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***Research Article***

# STUDIES ON POST HARVEST ROT OF APPLE (*MALUS DOMESTICA* BORKH)

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## ABSTRACT
Apples (*Malus domestica*) are important part of human diet because of their nutritional content hence, attempt should be made to keep them in consumable form. Isolation of various storage and shelf fungi associated with apple spoilage within the Badagry Zone of Lagos State, Nigeria was carried out. The associated fungi were cultured on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA). The isolated fungi were *Rhizopus* sp, *Aspergillus niger, Aspergillus terreus, Trichoderma* sp, and *Mucor* sp. The effect of the isolated fungi on the nutritional content of apple was examined and it was observed that for all the pathogens, there was an increase in the percentage moisture content - ranging from 82.46-84.48% as compared to the control which was 73.22%. For the percentage ash, fibre, protein, Vitamin C, fat and sugar contents, there was a reduction in the contents in the diseased samples when compared to the control. The lowest ash content was recorded in apple fruit attacked by *Aspergillus terreus*, while the highest was recorded in fruit attacked by *Rhizopus* sp. control. Lowest fibre content was in fruits attacked by *A. terreus* and the highest in those infected with *A. niger*. Of the isolated fungi, *Rhizopus* sp. caused the highest reduction in protein content, lowest reduction in *Trichoderma* sp. Lowest content of Vitamin C was in *A. terreus*, highest in *Trichoderma* sp. Fat content was lowest in *Mucor* sp., highest in *A. niger* Sugar content was lowest in *Trichoderma* sp. and highest in *Rhizopus* sp.

***Keywords:*** *Apple Spoilage, Fungi, Nutritional Content*

## INTRODUCTION
Apple belongs to the family Pomoideae (Korban and Skirvin, 1984). Apples are often eaten raw, but can also be found in many foods (especially desserts) and drinks. Apples can be canned or juiced; they are milled to produce apple cider (non-alcoholic, sweet cider) and filtered for apple juice. The juice can be fermented to make cider (alcoholic, hard cider), ciderkin, and vinegar. Health benefits of apple consumption are numerous because of the phytochemicals produced by such apple (Boyer and Liu, 2004). Postharvest diseases affect a wide variety of crops particularly in developing countries which lack sophisticated postharvest storage facilities (Jeffries and Jeger, 1990). Postharvest diseases of fruits and vegetables account for about 10-30% loss of food production mostly in developing countries (Ikhatua and Onwuatuogwu, 2012).

Fungal diseases play a major role in the wastage of long-stored apples (*Malus domestica*). Fungi can cause considerable post harvest losses of apple, depending on cultivar, season and production area among other factors (Valiuskaite *et al*., 2006). Infection by fungi and bacteria may occur during the growing season, at harvest time, during handling, storage, transport and marketing, or even after purchase by the consumer (Dennis, 1983). Due to the mechanical handling of apple fruits, its surface can harbor microorganisms which can attach to the surface or invade the fruits and multiply within its tissues (Oranusi and Wesley, 2012). Prominent postharvest diseases of apple caused by fungi include blue mould caused by *Penicillium expansum*; brown rot caused by *Monilinia fructigena*, Gloeosporium rot, caused by *G. album* and *G. frugtigenum* (Valiuskaite *et al*., 2006).

The spores of pathogens arrive at the surface of the fruit during the growing period when the natural resistance is high. The decay is initiated after a mechanical damage or decrease of apple resistance due to ripening (Dennis 1983). Spores of *Penicillium* species are present in the soil, on the fruit surface, in the air of the store etc. Its preferred hosts are pomiferous fruits. This species is responsible for blue mould rot, a major postharvest disease of apples worldwide (Baert *et al*., 2007; Schovánková and Opatová; 2011).

There are many devastating pathogens inducing fungal diseases, in particular *Penicillium expansum*, *Monilinia fructigena*, and *Gloeosporium* sp but apart from these pathogens there exist some other pathogens that affect the nutrient contents of apple and cause the post harvest spoilage of apple (Pierson *et al*., 1971).

The purpose of this research was to investigate fungal pathogens responsible for apple spoilage in the area under study and determine the effect(s) of the pathogens on the nutritional content of apple.

