.

MATERIALS AND METHODS

Sample collection: Fifteen different samples were purchased from 5 small private groceries at different zones of Addis Ababa. No specific selection criterium has been used except for the availability of the products in the visited groceries. The samples included Avocado *(Persea americana)*, Banana *(Musa sapietia)*, Grape fruit *(Citrus paradis)*, Guava *(Psidium quajava)*, Mango *(Mangifera indica)*, Orange *(Citrus sinensis)*, Papaya *(Carica papaya)*, Pineapple *(Ananas comosus)*, Tomato *(Lycopersicon esculentum)*, Carrot *(Daucot carota*), Cauliflower *(Bassica oleracia)*, Garlic *(Allium Ativum)*, Onion *(Allium ascalonicum)*, Kidney Bean *(Phasealus vulgaris)* and Potato *(Solanum tuberosum)*.

A portion of each sample was immediately submitted to the Public Health Bacteriology laboratory of National Research Institute of Health (NRIH) for the initial bacteriological analysis. The other portion of each fruit and vegetable samples, taken to the Food Technology Pilot Plant of Ethiopian Nutrition Institute (ENI) was processed by one or more preservation methods. All preserved samples were finally submitted to the NRIH lab for similar bacteriological analysis after 15 to 30 days of preservation. All samples were categorized into three groups on the basis of the application to similar preservation processes. Group I consists of the samples of papaya and guava only, both of which were preserved as jam or jelly. Group II consists of samples of the fruits avocado, banana, grape fruit, orange, mango and pineapple, all of which were preserved as squash and juice form. Group III consists of samples of the vegetables carrot, onion, potato, cauliflower, garlic, tomato and kidney bean preserved in 2% salt brine and packed in tight glass jars.

Sample preparation: Five field samples were required for the bacteriological analysis from each types of pre and post-preservation fruits and vegetables. In order to get a homogenous suspension of the microbes samples were prepared for easy pipetting. A standard process of sample preparation (10,15) was undertaken in the following way:- In the case of the solid portion of the unpreserved or preserved fruit and vegetable samples, all the 5 field samples were mixed together and 20 to 30 pieces were randomly picked up. The external surface of each piece was sterilized with denatured 95% ethanol thoroughly charred and with the flame of a bunsen burner. From the internal part of each of these samples small pieces were removed with sterile knife, spatula or spoon and transferred to a blender and aseptically blended. Then an 11 gm or 25 gm portion of the blended tissue was put into sterile flasks depending on the amount of available material. A diluent of 225 ml sterile saline was added onto the 25 mg sample unit and 99 ml of this diluent on the 11 gm sample unit. In both cases, the mixture was allowed to stand for 30 min. and vigorously shaken for 2 to 3 min. From these dilutions further serial dilutions of up to 10^{-4} were prepared. In the case of juice or liquid portions of the preserved or unpreserved fruit and vegetable sample an aliquot amount of 1 ml was added to 9 ml of the diluent to get the 10^{-1} dilution and further serial dilutions were prepared.

For each of the prepared sample the following bacteriological analyses were conducted:

1) Enumeration, isolation and identification of the indicator and spoilage organisms such as the mesophyllic aerobes and/or anaerobes, lactic acid bacteria, H_2S producers, thermophilic spore formers and molds/yeasts, and

2) Isolation and identification of specific food-borne disease causing pathogens.

