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Full Length Research Article

ASSESSMENT OF BACTERIAL QUALITY OF SOME FRUITS SOLD IN SELECTED MARKETS WITHIN KADUNA METROPOLIS

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ABSTRACT
Microorganisms are known to contaminate or destroy fruits there by reducing the quality and the profits derived from them. Consumption of raw fruits often lead to food related disease outbreaks. The purpose of the study was to isolate, and identify bacteria present in fruits that are commonly sold in three different markets within Kaduna metropolis. A total of 54 different fruit samples of avocados, apples, and Sour sops were purchased from Bakin Dogo, Station and Kawo markets. All samples were analyzed for the density of microorganisms present using standard plate count method. The frequency of occurrence of the different types of bacteria present on the fruit samples were also determined using standard microbiological methods. The composition of nutrient in each fruit sample was also analyzed using standard method. The results of the study showed that the microbial loads of all the fruits examined ranged between 1.02 x 10^5 ± 0.60 x 10^5 CFU/ml to 1.38x10^5 ± 0.30x10^5 CFU/ml and the differences were found to be significant ($P < 0.05$). The sour sops obtained from Bakin dogo market had the highest mean microbial load of 1.38x10^5 ± 0.30x10^5 CFU/ml, followed by Avocado (1.24 x 10^5 ± 0.07 x 10^5 cfu/ml) from Kawo. The least microbial load of 1.02x10^5 ± 0.60 x 10^5 CFU/ml was each for apples obtained from Bakin dogo and Station markets respectively. The species of bacteria isolated from the fruit samples were *Bacillus, Pseudomonas, Staphylococcus, Streptococcus, Escherichia coli,* and *Salmonella*. Of the 81(100 %) total isolates of bacteria the most frequently isolated species were *Staphylococcus* (35.8 %) followed by *Bacillus*, *Streptococcus* and *Escherichia coli* (29.2, 16,0 and 9.9 %) while the least frequently isolated species were *Pseudomonas* (6.2 %) and *Salmonella* (2.6 %). Soursop has high ash, fiber, protein and carbohydrate contents while avocado has high fat content. Reduction of risk for illnesses associated with fruit consumption can be achieved by adequate microbiological knowledge of fruits, and adopting proper hygienic ways of harvesting, processing, handling and storage.

Keywords: Assessment, bacteria, fruits, markets, Kaduna metropolis

INTRODUCTION

Fruits are excellent sources of nutrients and media for rapid microbial growth. They are usually subjected to natural contamination depending on the types of microorganisms present. Species of *Staphylococcus*, *Klebsiellas*, *Salmonella*, *Actinomycetes* and *Escherichia coli* have been reported to cause fruit spoilage (Eni *et al*., 2010). Some fruits can resist attack by microorganisms due to the antimicrobial activity of certain naturally occurring substances like essential oils, spices, lactoperoxidase present in them which provide protection against entry and subsequent spoilage by microorganisms (Singh *et al.,* 2013). Relative humidity and water activity affect the quality of fruits (Oluwole *et al*., 2012). When fruits with low water activity are stored in an environment of high humidity, water will transfer from the atmosphere to the fruit leading to the spoilage by viable flora (Singh *et al.,* 2013). There is also a relationship between temperature and humidity in general, the higher the temperature, the lower the relative humidity and vice versa (Neeraj and Sharma, 2007). However, the general quality of fruits depends on the success of preventing the entry of microorganisms and restricting their growth (Singh *et al.,* 2013). The present study examined the different types of bacteria present in some fruits obtained from three markets within Kaduna metropolis.

MATERIALS AND METHODS

Sample Collection

A total of 54 avocados, apples, and sour sops were randomly collected from different selling outlets in Bakin-dogo market, Station market and Kawo market with sterile gloves. Each sample was put in a sterile low density polythene bag and labelled. All samples were transported to the laboratory in the Department of Applied Science in special boxes for analysis.

Determination of Microbial Load of Fruit Samples

Twenty-five grams (25g) of each sample was weighed and washed in 100 ml of sterile distilled water. Ten -fold serial dilutions of each rinsed water was made and 1 ml of 10^{-2}, 10^{-4}, 10^{-5} dilutions were pipetted into sterile Petri-dishes. Sterile molten nutrient agar (45ºC) was added to each dish and swirled thoroughly to allow even distribution. The colonies were counted using a colony counter (Stuart Scientific, UK) after 24 h incubation at 37ºC (Cappuccino and Sherman, 2011).