## MATERIALS AND METHODS

### *Collection of Samples*

Diseased apple samples were collected from three different markets: Iyana Iba, Okoko and PPL markets all within Ojo Local Government area of Lagos State, Nigeria. The diseased samples manifested symptoms such as discoloration, rot, shriveling and stinking, unpleasant odour. The samples were kept in different bags and labeled appropriately.

### *Media*

Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were used. They were prepared according to the manufacturers' instruction.

### *Isolation of Fungi*

The method used to isolate fungi from rotted apple fruits was direct plating. The diseased apple fruit was cut from the rotten point into tiny pieces with a sterile razor blade. The tiny pieces of the diseased apple were surface sterilized.

The tiny pieces were picked with a sterilized inoculating needle and were put at the centre of the solidified media in sterile Petri dishes. The Petri dishes were incubated at a temperature of 25C-28$^0$C, observations of the fungal growth were made.

### *Identification of Fungal Isolates*

The workbench was disinfected with cotton wool soaked in absolute ethanol. The pure cultures preserved in McCartney bottles were sub cultured into freshly prepared media plates. After 48 hours, the cultural characteristics which include colour, pigmentation, elevation, margin and shape of the colony of each isolate was recorded. The isolates were put on slides and microscopic examination of isolates was done. The slides were labeled and a photomicrograph of each slide was taken with the photomicrograph microscopes to observe the nature of the hyphae, the mycelia, and spore apparatus of the organism. Reference was made to Onions *et al*., (1981); Collins *et al*., (1989); William and Dennis, (1990) for confirmation of identity.

### *Pathogenicity Test*

Healthy apple fruits were collected and surface sterilized. The workbench was also sterilized. Cylindrical discs were removed from the fresh apples with a sterile 4 mm cork borer. The cork borer was flamed red hot with a spirit lamp and allowed to cool before use.

About 4 mm mycelial discs of 5 days old cultures of the isolates were used to plug the holes created in the fruit respectively. The discs of the fruit in the cork borer was replaced and then sealed with vaseline jelly to make it air tight. They were then incubated in a humid chamber. The samples were checked every day to determine the effect of the pathogens on them.

### *Determination of Moisture Content*

Five grams of the crushed sample was placed inside a clean crucible which has been previously weighed and ignited. Then the crucible and sample was heated in an oven at 105$^0$C to a constant weight for 4-6 hours. The crucible was removed from the oven and cooled in a desiccator before weighing. The percentage moisture content of the sample was then calculated using the formula below:

$$\% \text{ Moisture} = \frac{100(W_1-W_2)}{W_1-W_0}$$

$W_0$ = Weight of the empty crucible in 'gm'

$W_1$ = Weight of the crucible dish and the sample before oven dried in g

$W_2$ = Weight of the crucible dish and the sample after oven dried in g

***Ash Content Determination***

Five grams (5.0g) of the crushed sample was weighed into porcelain crucible previously ignited and weighed. Organic matter was charred by igniting the material on a hot plate in the fume cupboard. The crucible were placed in the muffle furnace and maintained at $60^0$C for 3hrs. They were then cooled in a desiccator and the ash content was weighed immediately (AOAC, 2000). The percentage ash content was the calculated as follows:

$$\% \text{ Ash} = \frac{100 \text{ (Weight of crucible + ash) – weight of empty crucible)}}{\text{Sample weight}}$$

***Crude Fat Determination***

Five grams of crushed samples (5.0g) were put in thimbles and plugged with cotton wool. The thimbles were dried and inserted into Soxlet extractor system model HT2.The extraction was added to each cup. The cups were inserted into the position and 30-45 mins in rinsing position. The extractable fat was later evaporated to dryness in an oven at $105^0$C for 1hr cooled in a desiccator and weighed. The percentage fat in the sample was calculated.

***Crude Fiber Determination***

One gram (1.0g) of crushed samples or materials was placed inside a 250ml conical flask and 100ml of Trichloroacetic acid (TCA) solution (digestion reagent) was added. After the addition, the sample was boiled under reflux for 40mins commencing from the time of heating. The flask was removed and the content was filtered using Whatman No 4 filter paper.

