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Effect of chemical treatments on the microbial load of fruits and vegetables

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ABSTRACT

Effect of chemical treatments on the microbial load of fruits and vegetables was investigated. Fruits and vegetables purchased from street vendors were analyzed to determine their microbial load. Bacteria load ranged from 1.3×10^4–1.8×10^6 cfu/g while fungi load ranged from 3.0×10^4–5.2×10^4 cfu/g. Carrots had the highest bacterial and fungal load. Bacteria isolated included *Staphylococcus*, *Streptococcus*, *Bacillus*, *Pseudomonas*, *Escherichia*, *Salmonella* and *Klebsiella* while fungi were *Aspergillus*, *Rhizopus* and *Saccharomyces* with various percentage of occurrences. *Staphylococcus* was the most frequently isolated bacteria genera, varying between 60 and 100% in all cases. Treatment of fruits and vegetables with vinegar was found to significantly reduce their respective microbial load when compared with other solvent. This study revealed that fruits and vegetables were contaminated with different bacteria and fungi genera and the microbial load can be reduced when properly washed especially with vinegar.

INTRODUCTION

The importance of fruits (for example, apple, banana, pineapple, paw-paw, oranges) and vegetables (for example, lettuce, spinach, cabbage, cauliflower) to human cannot be over emphasized as they are high in fiber, vitamins, water, minerals, varying proportions of sugar, proteins and various phytochemicals such as flavonoid, saponin, tannin and anthocyanin (Gruda, 2005). Some phytochemicals are antibacterial, antifungal and antioxidant. They are vital for health and wellbeing, and as a result of this knowledge, consumption of fruits and vegetables has increased significantly in many countries (Kalia and Gupta, 2006). Hundreds of fruits and vegetables are commercially valuable as human food. In Nigeria, watermelon, pineapple, paw-paw and salad vegetables are usually sliced and packaged in poly-ethene bags and sold by street vendors. Consumption of these sliced fruits have increased because they are convenient, easily accessible, nutritious and especially cheaper than the whole fruits and vegetables (Halablab et al., 2011). However, sliced fruits and vegetables are widely exposed to microbial contamination (Nwachukwu et al., 2008) through contact with the soil, dust, water, or during handling like peeling, slicing or trimming. The increase in the consumption of sliced fruits and vegetables has been linked with a parallel increase in food-borne illnesses (Bagde and Tumane, 2011; Batz et al., 2012; Clark, 2012).

Among the groups of bacteria found in fruits and vegetables are coliform (Zhao et al., 1997). However, about two-third of the spoilage of these items is caused by mould of the genera *Penicillium*, *Aspergillus*, *Botrytis* and *Rhizopus* (Frazier and Westhoff, 1998). Micro-organisms capable of causing human illness such as *Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella* spp. have also been isolated in lettuce and salad vegetables (Francis et al., 1999). Contamination of fruits and vegetables takes place at all stages of fruit processing if proper sanitary and hygiene conditions are not maintained (Beuchat, 1995; De Roever, 1998). Outbreaks of salmonellosis have also

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been associated with consumption of cut watermelon in the United States (CDC, 2009).

Fruits and vegetables vendors generally do not follow good hygienic practices. There is no adequate storage conditions as fruits and vegetables are sold in trays, wheel barrows or on tables by vendors. Vendors who do not maintain personal hygiene can carry microorganism on their skin, hair, hands or cloths and may unintentionally contaminate fresh fruits or vegetables and create the opportunity to transmit food-borne illness. It therefore implies that to maximize all health benefits from adequate consumption of fruits and vegetables, handlers (vendors) should be free from microbial contamination. To this end, the present study was set out to investigate the microbial load of fruits and vegetables sold in South-eastern Nigeria and to determine the effect of chemical treatment on reducing such load.

MATERIALS AND METHODS

Sample collection

One hundred (100) samples each of fruits and vegetables were randomly obtained from different markets located in South-eastern Nigeria between 2011 and 2012. Each of the samples was placed in an individual sterile plastic bag and labeled appropriately. The samples were immediately transported in a container of ice packs to the laboratory and analyzed within 4 h.

