Microbial Spoilage and Effect on Nutritional Content of Carica Papaya Fruit

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Abstract:
The study was carried out to isolate microorganisms associated with Carica papaya with white rot symptoms in Enugu metropolis and assess their effect on nutritional value of the fruits. A total of 50 Carica papaya with white rot symptoms were obtained from vendors in Ogbete main market, Enugu, Enugu State, Nigeria. Cultures were done on nutrient agar and potato dextrose agar and the microbial isolates identified using standard technique. Disk susceptibility tests were also performed on microbial isolates. Bacterial species isolated were Bacillus subtilis at 15(30%) and Bacillus cereus at 10(20%) while fungi species isolated were Aspergillus niger at (20) (40%) and Alternaria spp at (5) (10%). The pathogen city test presumptively identified Aspergillus niger and Alternaria spp as organism responsible for the spoilage of Carica papaya. Sensitivity test confirmed that Bacillus subtilis and Bacillus cereus were sensitive to most of the tested antimicrobials while Aspergillus niger and Alternaria spp were susceptible to voriconazole at 100% and itraconazole at 100% and 80% respectively and completely resistant to clotrimazole and nystatin. Chemical analysis was carried out using standard methods of AOAC, (2010). The proximate composition of whole ripened Carica papaya fruit were moisture(77.05±1.12%), ash(1.11±0.00%), crude fiber(3.02±0.96%), lipid(0.31±0.07%) and carbohydrate(18.52±0.09%). There was significant difference (p<0.05) in proximate values between whole and infected Carica papaya fruit. Mineral composition of healthy ripened Carica Papaya fruit showed that Potassium (179.19±0.99%) was the most abundant mineral in the fruit followed by Mg(22.02±0.94%)Ca(3.89±0.46%)>Mn(2.63±0.74%)>Cu(1.94±0.78%)>Fe(0.23±0.78%)>Zn(0.12±0.09%). There was significant difference (p<0.05) in mineral values between whole and infected Carica papaya fruit. The results of this study showed that Carica papaya with white rot symptoms were contaminated with microbes and their nutritional contents depleted. Unwholesome pawpaw is often consumed by people and the presence of microbes in the fruit can lead to food poisoning, prudent intake of the fruit with signs of spoilage is advised.

Keywords: Carica papaya, microorganisms, proximate composition, mineral contents and antimicrobials

1. Introduction
Pawpaw (Carica papaya) is a popular fruit plant grown all over parts of the tropical and subtropical regions of the world. It is a fast growing but short-lived herbaceous plant with latex vessels in all its fruit. The Carica papaya (pawpaw) is a member of the small family “Caricaceae” allied to the “Passifloraceae”. This family comprises of Jamilla, Jacarata, Cyclicmorpha and Carica genera [36]. The only genus having species cultivated for their fruits is Carica. Nutritionally, papaya is a good source of calcium and an excellent source of vitamins A and C. Papaya also has several industrial uses. The pawpaw fruit contain minerals such as Na, K, Ca, Mg, P, Fe, Cu, Zn and Mn. It is also a source of carotenoids, thiamin, riboflavin, vitamin B6 and vitamin K [1].

Economically, Carica papaya is the most important species within the Caricaceae, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams, candies and as dried and crystallized fruit [46]. Green fruits and the leaves and flowers may also be used as a cooked vegetable [48]. The unripe green fruits can be eaten cooked usually in curries, stews and salads when combined with lettuce. Pawpaw fruits, apart from being taken as food also have some medicinal importance, the fruits, leaves; seeds and latex are used medicinally [18]. Its main medicinal use is as a digestive agent; it is prescribed for people who have difficulty digesting protein and is used to break up blood clots after surgery, which is due to the presence of enzyme papain in the plant’s latex [22, 33]. The latex from the trunk of the tree is also applied externally to speed the healing of wounds, ulcers, boils and warts [3]. One striking beneficial effect of the seeds of
Carica papaya is its ability to cure intestinal worms when chewed. The seeds have been used as vermifuge, thirst quencher, or pain alleviator [28, 34] reported that treatments of sickle cell diseases and poisoning related disorder is a possibility using seeds of Carica papaya. They are also used to expel worms and the flower may be taken as an infusion to induce menstruation. They have been used as a folk medicine to treat parasitic infections, E. coli, and other viral and bacterial infections.

