Advance Journal of Food Science and Technology 4(2): 97-102, 2012
ISSN: 2042-4876

Submitted: February 02, 2012 Accepted: March 06, 2012 Published: April 20, 2012

Microbiological Safety Assessment of Apple Fruits (*Malus domestica* Borkh) Sold in Owerri Imo State Nigeria

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Abstract: This study aimed at assessing the microbial colonizers, of apple fruits sold in Owerri to determine its safety for consumption. Apple fruits are dependable source of vitamins, it is rich in fiber, electrolytes, minerals and antioxidants and it is usually eaten fresh and raw, making the vitamins fully available for the body. The popularity and increased consumption of apple fruits therefore calls for necessary safety checks. Two hundred fresh and apparently healthy apple fruits were obtained from street vendors and shopping malls in major streets, motor parks and higher institutions in Owerri. The fruits were washed-out separately in 10 mL sterile distilled water to obtain suspensions which were assayed for total aerobic plate count, coliform count, and fungal count and for specific pathogens. A count of 3.4×10^5-4.5×10^7 cfu/mL was obtained for TAPC, while total coliform and total fungal counts ranges from 2.4×10^4-2.2×10^6 and 5.0×10^2-3.6×10^5 cfu/mL respectively. Twelve bacterial and seven fungal spp were isolated. The apple fruits sold in major busy spots in Owerri are contaminated, the presence of Shigella spp, *S. aureus*, Salmonella and *B. cereus* which are known pathogens calls for concern. Education of fruit vendors on food hygiene, adequate packaging/covering of apple fruits on display for sale and washing of fruits before consumption is advanced.

Key words: Adequate packaging, coliform, food hygiene, microbial colonizers, pathogen, safety

INTRODUCTION

The increasing understanding of the link between fruit intake and improved health coupled with the newly found nutritional values of apple (*Malus domestica*) has increased its popularity and thus consumption rate. Whole fruits and juices produced from apple are extensively used as health foods for its dependable source of vitamins, minerals, electrolytes, antioxidants and fiber. The fruits are usually eaten fresh and raw except for the seeds, making the nutritional values fully available for the body (Boyer and Liu, 2004; Avci *et al*., 2007; Wojdyto *et al*., 2008; Gerhauser, 2008; WAC, 2010; Hyson, 2011).

Evaluation of the antimicrobial, antimutagenic, antiinflamatory, anticarcinogenic, antioxidant, antidiabetic/osteoporosis properties and related qualities of apple fruits and juices have been reported (Rajav, 2005; Fratianni *et al*., 2007; Gerhauser, 2008; Fraternale *et al*., 2011; Hyson, 2011). Clinical observations have shown that apple consumption is associated with reduction in risk of cancer (Gallus *et al*., 2005; Michels *et al*., 2006; Theodoratou *et al*., 2007; American Institute for Cancer Research, 2010) Antioxidant activity of apple components is known to inhibit cancer cell proliferation, decrease lipid oxidation and lower cholesterol (Liu, 2003; Boyer and Liu, 2004; Schaefer *et al*., 2006; Ramos, 2007; He and Liu, 2007; Avci *et al*., 2007; American Institute for Cancer Research, 2010).

Apples are rich source of phytochemicals that have been reported to reduce risk of cardiovascular diseases, asthma; diabetes, cataracts, Alzheimer's disease/cognitive decline and pulmonary functions (Boyer and Liu, 2004; Song *et al*., 2005; Gercia *et al*., 2005; Chan *et al*., 2006; Chan and Shea, 2009; WAC, 2010; Hyson, 2011).

Despite the health benefits of fruits to healthy living, the contamination of these fruits had created another burden to consumers. Experts say fruits are reservoirs of disease causing germs. In recent years there has been an increase in the number of reported cases of food borne illness linked to fresh fruits (Dunn *et al*., 1995; Mensah *et al*., 1999, 2002; Buck *et al*., 2003; Eni *et al*., 2010). The surface of apple fruits habours microorganisms depending upon the mechanical handling of the fruits. Microbes can adhere to surface, invade/penetrate fruits surface and multiply within the tissue. Contamination could be from human handling, transport vehicles, insects, dust, and rinse water, harvesting equipment, soil, faeces, irrigation water, water used to apply fungicides and insecticides, manure, wild and domestic animals (Burnett and Beuchat, 2001; Buck *et al*., 2003). The diverse routes via which apple fruits and juices can be contaminated make it a veritable means for food borne disease and disease outbreak.