Microbiological analysis

Twenty-five grams (25 g) of each sample was aseptically collected and placed in a sterile blender to which 225 ml of distilled water was added and homogenized (Vanderzant and Splittstoesser, 1992). Subsequently, 1 ml of each fruit or vegetable suspension was serially (10 fold) diluted and 1 ml of the appropriate dilution was placed on solid medium following pour plate techniques and incubated at 37°C for 24–48 h for bacteria counts while Sabouraud dextrose agar (Oxoid) plates were incubated at 28°C for 72 h for fungi counts. The average of the counts was recorded as cfu/g. The media used for isolation included Nutrient agar, Mannitol salt agar, Salmonella-Shigella agar, Eosin methylene blue agar, MacConkey agar, blood agar and Sabouraud dextrose agar. Discrete colonies were picked and sub-cultured to obtain pure cultures. IMViC (indole, methyl red, voges proskauer, and citrate) test was performed on pink-red colonies on MacConkey agar and shiny-metallic green on eosin methylene blue agar to identify *Escherichia*. Colonies grown on mannitol salt agar were tested for coagulase reaction for identification of *Staphylococcus* and confirmed by cell morphology, Gram reaction, catalase activity. *Bacillus* sp. was determined based on spore stain, Gram stain, catalase test, motility test, nitrate reduction, anaerobic fermentation of glucose, voges proskauer test, production of acid from mannitol. Colonies with black centres on Salmonella-Shigella agar were inoculated onto Triple Sugar Iron agar (Oxoid) and subsequently biochemical and serological tests were performed for the identification of *Salmonella* sp. Gram reaction and biochemical tests such as catalase, oxidase, urease, citrate, methyl red test, Voges Proskauer, indole, sugar fermentation were performed to identify other bacteria (Buchanan and Gibbons, 1998) while fungal isolates were determined based on their cultural and microscopic morphology.

Chemical treatment of fruits and vegetables

A concentration of 1.5% of three chemicals (vinegar, sodium benzoate and potassium sorbate) was adopted (Eni et al., 2010) and prepared. Twenty-five gram (25 g) of each sample of fruits or vegetables was aseptically (cleaning surfaces with 70% alcohol) weighed out and placed in an individual sterile 500 ml glass beaker containing 225 ml of 1.5% concentration of each of the chemicals. The fruit or vegetable in the solution was washed by vigorously agitating the beaker. Subsequently, the fruit or vegetable sample in each of the solutions was allowed to stand for 10 min and thereafter 1 ml of the wash water was 10 fold serially diluted. Then 1 ml from the appropriate dilution was inoculated following pour plate method. The inoculated nutrient agar plates were incubated at 37°C for 24–48 h. The counts were from triplicate determinations.

RESULTS

Organisms isolated from fruits and vegetables samples included *Staphylococcus*, *Streptococcus*, *Bacillus*, *Klebsiella*, *Escherichia*, *Pseudomonas*, *Salmonella*, *Saccharomyces*, *Aspergillus*, *Rhizopus*. The percentage occurrences of the isolates are shown in Table 1. *Staphylococcus* occurred in all the samples and with a relatively very high percentage. The mean value of microbial load of fruit and vegetable samples is presented in Table 2. The bacteria load ranged from 1.3×10^4–1.8×10^6 cfu/g while fungi load ranged from 3.0×10^4–5.2×10^4 cfu/g. Table 3 shows the effects of the various chemical treatments on the microbial load of the fruits and vegetables. The treatment revealed that vinegar was relatively more effective than potassium sorbate and sodium benzoate.

DISCUSSION

The microbial quality of fruits and vegetables as well as

Table 1. Percentage occurrences of isolates (bacterial and fungal) in fruits and vegetables.

Microorganism detected	Pineapple	Apple	Watermelon	Carrot	Garden egg	Cucumber
	Values are given in percentage (%) of samples analysed					
Staphylococcus	90	60	70	100	100	80
Streptococcus	20	0	50	10	0	10
Bacillus	50	20	40	100	90	60
Pseudomonas	50	10	50	60	40	40
Escherichia	40	0	60	90	80	50
Salmonella	50	0	40	80	80	40
Klebsiella	10	0	10	20	10	10
Rhizopus	70	80	70	60	30	40
Aspergillus	30	30	30	50	30	30
Saccharomyces	70	40	60	60	50	40

Table 2. Total bacterial and fungal counts in fruit and vegetable samples.