The residue was washed six times with hot distilled water and once with petroleum spirit. The filter paper together with the residues were transferred into a porcelain crucible and oven dried at $100^0$C overnight in an oven.

The samples were cooled in a desiccator and weighed. The percentage crude fiber was determined by difference in weight during incineration as equivalent to the amount of crude fiber.

***Ascorbic Acid (Vitamin C)***

***Visual Titration Sample***

***Reagents***

Extracting solution 52, Trichloroacectic acid (TCA), 5g of TCA in 100ml of distilled water.

***Dye Solution:*** Fifty (50) mg of 2, 6-dichlorophenol-indophenol was weighed and 42mg of sodium bicarbonate was equally weighed and were dissolved in 100ml of hot (85-$95^0$C) distilled water; cooled and made to 200ml volume in volumetric flask.

Standard Ascorbic acid = 50mg of ascorbic acid was weighed and made up to 10ml with 5% TCA solution. 10ml was diluted to 100ml with 5 TCA (1ML = 0.1mg of ascorbic acid).

***Standardization of Dye***

Five (5) ml of standard ascorbic acid solution was taken plus 5ml of 5% TCA solution and was titrated with the dye solution (2, 6 dichlorophenol indophenol) to a pale pink endpoint which persisted for about 15secs

Dye factor = 0.5

***Titer***

***Preparation of Sample*:** Five grams of sample was weighed and blended with 80ml of 5 TCA solution, filtered and sample extract was made up to 100ml volume with extracting solution in a volumetric flask.

***Assay of Extract***

An aliquot (20ml) of the 5% TCA extract of the sample was measured into a beaker (150ml beaker) and the sample titrated with standard dye solution to a pink end point

The ascorbic acid content of the sample was calculated as given below:

$$\text{Mg of ascorbic acid} = \frac{\text{bcd x 100}}{\text{Ef}}$$

Where b = sample titer value

c = dye factor
d = volume of sample made up to 100ml
E = aliquot volume of sample taken for estimation
F = weight of sample used

***Crude Protein Determination***

For this determination, Kjeldahl nitrogen method was used. One gram (1.0gm) of sample was introduced into the digestion flask. Kjedahl catalyst (5 selenium tablets) tablets were added to the samples. Twenty milliliters of concentrated sulphuric acid (20ml) was added to each sample and fixed into the digester for 8hr until a clear solution was obtained. The cooled digest was transferred into 100ml volumetric flask and made up to the mark with distilled water.

The distillation apparatus was set and rinsed for ten minutes after boiling, 20ml of 4%$^{w}/_{v}$ boric acid was pipette into the conical flask, 5 drops of methyl red was added to each flask as an indicator and the sample were diluted with 75ml distilled water. Ten milliliter of the digest was made alkaline with 20ml of NaOH (20%$^{w}/_{v}$) sodium hydroxide and distilled. The steam exit of the distillator was closed and the change of colour of boric acid solution was timed.

The mixture was distilled for 15minutes (AOAC, 2000). The filtrate was then titrated against 0.1N HCl (hydrochloric acid solution) solution to an end point. Distilled water was used as blank. The percentage total Nitrogen was calculated as follows:

$$\% \text{ Total Nitrogen} = \frac{0.014 \text{ sample titre-blank titre } 0.1 \times 100}{\text{Sample weight}}$$

0.1N = Normality of acid
% Crude protein = % Total Nitrogen conversion factor
Where conversion factor = 6.25

## RESULTS AND DISCUSSION

***Results***

*Fungal Isolates*

The fungi isolated were *Rhizopus* sp., *Aspergillus niger, A. terreus, Trichoderma* sp. and *Mucor* sp.

*Pathogenicity Test*

The symptoms such as brown rots, lesions, scabs observed from the diseased fruit were the same with the apple fruit inoculated with pure isolates of fungi causing disease as they showed the same cultural characteristics when cultured on plate.