The enumeration activity is performed to estimate the microbial population per gram of each sample by using all the serially diluted preparations. This included first, the estimation of coliforms, faecal coliforms and other indicators by using three tubes of Lauryl sulphate Trypton broth (LSTB) and *Escherisnia Coli* broth (EC) per dilution factor and determination MPN (most probable number) of these organisms according to the AOAC (16) standard method. Secondly, the mesophyllic count was done by using pour plate method where molten and cooled (at 42^0C to 45^0C) plate count agar (PCA) is poured over 1 ml of each dilution in a pair of plates. One of the paired plates of each sample was incubated aerobically at 35 ± 2^0c for 48 hrs and the other plate of the pair was incubated in the same way using anaerobic gas jar for the anaerobic organisms. Thirdly, another 1 ml portion of each dilution was transferred into a pair of plates, over each of which was poured, molten and cooled potato dextrase agar (PDA) (OXOID) to count the total population of molds and or yeasts. One of each pair of these plates was incubated at room temperature (22^0c) for 5 to 7 days while the rest of the pairs were incubated at $35+2^0$C for 3 to 5 days. Fourthly, the lactic acid bacterial population was determined by using a 1 ml portion of each dilution in duplicate plates over which is poured, molten and cooled Rogossa agar media (DIFCO). All plates were incubated at 32^0c for 3 days to allow the growth of gram-positive, catalase-negative and non-spore forming rods, typical colonies for the lactic acid bacteria. Lastly, the H_2S-producing bacteria were also counted by using pour-plating technique with the sulphite agar medium and incubating different plates at $35+2^0$C both aerobically and anaerobically with anaerobic gas jar as well as at 55^0c for the thermophilic spore forming organisms. All of these incubations were done for 24 or 48 hours.

In all the above mentioned five enumeration activities, the plates with 30-300 typical colonies of each group were considered to count and multiply their averages with the dilution factors to get the total number of each organism per gram of the sample.

For the purpose of isolation and identification of each specific type of spoilage, indicator and pathogenic organisms we have used both direct and enrichment inoculation techniques. In the direct method, a loopful of undiluted blended or juice unit sample of each type was inoculated on general solid media like Blood agar, Glucose normal agar and MacConkey agar plates, as well as some selective media like Mannitol salt agar for *S.aureus;* Rogossa agar for *Lactobacilli*; Salmonella-Shigella agar for *Salmonella spp.*; *Bacillus cereus* selective agar; and perfringens agar and Wilkins Chalgrin Anaerobe agar for *Clostridium perfringens* and other anaerobic organisms (all of which were prepared from OXOID products). We used Thioglycholate, and Cooked Meat broth media for the enrichment of the anaerobic organisms while Kauffmann broth for enriching *Salmonella spp.*, and Tryptone Soy Yeast (TSY) broth for all the aerobic organisms. In both the direct and enrichment inoculation methods, the typical colonies were identified to the species or genera levels by using the standard biochemicals of both the Gram positive and Gram negative organisms.

RESULTS

The bacteriological analysis of 15 different types of fruit and vegetable samples gave a total of 25 species of 19 genera. These were isolated and identified as indicator organisms in 12%, spoilage in 60%, food-borne disease causing pathogens in 20% and normal contaminants in 8% of the total organisms. From the results

Table 1. Isolates from unpreserved samples of the three groups of fruits and vegetables

Group and names of Isolates	Number of samples with isolates

	Group I (n=2)		Group II (n=7)		Group III (n=6)		Total (n=15)	
	D	E	D	E	D	E	D (NO %)	E (No %)
Normal Contaminats								
1.S. Viridans	-	-	2	4	-	2	2(13.3)	6(40)
2.S.epidermis	1	-	1	3	-	4	2(13.3)	7(46.7)
Indicators								
1.Coliforms	-	-	2	4	4	6	6(40)	10(667)
2. E.Coil	1	2	4	5	6	6	11(73.3)	13(80)
3. S. faecalis	-	1	2	4	-	1	2()13.3)	6(40)
Spoilage								
1. Flavobacter	-	-	1	1	-	1	1(6.7)	2(13.3)
2. Micrococcus	-	-	1	2	-	3	1(6.7)	5(33.3)
3. Pseudomonas	-	-	-	1	2	2	2(13.3)	3(20.0)
4. Alkaligenes	-	-	-	1	1	1	1(6.7)	2(13.3)
5. Achromobacter	-	-	-	1	1	1	1(6.7)	2(13.3)
6. Bacillus	1	2	7	7	6	6	14(93.3)	15(100)
7. Interobacter	1	1	3	4	4	6	8(43.3)	11(73.3)
8. Serratia	-	-	6	1	4	3	10(66.7)	4(26.7)
9. Molds	-	-	6	1	4	3	10(66.7)	4(26.7)
Pathogens								
1. S. aureus	1	1	3	2	1	1	5(33.3)	4(26.7)
2. B.cereus	-	1	1	3	1	2	2(13.3)	6(40)
3. S.typhimurium	-	-	-	-	-	2	-------	2(13.3)
4.Shigella spp	-	-	-	-	1	-	1(6.7)	-------
5. S. pyogenes	-	-	-	-	1	-	1(6.7)	-------
Total 19	5	8	39	44	36	50	80(28.1)	102(35.8
		(2.8)		(15.4)		(17.5)		

n- number of samples of each group; D=direct, E=enrichment
- All percentages in the vertical column are out of 15 samples
- All % results in the horizontal column are out of the total 285 isolation possibilities

shown on table 1 and 2 one can observe the number of unpreserved and preserved samples of the three groups from which each of these organisms was obtained by both the direct and enrichment methods.