Isolation and Identification of Bacteria

Nutrient agar, MacConkey Agar, Eosin Methlene Blue agar, Salmonella-Shigella agar, peptone water (Oxoid, England), Methyl red voges-proskeur broth (Scharlau, Spain) and Simmons-citrate agar (Biomark, India) were prepared according to Manufacturer's instruction. The following media; MacConkey Agar, Eosin Methlene Blue agar Salmonella-Shigella agar, cetrimide agar nutrient and mannitol agar were each inoculated with 1 ml of the rinsed water using the Pour Plate Technique. The plates were allowed to solidify, inverted and incubated at 37ºC for 24 hours. Each colony was isolated in a pure form by sub-culturing. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed. Staphylococcus species appeared as white, yellow and creamy colonies, Salmonella species appeared as colorless rods, *E. coli* appeared as white glistering moist colonies *Streptococcus species* appeared milky with flattened raised colonies, *Bacillus* *species* appeared as flattened white fibroid colonies, *Pseudomonas* species appeared as whitish colonies turning media light green The identity of each isolate was confirmed by standard biochemical test using microgen identification kit**.** (Cappuccino and Sherman, 2011; Wogu, 2014).

Proximate Analysis of Fruits

Proximate analysis of fruit samples was performed in accordance with the method of AOAC, (2000). Fruit samples were washed, the skin and seeds were removed, and the remaining parts were homogenized in a blender to obtain 100 g pulp of each type of fruit. To determine the dry matter content, 2 g samples were dried in an oven at 105°C until constant weight. The samples were weighed before and after drying and the contents of dry matter were calculated. The protein content was determined in an Elementar Analyzer (Flash EA 1112 Series, Thermo Fisher Scientific, Sweden), by means of combustion of 25 mg samples. Aspartic acid (Thermo Fisher Scientific, Delft, The Netherlands) was used as a standard. The amount of protein was calculated by converting the amount of nitrogen by a factor 6.25. Crude fiber was determined gravimetrically after chemical digestion and solubilization of sample. The moisture content of the ground sample was determined by Thermo Gravimetric Analysis (TGA). The fat content was determined gravimetrically after extracting 1 g samples with petroleum ether (Sigma-Aldrich Chemicals Co., St. Louis, MO, USA) at 40–60°C for 1 hour using a Soxhlet equipment (SoxtecTM 2055, Foss, Höganäs-Helsingborg, Sweden) rapeseed oil was used as a standard. The ash content was determined by combustion of 2 g samples in silica crucibles in a muffle furnace (Carbolite, Sheffield, England) for 24 hours at 550°C. All determinations were performed at least in triplicate; the data are expressed as means ± standard deviations. Analysis of carbohydrate content was performed by high performance anion exchange chromatography (Dionex) with pulsed amperometric detection (HPAEC-PAD).

RESULTS

Determination of Microbial Load

In the present study, all the fruit samples analyzed were found to be contaminated. The microbial loads of fruits also varied significantly in each market. The mean microbial load ranged from $1.02x10^5 \pm 0.60x10^5$ CFU /ml - $1.38x10^5 \pm 0.30x10^5$ CFU /ml. Of all the fruits sampled from the various markets sour sop had the highest mean microbial load in Bakin Dogo ($1.38x10^5 \pm 0.30x10^5$ cfu/ml) followed by avocado and apple in Kawo ($1.24x10^5 \pm 0.07x10^5$ CFU /ml, and $1.17x10^5 \pm 0.16x10^5$ CFU /ml). Lower microbial loads were found in apples from Bakin Dogo and Station markets ($1.02x10^5 \pm 0.60x10^5$ CFU /ml), avocado in station market ($1.05x10^5 \pm 0.07x10^5$ CFU /ml and soursops in Kawo market ($1.19x10^5 \pm 0.20x10^5$ CFU /ml) respectively (Table 1).

Isolation and Identification of Bacteria from Fruit Samples

A total of six (6) species of bacteria; *Bacillus, Pseudomonas, Staphylococcus, Streptococcus, Escherichia coli* and *Salmonella* were identified based on cultural, morphological and biochemical characteristics of organisms (Table 2). *Staphylococcus* species appeared as white, yellow and creamy colonies, *Salmonella* species appeared as colorless rods, Coliforms such as *E. coli* appeared pink and cloudy as well as metallic green sheen, *Streptococcus* species appeared milky with flattened raised colonies, *Bacillus species* appeared as flattened white fibroid colonies, Pseudomonas species appeared as whitish colonies turning media to light green.

