Filamentous Fungi Associated with the Spoilage of Post-Harvest Sweet Orange Fruits (*Citrus Sinensis*) Sold in Awka Major Markets, Nigeria

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Abstract Sweet oranges are prone to spoilage by filamentous fungi as a result of their high levels of sugars and low P^H values. These fungi are known to produce toxins which are deleterious to human health. This study was therefore conducted to isolate, characterize and identify the filamentous fungi associated with the spoilage of sweet oranges sold in major Awka Markets, Nigeria. Thirty sweet orange fruits purchased from Eke-Awka, Temporary Site, Nodu, Ifite and Amaenyi Markets were used for the study. The average filamentous fungal count of the spoilt sweet orange fruits was 2.0×10^3 cfu/ml, 1.3×10^3 cfu/ml, 2.1×10^3 cfu/ml, 1.6×10^3 cfu/ml and 1.8×10^3 cfu/ml for the samples from Eke-Awka, Temporary Site, Nodu, Ifite and Amaenyi Markets respectively. The fungi were identified as *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor mucedo*, *Penicillium digitatum*, *Fusarium oxysporum* and *Aspergillus flavus*. The percentage distribution of the fungi was 27.5%, 22.5%, 15.0%, 10.0%, 7.5 and 17.5% for *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor mucedo*, *Penicillium digitatum*, *Fusarium oxysporum* and *Aspergillus flavus* respectively. *Aspergillus niger* caused the highest degree of spoilage. Good agricultural practices, adequate storage facilities and good handling practices must be put in place to reduce the incidence of these fungi in sweet oranges thereby minimizing their spoilage.

Keywords Filamentous Fungi, Spoilage, Sweet Orange Fruits, Major Awka Markets, Nigeria

1. Introduction

Sweet orange (*Citrus sinensis*) belongs to the family Rutaceae and is one of the major commercial fruit crops that is widely eaten fresh or taken as juice [1]. It is one of the most important winter fruit crops of the world and is mainly cultivated in the tropical and subtropical regions with a

Mediterranean climate in over 137 countries on six continents [2-4]. Sweet orange is an important fruit crop in international trade next to grapes.

In Nigeria, the Sweet orange-producing States are Benue, Taraba, Oyo, Imo, Ebonyi, Kwara, Kogi, Kaduna, Ogun, Ondo, Ekiti, Edo, Delta and Osun States [5]. Sweet orange is a major source of vitamins especially Vitamin C, Vitamin A and Vitamin B, calcium, potassium, magnesium, carotenoids, flavonoids, antioxidants, acids, essential oils, dietary fibres, folate, iron, manganese, zinc, sodium and iodine [6].

Sweet oranges have been reported to prevent cancer, asthma, obesity, arthritis, kidney stones, coronary heart diseases, high blood pressure and stroke and are also necessary for the maintenance of healthy mucus membranes, skin and vision [7-10].

One of the limiting factors that influence the fruits economic value is the relatively short shelf life caused by pathogens. In developing countries, where protection and proper handling of fresh sweet orange fruit is inadequate, losses during transit and storage can represent an excess of 50% of the harvested crops [11].

Spoilage of Sweet oranges refers to any changes in the condition of the fruits in which they become less palatable. These conditions may be accompanied by alterations in taste, smell, appearance or texture due to the activities of spoilage microorganisms [12]. The fruits contain high levels of sugars and their low P^H values make them particularly desirable to fungal spoilage.

Sweet oranges are susceptible to a large number of fungal diseases that can cause severe economic losses. These diseases include Sweet orange scab, Citrus black spot disease and Powdery mildew [13-15].

Many moulds and yeast are involved in the spoilage of sweet oranges fruits. These include *Aspergillus* spp, *Fusarium* spp, *Geotrichum* spp, *Penicillium* spp, *Rhizopus* spp, *Saccharomyces* spp, *Candida* spp and *Trichosporon* spp. These fungi render the fresh fruits unfit for human consumption by causing their deterioration, leading to the

reduction in quality and texture, off-flavour development and loss of nutrients hence the objective of this work was to isolate and identify the filamentous fungi associated with the spoilage of post-harvest sweet orange fruits sold in major Awka Markets, Nigeria in order to put in place control measures that would reduce the losses due to spoilage.