Table 3 indicates results of the enumeration values for the indicator organisms, mesophyllic aerobes, and molds/yeasts in the unpreserved fruits and vegetables of the three categories. Similarly, comparative results of the numbers of the three groups of samples with the isolated indicators spoilage, and pathogenic organisms between the pre and post-preservation analysis were shown in table 4.

It was also observed that the isolated organisms in two of the Group II samples were only among the indicator organisms after preservation, while in four samples of the same group spoilage organisms of the genus bacillus were

obtained by the enrichment method. *Enterobacters*, molds and *Serratia species* were also obtained from the preserved samples of Groups II and III, respectively.

DISCUSSION AND CONCLUSION

Fruits and vegetables can never be free of any micro-organisms, in whatever form they appear (1,2). The microflora of the surface of freshly harvested fruits and vegetables include both the normal surface flora and those from soil, water and perhaps plant pathogens (3-5). The most common genera of bacteria usually residing in such plant products *Pseudomonas, Alcaligens, Bacillus, Chromobacterium, Lactobacillus, Leuconostoc, Micrococcus, Sarcina, Serratia, Staphylococcus, Streptococcus,* and others, plus some members of the molds and few yeasts (1-5). If the surfaces of such plant products are

Table 2. Isolation possibilities of micro-organism from the post-preserved samples

Number of post-preserved samples								
	Group I		Group II		Group III		Total	
Isolates	D	E	D	E	D	E	D	E
Normal								
Contaminate	N	N	N	N	N	N	N	N
Indicators								
Coliforms	N	N	N	2	N	N	2	2
Others	N	N	N	N	N	N	N	N
Spoilage								
Bacillus	N	N	N	4	N	2	N	6
Enterobacter	N	N	3	N	N	N	3	N
Serratia	N	N	N	N	N	1	N	1
Molds	N	N	3	N	N	N	3	N
Others	N	N	N	N	N	N	N	N
Pathogens	N	N	N	N	N	N	N	N
Total	N	N	6	6	N	3	6	9

N = No growth

moist or their outer surfaces have been damaged, growth of some micro-organisms may take place between harvesting and processing or even during consumption. It is estimated that at least one fourth of all harvested fruits and vegetables are not consumed before some level of spoilage occurs. But adequate control of storage conditions like temperature and humidity or applying other efficient preservation methods can either eliminate or reduce the number of at least hazardous and spoilage organisms.

The overall result given in table 1 indicates the isolation of organisms of 19 genera giving a total number of 285 isolation possibilities, from the 15 fruit and vegetable samples. Out of these possibilities all organisms indicated in table 1 were found in 80 (28.1%) and 102 (35.4%) isolations by direct inoculation and enrichment methods, respectively, from the pre-preservation samples. Actually there were a total of 25 species members of these 19 genera whose total number of isolation possibilities was 375 from the same number of samples. Out of these 25 isolates 3 (12%) indicator organisms were obtained from the pre-preservation samples in 19 (5.1%) and 29 (7.7%) isolations by the direct and enrichment inoculating methods, respectively. Similarly, 15 (60%) species of the 25 organisms in the 19 genera, consisted of spoilage organisms the predominant of which included 7 members of the genus *Bacillus (B.lentus, B.pumilus, B.polymyxa, B.subtilis, B.nigrificans,* and *B.megaterium)*. These bacilli members were obtained in 14 (3.7%) and 15 (4%) isolations of the total 375 possibilities by the direct and enrichment culture methods, respectively. The rest of the other 8 genera members of the spoilage organisms were, all together, obtained in 34 (9.1%) isolations by direct and in 33 (8.8%) isolations by the enrichment culture methods from the pre-preservation samples. Five (20%) pathogenic organisms of the 25 total isolates were also obtained from these pre-preservation samples in 9 (2.4%) and in 12 (3.2%) isolations of the 375 possibilities by the direct method and enrichment methods, respectively.