Spoilage in pawpaw can also be referred to as rot or decay. Spoilt pawpaw fruits are characterized by excess softening, mycelia growth, loss of moisture, unpleasant odor, shrinkage and total drying up of water in the fruits [25]. The spoilage may be caused by microorganisms, insects and rodents attack, physical injury such as bruising, and freezing as well as chemical breakdown of the fruit may also lead to deterioration in quality of the fruit. The occurrence of spoilage in fruits by microorganisms depends on the types of organisms present and whether the fruit under its existing condition of storage can support the growth of any or all of them. Only certain species out of all the organisms present in a fruit will be able to thrive well and spoil it. Spoilage by microorganisms may be influenced by some qualities such as water content, pH value, temperature, texture and nutrient composition of the fruit [30]. Aspergillusniger, Aspergillusflavus, Rhizopusnigricans, Curvularialunata and Fusariumequseti have been reported to be responsible for the post-harvest losses in pawpaw in southern Nigeria [26].

Besides the losses in income to the pawpaw fruit markets, the fungi-spoilt fruits could also cause health hazards to the consumers due to their production of mycotoxins which are capable of inducing mycotoxicoses in man [24]. Thus, the aim of this study was to isolate and characterize microorganisms associated with the deterioration of pawpaw fruits and determine the pathogen city of the isolates and co-relate the presence of microbes on proximate and mineral content of the fruits.

2. Materials and Methods

2.1. Collection of Samples

50 infected and 10 non-infected fruits were collected in sterile bags from Ogbete main market, Enugu and taken to the Microbiology laboratory of the Department of Applied Microbiology and Brewing Enugu State University for isolation of the potential pathogenic agents.

2.2. Preparation of Samples

A sterile scapel blade was used to cut out a small portion of the diseased part and a homogenate was made by blending 1 g in 9ml of sterile normal saline. Serial dilutions of 10⁻¹ to 10⁻⁶ were made in sterile test tubes by several transfers of 1 ml of previously diluted samples from one dilution test tube to 9ml of sterile normal saline.

2.3. Isolation of Microorganism

Nutrient agar plates were used for isolation of bacteria while potato dextrose agar plates were used for isolation of fungi. Pour plates method were used in inoculation of bacteria and fungi by transferring 0.1 into sterile nutrient agar plates and potato dextrose agar plate. Inoculated nutrient plates were incubated as 37°C for 24 hours while PDA plates were incubated at room temperature for 24 (± 28°C) for 5 days. Pure isolates were obtained by selecting discrete colonies and having them sub-cultured on petri dishes containing freshly prepared nutrient agar media and potato dextrose agar media. Pure isolate was kept on nutrient agar and potato dextrose agar slant at 4°C in the refrigerator.

2.4. Identification of Bacteria Isolates

Gram’s staining techniques and biochemical test were used for the identification of Bacterial isolates according to Baker [24].

2.5. Identification of Fungal Isolates

The fungal growths that appeared were primarily identified using cultural and morphological features. The fungal isolates were identified by staining with lacto phenol cotton blue. 2 drops of lacto phenol cotton blue reagent was placed on a clean, grease-free glass slide. A small tuft of the fungus was obtained using sterile inoculating needle and transferred to the glass slide. A cover slip was placed over the preparation and examined under the microscope using magnification of X40.

2.5.1. Pathogen City Test

To ascertain pathogen city of isolates, 10 whole pawpaw fruits were properly washed with tap water, rinsed with distilled water and surface sterilized with 70% alcohol and rinsed in sterile distilled water according to [20]. With a 5mm diameter sterile cork borer, 2cm long cylindrical cores were removed from each fruit; discs of 7-day old cultures of each isolate were removed from agar plates and placed in bored holes on each fruit. Vaseline jelly was smeared to completely seal each hole. After a week of incubation at room temperature, the inoculated fruits were incised and observed for disease development. The colonies that appeared cultured and characterized were primarily identified using cultural and morphological features [17]. Rot symptoms developed with isolates were compared to the natural original rot [23].

2.6. Proximate Analysis

The method of AOAC (2016), AOAC (2007) and ISO 17025:2005 were used and the analysis involved the determination of the % constituents of the parameters; Moisture Content: 1 gram of the sample was weighed into a
crucible and dried in an oven for 1 hour at 105˚C. % moisture content loss = W1 – W2/ WT × 100/1. Ash content: 1 gram of the fruit sample was weighed and ashed using a muffle furnace at 500˚C. Ash content W2 – W1% Ash content W2 – W1/WT × 100/1. Crude Fiber Content: 2 grams of the fruit sample undergone acid treatment and base treatment and finally taken for ashing. Weight of fiber = weight of residue – weight of ash, % crude fiber = fiber weight/sample weight × 100/weight. Protein Content: 0.5 gram of the sample was weighed into kjethaldehyde flask for digestion, distillation and finally titration. TV × 0.0014 × 6.25 × DF × 100/Weight of sample. Fat Content (Lipid – Fat & Oil): 5 grams of the sample was weighed and the extraction of fat was done using soxhlet extraction method. Weight of oil W2-W1% lipids = W2 – W1/Weight of sample × 100/1

2.7. Mineral Analysis

The elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA 1995 (America Public Health Association) and the American Public Health Association (1998) 2111B, Direct Air – Acetylene Flame method. The digestion was carried out according to AOAC International (2007), Official methods of analysis 18th edition (2005) and method 960.52 (Micro-kjeldahl method) an (method 992.23 (Generic Combustion Method).