2. Materials and Methods

2.1. Samples Collection and Processing

Thirty sweet orange fruits were purchased from Eke-Awka, Temporary Site, Nodu, Ifite and Amaenyi Markets in Awka, Nigeria and kept for two weeks for spoilage to occur. Thirty healthy orange fruits were later purchased from the same Markets and used for the pathogenicity test (fifteen for the test and fifteen as controls) after the fungal isolation. All the samples were transported to the microbiology laboratory of Nnamdi Azikiwe University Awka in separate sterile polythene bags for the filamentous fungal isolation, characterization and identification.

2.2. Filamentous Fungal Isolation

The spoilt sweet orange fruits were surface-sterilized with cotton wool soaked in 70% alcohol and cut into small segments (3mm diameter) using sterile scalpels. The segments were separately homogenized and serially-diluted with sterile distilled water. One milliliter of each homogenized suspension was dispensed into a conical flask containing sterile potato dextrose agar (PDA) and 2.0 percent chloramphenicol.

The contents were properly mixed and dispensed aseptically into sterile petri dishes which were thereafter incubated at 28°C for seven days. The colonies that developed were subcultured onto sterile potato dextrose agar plates to obtain pure cultures and later stored on sterile PDA slants for characterization and identification.

2.3. Characterization and Identification of the Filamentous Fungi

The pure cultures of the filamentous fungi were identified on the basis of cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation using the slide culture technique and microscopic examination.

2.3.1. Slide Culture Technique

A small portion of the aerial mycelia was picked using a sterile inoculating needle and inoculated on a slide containing prepared potato dextrose agar, which was incubated at room temperature for 24 hours after which it was viewed under the microscope. The identity of the filamentous fungi was confirmed with the aid of a mycological atlas.

2.3.2. Microscopic Examination

Microscopic examination was also carried out using the lactophenol cotton blue stain. A drop of the stain was placed on a clean grease-free slide. A small portion of the filamentous fungal culture was emulsified in the stain after which the slide was covered with a coverslip avoiding bubbles. The slide was thereafter viewed under the microscope.

2.4. Pathogenicity Tests of the Filamentous Fungi

Each of the filamentous fungi was tested on healthy orange fruits for its ability to induce spoilage using the methods described by Baiyewu et al [16] and Chukwuka et al [17]. Thirty healthy oranges were used (fifteen as test samples and fifteen as control samples). The test samples were washed with tap water and rinsed with distilled water after which they were surface-sterilized with 70% ethanol. A sterile scalpel was used to make holes in each of the fruits after which the fungi were inoculated separately into the fruits and the core of the fruits replaced. The point of inoculation of the fruits was sealed with petroleum jelly to prevent contamination. The inoculated fruits were then placed in sterile polythene bags (one fruit per bag) and each moistened with wet balls of absorbent cotton wool to create a humid environment and incubated at 28°C for five days and observed for symptom development. The filamentous fungi were re-isolated from the inoculated fruits and compared with the original isolates. The decay rate of the fruits was also determined after two weeks of the inoculation of the fungi into the healthy oranges by measuring the rot diameter of each spoilt fruit.

3. Results

The average filamentous fungal counts of the spoilt sweet orange fruits are shown in Table 1. The counts were 2.0×10^3 cfu/ml, 1.3×10^3 cfu/ml, 2.1×10^3 cfu/ml, 1.6×10^3 cfu/ml and 1.8×10^3 cfu/ml for the fruits from Eke-Awka, Temporary Site, Nodu, Ifite and Amaenyi Markets respectively.

Table 1. Average filamentous fungal counts of the spoilt Sweet orange fruits

Market	Average Filamentous Fungal Count (cfu/ml)
Eke-Awka	2.0×10^3
Temporary site	1.3×10^3
Nodu	2.1×10^3
Ifite	1.6×10^3
Amaenyi	1.8×10^3

The colonial and microscopic characteristic of the filamentous fungi from the spoilt sweet orange fruits are presented in Table 2. The fungi were identified as *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium digitatum*, *Mucor mucedo*, *Fusarium oxysporum* and *Aspergillus flavus*.