In Nigeria, apple fruits are popularly displayed completely exposed for sales in shopping malls, along

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busy and major streets and hawked by street food vendors in motor parks and on busy roads with heavy traffic, security check points or at bad spots on the highways where motorists are forced to slow down. Fruits are often purchased as ready to eat and thus usually consumed without washing. This research aim at determining the microbial colonizers of surfaces of apple fruits sold in Owerri, to ascertain their health implication.

MATERIALS AND METHODS

Source of samples: Samples were collected from eight different sites identified to be the major sales location for apple fruits in Owerri and also locations considered as places where the fruits are bought as ready-to-eat for immediate consumption without washing or treatment of any sort. The sampling sites included four tertiary institutions of learning, two major motor parks and two major roads/busy streets.

Sample collection: Two hundred apparently fresh and healthy apple fruits were purchased comprising of twenty-five apples from each of the eight locations. Sampling sites were visited twice in two weeks within the months of July to October 2011, during which samples were obtained from shops and street vendors. Each sample was placed separately in sterile plastic bags and transported to the laboratory for processing within 1 h of collection.

Determination of microbial load: Each apple sample was rinsed out in 10 mL sterile peptone water (Fluka, Germany). The resultant homogenate was diluted 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. The pour plate technique was adopted in plating aliquots of 0.2 mL of the dilutions in duplicate onto different media plates; Nutrient agar and plate count agar (Oxoid, England) for total aerobic plate count, Eosin Methylene Blue (EMB) agar (Oxoid) and MacConkey agar (Fluka) for coliform count, Sabouraud-Dextrose agar (Fluka) for fungal count. All the media were prepared according to the manufacturer's instruction. Plates were incubated for 24 h at 37ºC. Sabouraud Dextrose agar was however, left at room temperature after initial four hours incubation. Colonies were counted after the incubation time using Colony counter (Stuart Scientific, UK).

Isolation of microorganisms: Sample homogenate 0.2 mL was plated using spread plate technique onto Mannitol salt agar (Oxoid) for isolation of *S. aureus*, EMB for isolation of *E. coli* and other coliforms, Sabouraud Dextrose agar for fungal isolation. Salmonella-Shigella agar (Fluka) was inoculated for isolation of salmonellae after initial pre-enrichment of sample homogenate in Selenite-F broth for 24 h. Inoculation was also made onto Nutrient agar. All inoculated plates were allowed to "dry", inverted and incubated at 37ºC for 24 h. Sabouraud Dextrose agar plates was however, incubated at 28ºC for 72 h. Distinct discrete colonies on the different media were isolated and purified on nutrient agar by repeated sub-culturing. Further characterization of pure cultures stored on agar slants at 4ºC was by the methods described by Speck (1976)

Characterization of isolates:

Coliform organisms: The procedure of Speck (1976) for confirmation of coliforms was adopted. Characteristic colonies on EMB were inoculated into Lactose broth with Durham tubes. The presence of gas after 24-48 h incubation at 37 and 44ºC constitute positive presumptive test. Confirmatory test was by plating out positive presumptive test broth on EMB, incubation at 37 and 44ºC for typical colonies of *E. coli* appearing bluish black with greenish metallic sheen or brownish mucoid colonies characteristics of *E. aerogenes*. Production of gas in Lactose broth of these typical colonies constitute completed test. Further confirmatory tests were by methods of Speck (1976)

Fungal isolates: Identification was based on their macroscopic and microscopic characteristics as seen in culture morphological characteristics, needle mount and slide culture. Reference was made to standard identification keys and atlas (Fawole and Oso, 1986; Tsuneo, 2010).

Bacterial isolates: Typical colonies stored on nutrient agar slants at 4ºC were Gram stained and confirmed (Speck, 1976). Cultural characteristics and biochemical tests-IMVIC test, carbohydrate utilization, reaction on TSI, gelatin liquefaction, nitrate reduction, motility, Oxidase and Urease production were carried out. Confirmation of typical *S. aureus* colonies on Mannitol salt agar was on the basis of the results of Catalase, Coagulase, Phosphatase production, nitrate reduction and carbohydrate utilization (Oranusi *et al*., 2002).