2.8. Statistical Analysis

The SPSS version 21.00 was used for the data analysis, statistically significant different groups was calculated with the One-way Analysis of variance (ANOVA). The Duncan test was used for multiple comparisons, and level of statistical significance was set at p ≤ 0.05

3. Result

3.1. Occurrence of Microorganism in Carica Papaya with White Rot Symptoms

Out of 50 samples with the white rot symptoms, bacterial species isolated were Bacillus subtilis at 15 (30%) and Bacillus cereus at 10 (20%). The fungal species isolated were Aspergillus niger at 20 (40%) and Alternaria spp at 5 (10%) (Table 1).

3.2. Growth and Morphological Characteristics of Fungi

Aspergillus niger were thick septate, hyphae with conidia borne in chains from the sterigmata while Alternaria spp were erect conidiophores, hyphae and cylindrical conidia (Table 2).

3.3. Cultural, Microscopic and Biochemical Test of Bacteria Isolates

Bacillus species were smooth white slightly raised, small sized colonies on nutrient agar. There were variations in the biochemical reactions and both were Gram positive rods (Table 3).

3.4. Antibiotic Sensitivity Pattern of Bacteria Isolates

The bacteria isolates were sensitive to all antimicrobial agents tested (Table 4).

3.5. Antifungal Susceptibility Pattern of the Fungal Isolates

Fungi isolates showed varied susceptibility to the antifungal agents tested (Table 5).

3.6. Pathogenicity Test of Isolates

Aspergillus niger and Alternaria spp were capable of causing spoilage on healthy Carica papaya fruit by the 5th day of inoculation. Signs of spoilage showed a white rot at the point of the inoculation and brownish deterioration. Bacillus spp did not show signs of spoilage on Carica papaya fruit (Table 6).

3.7. Proximate Analysis of Carica Papaya

Proximate analysis showed that proximate contents were lower in Carica Papaya with white rot than in whole fruits. There was significant difference (p < 0.05) between proximate contents of Carica Papaya with white rot than in whole fruits (Table 7).

3.8. Mineral Analysis of Carica Papaya

Potassium was the most abundant mineral in the fruit followed by Mg > Ca > Mn > Cu > Fe > Zn. There was significant difference (p < 0.05) between mineral contents of Carica Papaya with white rot than in whole fruits (Table 8).
Table 1: Occurrence of Microorganisms Present in Carica Papaya with White Rot Symptoms

| Number of Sample Collected | Organism isolated | Names of organisms | Occurrence (%) |
|----------------------------|-------------------|--------------------|----------------|
| 50                         | Fungi             | Aspergillusniger   | 20 (40%)       |
| 50                         | Bacteria          | Bacillus subtilis  | 15 (30%)       |
| 50                         | Bacteria          | Bacillus cereus    | 10 (20%)       |
| 50                         | Fungi             | Alternaria spp     | 5 (10%)        |

Table 2: Cultural and Microscopic Characteristics of Fungi Isolates from Carica Papaya

| Sample | Cultural Characteristics | Microscopic Characteristics | Organism Identified |
|--------|--------------------------|-----------------------------|---------------------|
| A      | Black fluffy growth with on PDA plates | Thick Septate hyphae with conidia borne in chains from the sterigma | Aspergillusniger |
| B      | Grey and flat white colonies on PDA plate | Erect condiophores, septate; hyphae and cylindrical conidia | Alternaria spp |

Table 3: Cultural, Microscopic and Biochemical Test of Bacteria Isolates from Carica Papaya

Key: + = Positive  - = Negative

Table 4: Antibiotic Susceptibility Pattern of the Isolates from Carica papaya Fruit

S = Susceptibility; I = Intermediate; R = Resistant
Table 5: Antifungal Susceptibility Pattern of the Fungal Isolates from Carica papaya Fruit.