The deterioration began from the point of injury and from there it spread slowly to the rest of the apple fruit. After one week of inoculation with daily checking, it was observed that the symptoms observed on the inoculated fruits are similar with that of the diseased fruit samples collected from the markets originally.

*Effect of Fungal Infection on Nutrient Composition of Apple*

From the analyses of the effect of isolated pathogens on the nutritional content of the apple fruit (Table 1), it was observed that for all the pathogens, there was an increase in the percentage moisture content - ranging from 82.46-84.48% as compared to the control which is 73.22% - For the percentage ash, fibre, protein, Vitamin C, fat and sugar contents, there was a reduction in the contents in the diseased samples when compared to the control.

The lowest ash content was recorded in apple fruit attacked by *Aspergillus terreus* (0.06%), while the highest was recorded in fruit attacked by *Rhizopus* sp. (0.14%). Lowest fibre content was in fruits attacked by *A. terreus* (0.28%) and the highest in apple fruit infected with *A. niger* (0.60%). Of the isolated fungi, *Rhizopus* sp. caused the highest reduction in protein content (1.26%), lowest reduction in *Trichoderma* sp.

Lowest content of Vitamin C was in *A. terreus* (5.11%), highest in *Trichoderma* sp. Fat content was lowest (2.26%) in *Mucor* sp., highest in *A. niger* (2.62%) while the control was 5.70%. Sugar content was lowest in *Trichoderma* sp. (4.17%) and highest in *Rhizopus* sp. (6.81%) control was 8.93%.

**Table 1: Effect of Fungal Infection on Nutrient Composition of Apple**

| Fungal Isolates | Moisture Content (%) | Ash Content (%) | Fibre (%) | Protein (%) | Vitamin C (%) | Fat (%) | Sugar (%) |
|---|---|---|---|---|---|---|---|
| Control | 73.22 | 0.28 | 1.00 | 3.10 | 7.77 | 5.70 | 8.93 |
| *Trichoderma* sp. | 84.12 | 0.12 | 0.50 | 1.84 | 6.81 | 2.44 | 4.17 |
| *Rhizopus* sp. | 82.46 | 0.14 | 0.40 | 1.26 | 6.36 | 2.56 | 6.81 |
| *Aspergillus Niger* | 83.56 | 0.08 | 0.60 | 1.37 | 5.62 | 2.62 | 6.15 |
| *Aspergillus Terreus* | 85.52 | 0.06 | 0.20 | 1.44 | 5.11 | 2.38 | 5.29 |
| *Mucor* sp. | 84.48 | 0.10 | 0.40 | 1.62 | 6.51 | 2.26 | 4.63 |

***Discussion***

Apple is an important part of human diet because of its nutritional content, but the effect of deterioration of apple does not only produces unpleasant odour, lesions and symptoms but it also reduces the nutrient composition of the apple and different fungus produces different type of contamination that reduces the nutrient composition of the apple fruit.

From this research work, *Rhizopus* sp, *Aspergillus niger*, *Aspergillus terreus, Trichoderma* sp. and *Mucor* sp from diseased apple fruit were isolated. Due to the shelf life of apple, no visible changes took place between the first three days after inoculation for the pathogenicity tests.

Fruits are susceptible to attack by pathogenic fungi due to low pH, high moisture content and nutrient composition which make them rot and may also make them unfit for consumption by the production of mycotoxins (Philips, 1984).

For an organism to cause infection, it must have the ability to break down the natural defence mechanisms of the host (Norris and Ribbons, 1971). Fungi have been documented to penetrate host tissue through natural openings such as lenticels, stomata and through the unbroken epidermis by means of appresorium or germ tube (Norris and Ribbons, 1971).

This work revealed that *Aspergillus niger*, *Rhizopus* sp, *Aspergillus terrues,* and *Mucor* sp are causative agent in the deterioration of apple fruit from different areas in the Badagry axis of Lagos State, Nigeria. *Aspergillus niger*, *Rhizopus* sp, *Aspergillus terrues, Penicilium chrysogenum, Mucor* sp have been previously reported (Tournas, 2005; Badosa *et al.,* 2008) as common environmental contaminants. Some of these fungi were also isolated in this study. The isolates are the major cause of the perceived offensive odour, change in colour, and also change in the nutrient and chemical composition of the apple fruit.