Table 2. Colonial and Microscopic Characteristics of the Filamentous fungi from the spoilt Sweet Orange Fruits

Isolate	Colonial features	Microscopic features	Identify
1	Colonies were black in colour	Conidiophores were unbranched with rounded ends that bore conidia	<i>Aspergillus niger</i>
2	Colonies were white in colour and cottony	Sporangia containing spores were seen. Rhizoids were also present	<i>Rhizopus stolonifer</i>
3	Colonies were green in colour	Brush-like Conidiophores bearing Conidia were present	<i>Penicillium digitatum</i>
4	Colonies were cottony and black in colour	Sporangia bearing spores were seen. Rhizoids were absent	<i>Mucor mucedo</i>
5	Colonies were pink in colour	Conidia were spindle shaped	<i>Fusarium oxysporum</i>
6	Colonies were white in colour at first and later black. The reverse side was pale yellow	Hyphae were septate, simple and thick-walled. Conidiophores bearing conidial heads containing conidia were seen	<i>Aspergillus flavus</i>

The occurrence of the filamentous fungi in the Sweet orange fruits is shown in Table 3. *Aspergillus niger* were isolated from the fruits from all the markets, *Rhizopus stolonifer* from the fruits from Eke-Awka, Nodu, Ifite and Amaenyi markets while *Mucor mucedo* were isolated from the fruits from all but Nodu market. In addition, *Penicillium digitatum* were isolated from Nodu, Ifite and Amaenyi markets, *Fusarium oxysporum* from the fruits from Temporary site and Ifite markets while *Aspergillus flavus* were isolated from the fruits from Eke-Awka, Nodu and Amaenyi markets.

Table 3. Occurrence of the filamentous fungi in the spoilt Sweet orange fruits

Market	<i>Aspergillus niger</i>	<i>Rhizopus stolonifer</i>	<i>Mucor mucedo</i>	<i>Penicillium digitatum</i>	<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>
Eke-Awka	+	+	+	-	-	+
Temporary Site	+	-	+	-	+	-
Nodu	+	+	-	+	-	+
Ifite	+	+	+	+	+	-
Amaenyi	+	+	+	+	-	+

+ = detected

= not detected

The distribution of the filamentous fungi in relation to the markets is presented in Table 4. The percentage distribution of 25.5%, 10.0%, 37.5%, 12.5% and 15.0% were obtained for the samples from Eke-Awka, Temporary site, Nodu, Ifite and Amaenyi markets respectively.

Table 4. Distribution of the filamentous fungi in relation to the Markets

Markets	<i>Aspergillus niger</i> (n)	<i>Rhizopus stolonifer</i> (n)	<i>Mucor mucedo</i> (n)	<i>Penicillium digitatum</i> (n)	<i>Fusarium oxysporum</i> (n)	<i>Aspergillus flavus</i> (n)	% Distribution
Eke-Awka	2	3	3	0	0	2	25.0
Temporary Site	1	0	1	0	2	0	10.0
Nodu	5	4	0	2	0	4	37.5
Ifite	1	1	1	1	1	0	12.5
Amaenyi	2	1	1	1	0	1	15.0

n= number of isolates

The percentage distribution of the filamentous fungi in the spoilt Sweet orange fruits is presented in Table 5. The percentage distribution were 27.5%, 22.5%, 15.0%, 10.0%, 7.5% and 17.5% for *Aspergillus niger*, *Rhizopus stolonifer*, *Mucor mucedo*, *Penicillium digitatum*, *Fusarium oxysporum* and *Aspergillus flavus* respectively

Table 5. Percentage Distribution of the filamentous fungi isolated from the spoilt sweet orange fruits

Filamentous fungi	No. of isolates	Distribution (%)
<i>Aspergillus niger</i>	11	27.5
<i>Rhizopus stolonifer</i>	9	22.5
<i>Mucor mucedo</i>	6	15.0
<i>Penicillium digitatum</i>	4	10.0
<i>Fusarium oxysporum</i>	3	7.5
<i>Aspergillus flavus</i>	7	17.5

The decay rate of the filamentous fungi in the healthy Sweet orange fruits is presented in Table 6. The rot diameter of *Aspergillus niger* was 35mm, that of *Rhizopus stolonifer* 30mm while *Mucor mucedo*, *Penicillium digitatum*, *Fusarium oxysporum* and *Aspergillus flavus* had 25mm, 20mm, 10mm and 28mm respectively.