| Organism inoculated | Presence of Pathogenicity | Number of days spoilage occurred | Observation |
|---------------------|---------------------------|----------------------------------|-------------|
| Aspergillus spp      | +                         | 5                                | White rot with rough edges |
| Alternaria spp       | +                         | 5                                | Brownish decay with rough edges |
| Bacillus subtilis    | -                         | -                                | -            |
| Bacillus cereus      | -                         | -                                | -            |

Table 6: Pathogenicity Test of Isolates on Carica Papaya Fruits

+ = Detected; - = Undetected

Table 7: Proximate Analysis of Ripened Whole and Ripened Carica Papaya with White Rot

| Proximate content (%) | Ripe whole Carica papaya (%) | Carica papaya with white rot (%) |
|-----------------------|------------------------------|----------------------------------|
| % Moisture            | 77.05±1.12d                  | 72.02±0.99e                      |
| % Ash                 | 1.11±0.00a                   | 1.06±0.12b                      |
| % Crude Fiber         | 3.02±0.96b                  | 3.02±0.51c                      |
| % Protein             | -                            | 0.11±0.01a                      |
| % Lipid               | 0.31±0.07a                   | 0.29±0.02ab                     |
| % Carbohydrate        | 18.52±0.09c                  | 15.32±0.38d                     |

Table 8: Mineral Analysis of Ripened Whole and Ripened carica Papaya with White Rot

| Mineral         | Composition for Ripped Infected Carica Papaya (Mg/G) |
|-----------------|-------------------------------------------------------|
| Calcium (Ca)    | 3.89±0.46       | 3.25±0.41       |
| Copper (Cu)     | 1.94±0.78       | 1.32±0.16       |
| Manganese (Mn)  | 2.63±0.74       | 1.87±0.02       |
| Magnesium (Mg)  | 22.02±0.94      | 21.08±1.86      |
| Zinc (Zn)       | 0.12±0.02       | 0.10±0.04       |
| Potassium (K)   | 179.19±0.99     | 176.85±3.52     |
| Iron (Fe)       | 0.23±0.01       | 0.22±0.01       |

4. Discussion

Since most fruits and vegetables contain high level of water and nutrients, they serve as good substrates that support the growth of spoilage and pathogenic microorganisms [5]. In the study, out of 50 Carica papaya fruits with white rot, fungi spp isolated were Aspergillus niger at 20(40%) and Alternaria spp at 5 (10%) while bacterial species were Bacillus subtilis at 15(30%), and Bacillus cereus at 10(20 %)(Table 1). This is in line with the work of [14] who isolated A.
niger from diseased pawpaw fruit. This is also in agreement with the works of [42] who recorded the frequency of A. niger in some selected fruits in which pawpaw was inclusive at 38%. Similar results were observed in the works of [31, 14] who recorded a high percentage occurrence of A. niger in spoilt pawpaw sold at south western Nigeria; to be 82% and 88% for 2000 and 2001 respectively. The present study is in contrast to the work of [20] in which A. niger was recorded as the least predominant organism among other fungi isolated from the partly spoilt pawpaw fruit in Oyo state Nigeria. The prevalence of the fungi species in this present work supports the work of [25] who isolated A. niger and Alternaria spp amongst other fungi in some fruits and vegetables from selected markets in Lagos State Nigeria. [43] implicated fungi as contaminants of pawpaw fruits. Gupta and Pathak, (2004) also isolated Aspergillus spp and Fusarium spp from post-harvest pawpaw fruits in southern Nigeria.

In the study, bacterial spp isolated were in line with the findings of [5] who indicated that (Gram- positive) organisms like Bacillus species may be responsible for initiating and causing spoilage in cucumber, garden egg and pawpaw fruits. The low prevalence of Bacillus spp in the present study can be compared to that of [49] who found Bacillus spp occurring at least at 10% when compared to other microorganisms associated with spoilage of garden egg, cucumber, and pawpaw in makurdi metropolis, Benue State. Bacillus spp are pathogenic and can cause food poisoning such as endocarditis and bacteremia. Their presence in some fruits samples could be as a result that fruits are sold openly in markets thereby exposing them to the spores of these organisms and are dormant to the lethal effects of heat, drying and ultraviolet radiation [25]. In the study, Aspergillus niger and Alternaria spp were capable of causing spoilage on healthy Carica papaya fruit indicating that they are pathogenic cause of spoilage on the fruit. Zitter, (1985) also observed that fungi cause spoilage of fruits through mechanical injuries such as bruises and wounds on fruits (Table 6).