Postharvest fungal infection of apple reduces the nutrient composition of apple; therefore, methods of preservation must be properly adhered to in order to prevent fungi from infestation on apple fruit. Proper handling, packaging, storage, and transportation of apple fruit will reduce the chance of fungal infection in apple fruit around the Badagry zone of Lagos since these pathogens have been discovered to be common environmental contaminants.

**REFERENCES**

**Association of Official Analytical Chemists International (AOAC) (2000).** *Official Methods of Analysis*, 17th edition (AOAC International, Gaithersburg, MD, USA).

**Badosa ER, Trias D, Pares MP and Montesinos E (2008)**. Microbiological quality of fresh fruit and vegetable products in Catalinia (Spain) using normalized Plate-Counting methods and Real Time Polymerase Chain Reaction (QPCR). *Journal of the Science of Food and Agriculture* **88** 605-611.

**Baert K, Devlieghere F, Flyps H, Oosterlinck M, Ahmed MM, Rajkovi A, Verlinden B, Nicola B, Debevere J and De Meulenaer B (2007)**. Influence of storage conditions of apples on growth and patulin production by *Penicillium expansum. International Journal of Food Microbiology* **119** 170-181.

**Boyer J and Liu RH (2004)**. Apple phytochemicals and their health benefits. *Nutrition Journal* **3**(1) 289-303.
**Collins CH, Patricia ML and Grange JM (1989)**. *Collins and Lynes Microbiological Methods* (edition 6th), (Butterworths, England) 409.
**Dennis C (1983)**. *Post-harvest Pathology of Fruits and Vegetables*, (London, Academic Press Inc) 264.
**Ikhatua MI and Onwuatuogwu CM (2012)**. Cross infectivity of *Brachysporium* sp. from *Dacryodes edulis* and its associated organism on some pomaceous fruits. *Archives of Applied Science Research* **4**(3) 1470-1475.
**Jeffries P and Jeger MJ (1990)**. The biological control of postharvest diseases of fruit. *Biocontrol News and Information* **11** 333-336.
**Korban SS and Skirvin H (1984)**. Nomenclature of the cultivate apple. *Horticultural Science* **19** 177-180.
**Norris JR and Ribbons DW (1971)**. *Methods in Microbiology*, (Academic Press, London, New York) 695.
**Onions AHS, Allsopp D and Eggins HOW (1981)**. *Smith's Introduction to Industrial Mycology* (edition 7th) (Edward Arnold, Bedford Square, London, UK) 398.
**Oranusi US and Wesley B (2012)**. Microbiological Safety Assessment of Apple Fruits (*Malus domestica* Borkh) Sold in Owerri Imo State Nigeria. *Advance Journal of Food Science and Technology* **4**(2) 97-102.
**Phillips DJ (1984).** Mycotoxins as a postharvest problem. *Post-Harvest Pathology of Fruits and Vegetables: Post-harvest Losses in Perishable Crops*. In: Moline, H.E. (edition), (Agricultural Experimental Station, University of Califonia, Berkeley Publications, NE) 50-54.
**Pierson CF, Ceponis MJ and McColloch LP (1971)**. Market Diseases of Apples, Pears, and Quinces**,** *Agriculture Handbook* 376, (U.S. Department of Agriculture, Agricultural Research Service, Washington, DC).
**Schovánková J and Opatová H (2011)**. Changes in phenols composition and activity of phenylalanine-ammonia lyase in apples after fungal infections. *Horticultural Science* (Prague) **38** 1–10.
**Tournas VH (2005)**. Moulds and Yeasts in fresh and minimally processed vegetables and sprouts. *International Journal of Food Microbiology* **99** 71-77.
**Valiuskaite A, Kvikliene N, Kviklys D and Lanauskas J (2006)**. Post-harvest fruit rot incidence depending on apple maturity. *Agronomy Research* **4**(Special Issue) 427-431.
**William CF and Dennis CW (1990)**. *Food Microbiology*, 4th edition. (Tata McGraw-Hill Publishing Company Ltd) 412-416.