On the other hand, table 2 shows that all members of the 19 genera were obtained from the post-preservation samples in only 6 (2.1%) and 9 (3.2%) isolations of the 285 possibilities by the direct and enrichment inoculation, respectively. It can be observed that only 2 (0.7%) isolation of the single indicator organisms by enrichment method among the spoilage genera only 6 (2.1) isolations by direct inoculation, and 7 (2.5%) isolations by enrichment culture method were obtained from the post-preservation samples. Interestingly no pathogenic organisms were obtained out of the 285 or 375 possibilities of isolations by any of the culture methods from these post-preservation fruit and vegetable samples. This shows that the reduction rates of isolation with the help of the preservation methods were 100% and 94% for the indicators; 85% and 87% for the spoilage organisms; and 100% and 100% for the pathogenic organisms when examined by the direct and enrichment culture methods of each group, respectively.

All of the above results, thus, show some agreement with those of other workers elsewhere (13-19) with significant (X^2=32.89, P<0.001) reduction or elimination of isolation possibilities of all the 19 genera after

the preservation processes have been applied to each sample. One can also observe that there is, in both the pre and post-preservation
samples, a significantly

Table 3. Load of micro-organisms per gram weight of pre and post-preserved fruit and vegetable samples

–	Total number of micro-organisms during							
	Pre-preservation				Post-preservation			
	TPC				TPC			
Type of samples	PCC	AeO_2	AnO_2	PDA	PCC	AeO_2	AnO_2	PDA
Group I								
Papaya	2,400	TMC	67000	500000	Nil	30	Nil	30
Guava	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Group II								
Avocado	2,400	TMC	2,000,000	29000	Nil	30	Nil	Nil
Banana	470	6000000	900	100	Nil	Nil	30	Nil
Grape fruit	Nil	200	Nil	100	Nil	Nil	Nil	Nil
Orange	Nil	Nil	Nil	30000	Nil	Nil	Nil	Nil
Pineapple	2400	5000	100	200	300	Nil	Nil	Nil
Mango	30	300	Nil	Nil	Nil	Nil	Nil	Nil
Group III								
Onion	2400	200	Nil	1500	30	Nil	Nil	30
Potatoe	2400	1300	300	Nil	Nil	Nil	Nil	Nil
Tomatoe	300	Nil	Nil	50000	Nil	Nil	Nil	30
Cauliflower	2400	200	34000	30000	30	Nil	30	Nil
Garlic	Nil	30	Nil	30	Nil	Nil	Nil	Nil
Carrot	460	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Kidney bean	2400	4500	500	50	Nil	Nil	Nil	Nil

PCC = Presumptive coliform, TPC = Total plate count, PDAC = Potatoes agar count for molds/yeasts,
AeO_2 = Aerobic incubation, AnO_2 = Anaerobi incubation, TMC = Too many to count, Nil = No growth of organisms
N.B.- The acceptable limits for fruits and vegetables (11) are
1. PCC = 10-10^2 coliform/gm 2. TPC = 10^4-10^2 organisms/gm 3. PDAC = 10^2-10^4 molds/gm

(X^2=6.55, P<0.5) increased rate of isolation possibilities by the enrichment method over that of the direct culture method.

It can be realised from tables 1 to 3 that significantly (X^2=301.79, P<0.001) more dominant organisms in the fresh unpreserved
fruits and vegetables were the members of the spoilage organisms of the genera *Bacillus, Enterobacter* and molds isolated, respectively in 26 (9.1%), 11 (3.9%) and 4 (1.4%) isolations of the total 285 possibilities by the enrichment methods. These agree with the fact that fruits and vegetables without preservation methods are highly perishable food products spoiled usually
by bacterial soft rots, various mold rots, coloured rots and slimness or souring tastes all caused by the above mentioned organisms (10).