The occurrence of fungal spoilage of fruits and vegetables is also recognized as a source of potential health hazard to man and animals due to the production of mycotoxin which are capable of causing mycotoxicosis in man following ingestion and inhalation [14]. The presence of these microorganisms in Carica papaya fruits is a public health concern because of the health risk they pose. Bacterial isolates were sensitive to antibiotics tested (Table 4). Among the fungi isolates while the fungi isolate Aspergillus niger showed 100% resistance to nystatin, caposofungin and clotrimazole while Alternaria spp showed 100% resistance to fluconazole, nystatin, vosaconazole and clotrimazole (Table 5).

In the study, the proximate analysis of ripe Carica papaya fruit showed that Carica papaya had high moisture content and thus supports the growth of microorganisms especially Aspergillus spp [5]. This is similar to the work of [19] who reported high moisture content of Carica papaya at 89.21%/100g thus accounting for their high perishable nature and short life span under normal storage conditions. Comparable studies were also observed by USDA National Nutrient Database for Standard Reference Release 28 (2017) at 88.06/100g. The work of Awe et al., (2013) recorded very high moisture content of 91.4±0.2 which is higher than that obtained in the present study (Table 7). The study showed a low value for ash content at1.1±0.00%. Higher results were obtained by [19, 38] at 2.83%/100g and 2.44%/100g respectively. The study does not support the works of [12, 39] who recorded lower ash content of 0.43±0.2 and 0.27±0.03 respectively. The report is not also in conformity with the work of [31] who recorded a very high ash content of 5.8±0.2. In the study, crude fiber content was obtained at 3.02±0.96%. This is not in line with the work of [19] who found crude fiber present at 6.18%/100g while [41] recorded lower crude fiber content at 1.23±0.01%. Both results are not in conformity with the present study. The presence of high crude fiber (high) in papaya indicates its potential for use in animal feeds formulation, since fiber makes for good bowel movement as well as aiding nutrient absorption [37]. The non-significant result of healthy Carica papaya at 0.00% (-)/0.5g in protein contentin the present study is comparable to very low protein content value at 0.47%/100g recorded by the United States of department of Agriculture data base of nutritional components of fruits (2017). Low values recorded in the study also corresponds with the results of [12, 19] at 0.12%/100g and 0.29%/100g respectively. This is a probable indication that the fear of accumulation due to consumption of the fruits do not arise. This study also showed that Carica papaya fruit is a poor source of protein as reported in the work of [39] with protein content of 0.94±0.03%. Very ripe papaya is not a very good source of protein and has its highest value at 0.29% for the pulp [19]. This report is also in line with the work of [29] who reported that very ripe papaya is not a recognized high source of protein and there is reduction in protein content from one ripening stage to another. From the study, the lipid content in Carica papaya was 0.31±0.07%. This corresponds to results observed by [19] with lipid content 0.35%. This report is in contrast with the works of [41, 39] who recorded the lipid contents of Carica papayaat 0.65±0.01% and 0.78±0.03% respectively. In the study, the carbohydrate content of Carica papaya was 18.52±0.09% and it is in line with the work of [41] who found carbohydrate content of papaya at 18.2±2.5. Carica papaya fruits contain reasonable amounts of carbohydrate, which gives an account of their high caloric value and indication [39]. Papaya fruit is rich in carbohydrates (42.28% starch and 15.15% sugar in pulp), [16, 35]. The results of the study were significantly higher than results obtained by [38, 19], however previous studies by [31] found higher carbohydrate content of ripe papaya at 70.7g/100g and it is not in line with this work in which the carbohydrate content was found moderate. Lower values were recorded in proximate contents of infected Carica papaya and there was significant difference (p<0.05) between proximate contents of whole and infected fruits. This could be attributed to the activities of microorganisms in the fruit which depleted the nutrients.

In the study, there was also a reduction in mineral contents of infected Carica papaya fruit when compared to the whole fruits (Table 8). This could be due to the utilization of mineral elements by microorganisms present in the fruit. There was significant difference (p<0.05) between mineral contents of whole and infected fruits. Potassium was the predominant element among mineral contents analyzed while iron was the least. This is in line with the work of [40, 31].
5. Conclusions

From the study, microorganisms were present in Carica papaya with white rot which are often consumed by people. The presence of Bacillus spp is of public health importance as its presence can lead to food poisoning and intoxication while Aspergillus niger produce toxic metabolites such as malformins, naphthopyrones and mycotoxins (ochratoxins) which are known to be hepato-carcinogenic and nephrogenic. Fungal isolates were pathogenic cause of spoilage of the fruit. The mineral and proximate contents of Carica papaya were depleted due to the activities of microorganisms. Carica papaya is a rich source of nutrients and vitamins and are is used for body building and repair of worn out tissues. Therefore, adequate preservative methods need to be taken to avoid waste and routine inspection of post-harvest pawpaw fruits by food regulatory agencies recommended.

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