RESULTS

Total aerobic plate count, coliform count and fungal counts are shown in Table 1. Aerobic plate count in most of the samples was high $\geq 10^6$ cfu/sample. Samples from Wetheral road and Alvan Ikoku College of Education had

Table 1: Mean microbial load of apple fruits sold in Owerri, Nigeria

	Microbial load cfu/mL		
Sample site	TAPC	TCC	TFC
Douglass road	2.2×10^7	1.2×10^6	1.2×10^4
Wetheral road	3.4×10^5	6.1×10^4	1.0×10^4
ITC park	4.5×10^7	2.2×10^5	1.0×10^3
Okigwe motor park	8.4×10^6	2.2×10^6	2.1×10^4
FUTO	9.2×10^5	2.4×10^4	2.2×10^3
IMSU	3.5×10^6	5.0×10^4	5.0×10^2
Federal polytec. nekede	4.8×10^6	3.4×10^6	3.2×10^5
Alvan, college of education	5.3×10^5	4.6×10^5	6.3×10^4

TAPC: Total aerobic plate count; TCC: Total coliform count; TFC: Total fungal count; FUTO: Federal University of Technology Owerri; IMSU: Imo State University

Table 2: Microorganisms isolated from apple fruits sold in Owerri, Nigeria

Sample site	Organisms isolated
Douglass road	Klebsiella spp. Shigella spp. Bacillus spp. *S. aureus*, Penicillium spp. *Aspergillus niger*
Wetheral road	*Pseudomonas aeruginosa*, *S. aureus*, Bacillus spp. Penicillium spp.
ITC park	Rhizopus spp. *Bacillus cereus*, *S. aureus*, *E. coli*, *S. epidermides*, Fusarium spp. Penicillium spp. *Aspergillus niger*
Okigwe motor park	*S. aureus*, Klebsiella spp. Enterococcus, Bacillus spp. Salmonella, *Aspergillus niger*
FUTO	E. coli, S. aureus, *Bacillus cereus*, Bacillus spp. *S. Cerevisiae*, Mucor spp. Aspergillus spp.
IMSU	Micrococcus spp. *Bacillus subtilis*, Aeromonas spp. Penicillium spp. *S. Cerevisiae*
Federal polytec. nekede	Proteus spp. *S. aureus*, Enterobacter spp. Bacillus spp. Cladosporium spp. Mucor spp. *A. Niger*
Alvan, college of education.	Bacillus spp. Aerococcus, Salmonella spp. *S. aureus*, *A. niger*, Fusarium spp. Penicillium spp.

FUTO: Federal University of Technology Owerri; IMSU: Imo State University

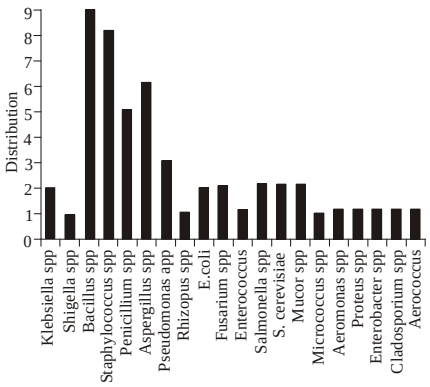

Fig. 1: Distribution of microorganisms isolated from apple fruits in Owerri, Nigeria

the lowest aerobic plate counts of 10^5 cfu/sample. Coliform counts of the order 10^6 were recorded for samples from Douglass road, Okigwe Motor Park and Federal Polytechnic Nekede. Fungal count of $\leq 10^4$ cfu/sample was recorded from all the sampling locations except however, for samples from Federal Polytechnic Nekede with a count of 3.2×10^5 cfu/sample.

Table 2 shows that Bacillus spp and *S. aureus* was isolated from samples in all the locations. Shigella spp, Samonella spp, *E. coli* was detected in some samples. Fungi, specifically Aspergillus and Penicillium spp were the major fungal contaminants. Fusarium spp, Mucor spp and Cladosporium spp and the yeast *S. cerevisiae* were also isolated (Table 2). The distribution of microorganisms isolated from apple in all the sample locations show the order Bacillius spp, > Staphylococcus spp > Aspergillus spp > Penicillum spp > Pseudomonas (Fig. 1).