Considering the results of enumeration of total number of organisms in each of the unpreserved and preserved samples, one can observe from table 3 that 10 (66.6%) of the pre-preservation samples showed presumptive coliform counts to be very high above the maximum limit of

acceptable range (10-10^2 molds/gm), for fresh fruits and vegetables (11). Similarly, 6(40%) of these pre-preservation samples also showed high total mold counts above the limits of acceptable ranges (10^2-10^4 molds/gm), while only 4
(26.7%) samples showed too many counts of mesophyllic aerobic organisms above the limits of acceptable ranges (10^4-10^6 organisms/gm). These results indicate an agreement with the fact that unpreserved fruits and vegetables are more susceptible to indicator and spoilage organisms. It has been suggested that such high

microbial contamination can acquired from various sources like poorly sanitized environmental conditions and poor handling of the product during harvest collection, transportation and marketing activities.

To compare the different preservation methods, we have tried to analyze the effectivity of each method. In table 4 it is shown that in the case of samples of Group I, preserved by making jam or jelly and the majority of Group III samples, preserved by the preserve method, the

Table 4. The number of micro-organisms isolated from pre and post-preservation fruit and vegetable samples

	Number of group isolates in samples during													
	Pre-preservation analysis						Post-preservation analysis						Total	
	Indicator N=3		Spoilage N=9		PAthogen N=5		Indicator N=3		Spoilage N=9		PAthogen N=5		N=17	
Types of samples	D	E	D	E	D	E	D	E	D	E	D	E	D(%)	E(%)
Group I														
Papaya	1	2	1	1	1	2	-	-	-	-	-	-	3(18)	5(29)
Guava	-	1	1	2	-	-	-	-	-	-	-	-	1(16)	3(18)
Group II														
Avovcado	2	3	3	5	1	1	-	1	3	1	-	-	9(3)	11(65)
Banana	1	3	4	1	-	-	-	-	1	1	-	-	6(35)	5(29)
Orange	1	1	4	6	1	2	-	-	1	1	-	-	7(41)	10(59)
Pineapple	2	2	5	7	-	-	-	-	-	-	-	-	7(41)	9(35)
Grape firut	-	-	2	2	-	-	-	-	-	-	-	-	2(12)	2(12)
Mango	2	1	2	1	1	2	-	1	1	1	-	-	6(35)	6(35)
Group III														
Onion	2	3	2	3	1	1	-	-	-	-	-	-	5(29)	7(41)
Potatoe	1	2	4	5	-	-	-	-	-	2	-	-	5(29)	9(53)
Tomatoe	1	2	3	6	1	1	-	-	-	-	-	-	5(29)	9(53)
Cauliflower	2	2	3	6	-	1	-	-	-	-	-	-	5(39)	9(53)
Garlic	-	+	-	1	-	-	-	-	-	-	-	-	- - - -	2(12)
Carrot	2	2	2	4	1	1	-	-	-	-	-	-	5(29)	7(41)
Kidney bean	2	2	4	4	1	1	-	-	-	-	-	-	7(41)	8(47)
Total	9	16	40	55	8	12	-	2	6	7	-	-	73(25.6)	102(35.7)

N = number of organisms of each group D = direct method of inoculation E = enrichment method
(%) = percentage of organisms in each sample.

rates of reduction were 100% effective in both the direct and enrichment culture methods. On the other hand, the rates of reduction of the number of organisms in Group II samples, preserved by juice and squash formation were shown to be effective in only 10% and 82% respectively, by both culture methods.

In summary, the overall results of this preliminary survey indicate that the indicator, spoilage and pathogenic organisms are highly liable to contaminate unpreserved fruits and vegetables but can effectively be reduced or eliminated with all simple preservation methods
at a rate of 100% or slightly lower. It was also
observed that 8 (53.3%) of the unpreserved fruits and vegetables have encountered at least a single pathogenic organism; 9 (60%) samples at
least 2 indicators; and 11 (73.3%) of the sample have 3 or more spoilage organisms. But there was not any single sample that was free of at least one individual species of the normal contaminant organisms. One could also realise

from this work that the vegetables of Group III samples seem to be more susceptible to all groups of micro-organisms. This was proved by the observed results of 50 (17.5%) isolation of the total 285 isolations possibilities by the enrichment culture method. This is comparable to 44 (15.4%) and 8 (2.8%) isolation of Group II and Group I fruit samples, respectively.