Frequency of Occurrence of Bacteria in Fruit Samples

Of the 81(100 %) A total of 81(100 %) bacterial species were isolated from fruit samples. The most frequent isolated species was *Staphylococcus* (35.8 %) with higher frequency of occurrence in Station market (55.2 %) followed by *Bacillus species* (29.2 %) with higher frequency of occurrence in Bakin Dogo market (37.5 %), *Streptococcus* (16.0 %) with higher frequency of occurrence in Kawo market (61.5 %) and *Escherichia coli* (9.9 %) with higher frequency of occurrence in Station market (62.5 %).The least frequently isolated bacteria were Species of *Pseudomonas* (6.2 %**)** and *Salmonella* (2.6 %) with higher frequency of occurrence in Kawo markets (60 % and 100 %) respectively. *Pseudomonas* was detected in the fruits obtained from Station and Kawo market and none from Bakin Dogo while *Salmonella* was only detected in fruits obtained from Kawo market (Table 2)

Proximate Composition of Fruit Samples

Results of the proximate composition (moisture, dry matter, ash content, crude fiber, fat content, crude protein, and carbohydrate) of Avocado, Soursop and Apple are presented in Table 3. Significant ($p<0.05$) differences were observed between the proximate parameters of the three different fruits. Apple had the highest moisture content (68.77 %) while the least moisture content was recorded in soursop (11.94%). Correspondingly a least dry matter was observed in apple (18.33%) as against soursop (28.33%) with higher dry matter. Soursop sample had the highest ash content (5.94%) and the least ash content was found in apple (0.58%). Similarly, soursop had higher crude fibre (11.93%) while apple and avocado had lower crude fiber values (1.83%, 4.16%). The fat content in the avocado fruit (18.07%) is higher than in the other fruit samples (6.89 %, 0.9 %). It was observed that soursop fruit was the richest in protein (17.8%) while apple was the lowest (0.97%). It was also detected that soursop sample had the highest carbohydrate content (40.68%).

DISCUSSION

The variation in microbial loads of fruits in the study areas could be due to contamination by microorganism from soil, irrigation water, the environment during transportation, washing/rinsing water or handling by processors or may be part of the natural flora of the fruits as reported by other authors (Ofor *et al*., 2009: Singh *et al.,* 2013). In addition, the different species of bacteria isolated from the fruit samples could be due to poor storage conditions as well as handling and processing of the fruits (Eni *et al.,* 2010; Singh *et al.,* 2013). Similar species of bacteria were also isolated by many researchers in an attempt to determine or identify microbial populations present in fruits or responsible for their spoilage (Uzeh *et al*., 2009; Bukar *et al.,* 2010). The presence of *Escherichia coli* and *Salmonella species* indicate contamination by fecal matter (Musa *et al*., 2016). Moreover, species of *Staphylococci* and other pathogenic and opportunistic species of bacteria like *E. coli, Salmonella* and *Streptococci* detected in the fruit samples could lead to food borne illnesses since the fruits are usually consumed raw (Bukar *et al.,* 2010; Eni *et al.,* 2010).

The low moisture content in soursop (11.94%), implies that they will have good storage qualities (Edema and Oklemen, 2000).

The ash content though quite low in the Apple sample (0.58%) is significantly important in foods as they account for the mineral constituents but must not be too much (Oluwole *et al*., 2012). However, the higher ash, fiber and protein contents in soursop, could be attributed to its low moisture and the decrease in moisture content of fruits has been reported to affect the concentration of nutrients (Sing, 2001). The spongy mass of fiber helps to satisfy the appetite and it also assists in moving food through the alimentary canal by aiding the muscular action of the intestine thus preventing constipation (Edem, *et al*., 2009). Avocado fruit is richer in fat content (18.07%). The essence of fats in foods cannot be over emphasized as they are used up by the cells of organs and glands to provide energy, synthesis of some of their secretions, fuel the body, insulate nervous system, building blocks of hormones and help absorb some vitamins (Okpero, 2011). Soursop fruit among two other fruits is a better source of protein and carbohydrate. Carbohydrates provide source of energy to the body while proteins are important in food as they help in the growth and development of the body (Okpero, 2011).