DISCUSSION

The apple fruits on sale as ready-to-eat in major and busy spots in Owerri are contaminated. This could be explained by the fact that microorganisms form part of the epiphytic flora of fruits and many will be present at the time of consumption (EC-SCF, 2002). The microorganisms present in raw fruits and vegetables are a direct reflection of the environment through which the product has passed. The extent of microbial contamination depends on the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce (EC-SCF, 2002; Buck *et al*., 2003; Ray and Bhunia, 2007).

The microorganisms reported for this work corroborate the findings of other studies on fruits and vegetables (Abadias *et al*., 2008; Uzeh *et al*., 2009; Bucker *et al*., 2010; Eni *et al*., 2010; Ukwo *et al*., 2011; Joceyln *et al*., 2012).

The total aerobic plate count ranges from 10^5-10^7 cfu/sample this qualify the fruits as "Average" and "Poor" for human consumption. Hazard Analysis and Critical Control Point-Total Quality Management (HACCP-TQM) Technical Guidelines rates microbial quality for raw foods containing aerobic plate count of $<10^4$ cfu/g as "Good", 10^4-5×10^6 "Average", 5×10^6-5×10^7 "Poor" and $>5\times10^7$ cfu/g "Spoilt" (EC-SCF, 2002; Aycicek *et al*., 2006).

The coliform counts reported in this work is higher than the report of De Giusti *et al*. (2010), it is however, lower than the findings of Viswanathan and Kaur (2001) and it is in agreement with the reports of Aycicek *et al.* (2006), Bagci and Temiz (2011), Ukwo *et al*. (2011), Mahuya *et al.* (2011) and Joceyln *et al.* (2012). Coliforms are indicator organisms and counts of 10^4-10^6 cfu/sample reported in this work are a cause for concern, since the fruits are usually consumed without further processing.

Some of the bacterial isolates from apple fruits are Gram negatives and non pathogenic, however, the presence *E. coli*, Salmonella spp, Shigella spp which are often associated with poor sanitary practices indicate that they put a pointer to a potential risk of food borne illness to consumers (Aycicek *et al*., 2006; Oranusi *et al*., 2006, 2007; Eni *et al*., 2010). *Staphylococcus aureus* and *B. cereus* are common food contaminants from Man and the environment, their presence in food however, need to be controlled because they have been reported as cause of major food borne illnesses (Mudgil *et al*., 2004; Oranusi *et al*., 2004, 2006a, 2006b, 2007).

The fungal isolates of apple fruits in this study Aspergillus spp; Penicillium spp, Rhizopus, Mucor spp are common environmental contaminants, they have been reported by other researchers (Tournas, 2005; Badosa *et al*., 2008). They are known to be the major cause of spoilage of fruits and vegetables (ICMSF, 1998). Some of these fungi have been reported to produce mycotoxins and are implicated in cases of mycoses (Tournas, 2005; Katherine *et al*., 2006).

Raw fruits and vegetables are known potential for a wide range of microorganisms, including human pathogens (EC-SCF, 2002). The survival or growth of these organisms on intact fruit surfaces will be dependent on the extrinsic factors of available nutrient, temperature, presence of scales and fibres, gaseous atmosphere, mechanical handling and moisture. The apple fruits on display for sale are often visited by many hands of the customers and by the vendors. These individuals pick and drop as many apple fruits as are available, to enable them make a choice. Poor handling by unhygienic hands is a factor contributing to the high microbial load. The dusty environments of the motor parks, busy roads and campuses/institutions, coupled with water of questionable quality which often is used to sprinkle the fruits to keep fresh are contributing factors that could aid the survival and possible multiplication of contaminants on fruit surfaces.

The present study revealed the potential hazard of ready to eat apple fruits. Therefore, the result highlighted the importance of proper washing before consumption. Nigeria with a population of over 150 million people is fast on the lane of joining the League of Nations with high apple consumption rate, more research in this direction is apt to generate data urgently needed to conduct effective risk assessment of the microbial hazards associated with apple fruit surface colonizers. Buck *et al*. (2003) noted that the chain of production of fruits from planting to consumption lack effective antimicrobial treatment at any step such that pathogens introduced at any point may be present on the final food product. Simple treatment may remove only portion of pathogenic microorganisms if present, use of disinfectants and proper rinsing thereafter, effective HACCP application, education of vendors on food safety, public enlightenment for consumers and all concerned from production to transportation and marketing would help to prevent possible food borne illness associated with consumption of unwholesome fresh apple fruits.

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