Sample	Bacterial count (cfu/g)	Fungal count (cfu/g)
Watermelon	8.3×10^5	4.1×10^4
Apple	4.5×10^5	3.8×10^4
Pineapple	3.5×10^5	3.6×10^4
Carrot	1.8×10^6	5.2×10^4
Cucumber	3.2×10^5	5.1×10^4
Garden egg	1.3×10^4	3.0×10^4

Table 3. Effect of chemicals on microbial load of fruits and vegetables.

Sample	Chemical	Total viable count/g$\times10^3$
Garden Egg	Vinegar	3.1 ± 0.4^a
	Sodium benzoate	3.2 ± 0.5^a
	Potassium sorbate	3.2 ± 0.6^a
Cucumber	Vinegar	2.9 ± 0.5^a
	Sodium benzoate	3.2 ± 0.4^b
	Potassium sorbate	3.2 ± 0.4^b
Apple	Vinegar	5.9 ± 0.7^a
	Sodium benzoate	6.6 ± 0.8^b
	Potassium sorbate	7.3 ± 0.1^c
Carrot	Vinegar	5.2 ± 0.6^a
	Sodium benzoate	5.7 ± 0.8^b
	Potassium sorbate	6.6 ± 0.7^c
Watermelon	Vinegar	2.7 ± 0.8^a
	Sodium benzoate	3.0 ± 0.5^b
	Potassium sorbate	3.6 ± 0.5^c
Pineapple	Vinegar	3.2 ± 0.5^a
	Sodium benzoate	3.3 ± 0.4^a
	Potassium sorbate	3.5 ± 0.4^b

Values followed by same alphabet are not significantly different ($p<0.5$). Values are means ± standard deviation of triplicate determinations.

the effects of chemical treatment on the microbial load were investigated. Bacteria isolated included *Bacillus*, *Escherichia*, *Klebsiella*, *Staphylococcus*, *Streptococcus*, *Salmonella* and *Pseudomonas* with various percentages of occurrences. *Rhizopus*, *Aspergillus*, *Saccharomyces* were fungi isolated from fruits and vegetables. Similar organisms were isolated from fruits or vegetables (Nwachukwu et al., 2008; Ankita, 2010; Eni et al., 2010). Fungi are known to cause spoilage of fruits and vegetables. These microorganisms could contaminate the fruits and vegetables through the use of manure in the farm, vehicles used in transporting the fruits, from other fruits and vegetables, spores from the atmosphere, water use in washing the fruits and vegetables or from the vendor and from the use of dirty processing utensils like trays and knives (Beuchat, 1995; De Roever, 1998; Ray and Bhunia, 2007). The high frequency of *Staphylococcus* could be contaminant from hands or skin during handling or washing of human Food-borne pathogens commonly detected in fresh fruits and vegetables which include *Salmonella*, *Shigella*, *E*. *coli*, *Streptococcus* and coliform bacteria. In addition to that, man had been reported as the main source (Tambekar and Mundhaba, 2006). The presence of these microorganisms in fruits and vegetables creates a public health concern.

The results of the effect of chemical treatments on microbial load of fruits and vegetables revealed that each of the chemicals reduced microbial count to various degrees. Vinegar reduced the microbial count more than sodium benzoate and potassium sorbate. Shalaby and El-Raliman (2006) reported that potassium sorbate was not effective against many bacteria. This could be as a result of vinegar being acidic in nature and bactericidal (Entani et al., 1998), while sodium benzoate and potassium sorbate are alkaline and bacteriostatic in nature (Ostergaard, 1994). In terms of toxicity, potassium sorbate, sodium benzoate and vinegar used in this study are generally recognized as safe (Frazier and Westhoff, 1998). It can be deduced from this study that soaking fruits and vegetables in appropriate concentration of vinegar for at least ten minutes will help to reduce the level of microorganisms.

This study has shown that the fruits and vegetables were contaminated with microorganisms which could be of public health importance. The microorganisms were present in various quantities and distributions depending on the fruit or vegetable. Good sanitary measures should be adopted while handling fruits and vegetables to limit the level of microbial contamination. The use of vinegar in washing fruits or vegetables is recommended.

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