Conclusion

In conclusion, all the fruits obtained from Bakin Dogo, Station and Kawo markets were found to be contaminated with mean microbial load that ranged from $1.02x10^5 \pm 0.60x10^5$ CFU/ml to $1.38x10^5 \pm 0.30x10^5$ CFU/ml. Soursop had the highest mean microbial load in Bakin Dogo ($1.38x10^5 \pm 0.30x10^5$ CFU/ml). The least microbial loads of $1.02x10^5 \pm 0.60x10^5$ mg/ml were found in apples obtained from Bakin Dogo and Station markets. The species of bacteria isolated from the fruit samples were *Bacillus, Pseudomonas, Staphylococcus, Streptococcus, Escherichia coli* and *Salmonella.* The most frequently isolated species of bacteria was *Staphylococcus* (35.8 %) and the least frequently isolated species was *Salmonella* (2.6 %)**.** Soursop fruit had the highest ash, fiber, protein and carbohydrate contents and lower in moisture content. Avocado was higher in fat content and apple was lower in fat content. This may affect the quality of fruits. Reduction of risk for illnesses associated with consumption of these fruits can be achieved by adequate microbiological knowledge and storage conditions as well as adoption of proper hygienic ways of harvesting and processing of fruits.

Table 1: Total Plate Count of Fruits Sampled from Three Different Markets

Fruits	Markets	Mean±Standard Deviation (CFU /ml)
Avocado	Bakin-Dogo	$1.18x10^5 \pm 0.14x10^5$
	Station	$1.05x10^5 \pm 0.07x10^5$
	Kawo	$1.24x10^5 \pm 0.07x10^5$
Apple	Bakin-Dogo	$1.02x10^5 \pm 0.60x10^5$
	Station	$1.02x10^5 \pm 0.60x10^5$
	Kawo	$1.17x10^5 \pm 0.16x10^5$
Sour Sop	Bakin-Dogo	$1.38x10^5 \pm 0.30x10^5$
	Station	$1.22x10^5 \pm 0.08x10^5$
	Kawo	$1.19x10^5 \pm 0.20x10^5$

Values are means ± Standard deviation at p<0.05

Table 2: Frequency of Occurrence of Bacteria in fruits Obtained from Three Markets

		Market		
		Bakin Dogo	Station	Kawo
Bacteria	Number isolated	Number of Occurrence (%)	Number of Occurrence (%)	Number Of Occurrence (%)
Staphylococcus aureus	**29(35.8)**	**8 (27.6)**	**16 (55.2)**	**5 (17.2)**
Streptococcus species	**13 (16.0)**	**3 (23.1)**	**2 (15.3)**	**8 (61.5)**
Pseudomonas species	**5(6.2)**	**0**	**2 (40)**	**3 (60)**
Bacillus species	**24(29.2)**	**9 (37.5)**	**5 (20.8)**	**10 (10.7)**
Escherichia coli	**8(9.9)**	**1 (12.5)**	**5 (62.5)**	**2 (25.0)**
Salmonella species	**2(2.6)**	**0**	**0**	**2 (100)**
Total	**81 (100)**	**21(25.9)**	**30(37.0)**	**30 (37.0)**

Table 3: Proximate analysis of Avocado, Soursop and Apple

Parameter	Avocado	Soursop	Apple
Moisture content	64.63 ± 3.77^a	11.94 ± 0.14^b	68.77 ± 3.04^a
Dry matter	24.67 ± 2.52^a	28.33 ± 2.52^a	18.33 ± 1.53^a
Ash content	1.47 ± 0.25^a	5.94 ± 0.21^b	0.58 ± 0.63^a
Crude fiber	4.16 ± 0.34^a	11.93 ± 0.45^b	1.83 ± 0.57^c
Fat content	18.07 ± 0.29^a	6.89 ± 0.22^b	0.9 ± 0.26^c
Crude Protein	2.36 ± 0.06^a	17.8 ± 1.13^b	0.97 ± 0.12^a
Carbohydrate	4.58 ± 0.03^a	40.68 ± 1.64^b	15.87 ± 0.6^c
Total	17.13 ± 21.62	17.65 ± 12.06	15.32 ± 23.52

Mean ± SD of triplicate determinants, values with different letter across row are statistically significant with p<0.05

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