Finally the cumulative effect of preserving these products could be summarized as to have no resisting pathogen against all the preservation methods, while 4 (44.4%) of the spoilage and only 1 (33.3%) of the indicator genera were able to resist preservation in 13 (86.7%) and 2 (13.3%) of the 15 samples, respectively. Similarly, it could be also be concluded from this study that all of the preservation methods applied to all the examined samples have shown a 100% effectivity in reducing the numbers of indicator mesophyllic aerobes, and spoilage molds that were observed in the pre-preservation samples have also resulted in the growth of none or few of these organisms/gm of the samples.

In conclusion, although the results of this preliminary study are adequate to indicate the effectivity of these preservation methods for such essential plant products, the study has to be confirmed by extending it to a large, scale. Preservation development future work should also utilize better experimental designs concerning the number, size, kinds and collection sites of the samples. It would then be possible to prepare a simple, comprehensive and acceptable manual for home, hotel, cottage, and hospital-level preservation techniques.

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REFERENCES

1. Faerbroth, R.W. and Geoff Taylor. A textbook of Bacteriology. 9th edition. London. 1962; 457-488.
2. Mundt, J.O., Graham, W.F. and McCarty, T.E. Spherical Lactic Acid producing Bacteria of Southern-grown rawand processed vegetables. Appl. Microbiology. 1967; 15:1303-1308.
3. Tomkins, R.G. The microbiological problems in the preserva

tion of fresh fruits and vegetables. J. Sci. Food Agr. 1951; 2:381-386.
4. Fraizer, W.C. and Westhoff, D.C. Food Microbiology. 3rd edition. Tata Mcgraw-Hill, New Delhi. 1981; 194-217.
5. Banawrt, G.J. Basic Food Microbiology. 3rd edition, AVI Publishing Co., Inc., Westport, Connecticut. 1983; 422-425.
6. Geyid, A. and Gebre Yohannes. A Compiled Laboratory Manual in Public Health Bacteriology. NRIH, Addis Ababa. 1982; 115-123.
7. Von Schelhorn, M. Control of micro-organisms causing spoilage of fruit and vegetable products. Adv. Food Res. 1951; 3:429-482.
8. Salle, A.J. Fundamental Principles of Bacteriology. 5th edition, Mcgraw-Hill Book Co.,Inc., New York. 1961; 546-629.
9. WHO/FAO Expert Committee Report. Microbiological aspects of food hygiene. WHO, Geneva. Technical Report Series. 1976; 6-103.
10. Sharp, J.M. Recommended methods for the microbiological examination of foods. 2nd edition, The American Public Health Association Inc., Washington, D.C. 1966.
11. FAO/Food and Nutrition Paper. Manual of food quality control microbiological analysis. Rome. 1979.
12. Geldreich, E.C. and Bordner, R.H. Faecal contamination of fruits and vegetables during cultivation and processing for market. A review. J. Milk Food Technol. 1971; 34:184-195
13. Berry, J.M., Witter, L.D. and Folinazzo, J.F. Growth characteristics of spoilage organisms in orange juice and concentrate. Food Technol. 1986; 10:553-556.
14. Salunkhe, D.K. Development in technology of storage and handling of fresh fruits and vegetables. Crit. Rev. Food Technol. April 1974; 15-54.
15. Von Schelhorn, M. Control of micro-organisms causing spoilage of fruit and vegetable products. Adv. Food Res. 1951; 3:429-482.
16. Hays, G.L. and Riester, D.W. The control of "Off-Odour" spoilage in frozen concentrated orange juice. Food Technol. 1952; 6:386-398.
17. Hucker, G.J., Brooks, R.F. and Emery, A.J. The source of bacteria in processing and their significance in frozen vegetables. Food Technol. 1952; 6:147-155.
18. Murdock D.I. and Hatcher Jr., W.S. Growth of micro-organisms in chilled orange juice. J. Milk Food Technol. 1975; 38:393-396.
19. Rushing, N.D. and Senn, V.J. Shelf-life of chilled orange juice with heat treatment and preservatives. Food Technol. 1964; 18:112-114.