Table 6. Decay rate of the filamentous fungi in the Sweet Orange fruits

Filamentous fungi	Rot Diameter (mm)
<i>Aspergillus niger</i>	35
<i>Rhizopus stolonifer</i>	30
<i>Mucor mucedo</i>	25
<i>Penicillium digitatum</i>	20
<i>Fusarium oxysporum</i>	10
<i>Aspergillus flavus</i>	28

4. Discussion

The filamentous fungi associated with the spoilage of Sweet orange sold in Awka major Markets, Nigeria were studied. The average counts ranged between 1.3×10^3 cfu/ml and 2.1×10^3 cfu/ml. The sample from Nodu Market had the highest count of 2.1×10^3 cfu/ml while those from Temporary Site market had the least count of 1.3×10^3 cfu/ml (Table 1). The filamentous fungi isolated from the spoilt orange fruits were identified as *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium digitatum*, *Mucor mucedo*, *Fusarium oxysporum* and *Aspergillus flavus* (Table 2). Ojo et al [18] also isolated *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium digitatum* and *Rhizopus stolonifer* from the rotted orange fruits displayed for sale in five different market places in five major cities in Oyo State in South Western Nigeria. Akintobi et al [19] also isolated *Aspergillus niger*, *Penicillium digitatum* and *Rhizopus stolonifer* from deteriorating citrus fruits in Ibadan, Southwestern Nigeria.

Mohammed et al [20] also isolated *Fusarium oxysporum*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium*

digitatum from decayed sweet orange fruits collected from Lapai, Niger State, Nigeria. Tafinta et al [21] also isolated *Aspergillus niger*, and *Rhizopus stolonifer* from sweet orange fruits in Sokoto State.

Aspergillus niger were isolated from the samples from all the markets while *Rhizopus stolonifer* were isolated from the samples from all except the Temporary Site Market. *Mucor mucedo* were isolated from the samples from all except Nodu market, *Penicillium digitatum* were isolated from Nodu, Ifite and Amaenyi markets, *Fusarium oxysporum* were isolated from the samples from Temporary site and Ifite markets while *Aspergillus flavus* were isolated from the samples from Eke-Awka, Nodu and Amaenyi markets (Table 3).

The distribution of the filamentous fungi in relation to the markets showed that 25.0%, 10.0%, 37.5%, 12.5% and 15.0% of the fungi were isolated from the samples from Eke-Awka, Temporary Site, Nodu, Ifite and Amaenyi markets (Table 4).

Aspergillus niger had the highest percentage distribution of 27.5%, *Rhizopus stolonifer* (22.5%), *Mucor mucedo* (15.0%), *Penicillium digitatum* (10.0%), *Fusarium oxysporum* (7.5%) and *Aspergillus flavus* (17.5%) (Table 4).

Tafinta et al [21] however reported the percentage distribution of 17% and 36% for *Aspergillus niger* and *Rhizopus stolonifer* in the sweet orange samples they studied in Sokoto. Akintobi et al [19] reported a frequency of occurrence of 50%, 10% and 50% for *Aspergillus niger*, *Penicillium digitatum* and *Rhizopus stolonifer* respectively in their orange samples from Ibadan South western Nigeria.

The decay rate of the filamentous fungi in the sweet orange fruits showed that *Aspergillus niger* produced the highest rot with a diameter of 35mm, *Rhizopus stolonifer* (30mm), *Mucor mucedo* (25mm), *Penicillium digitatum* (20mm), *Aspergillus flavus* (28mm) while *Fusarium oxysporum* produced the lowest rot with a diameter of 10mm. Tafinta et al [21] however reported that *Rhizopus stolonifer* was the most active fungi that caused the rot of the orange fruits they studied, with a rot diameter of 45mm while *Aspergillus niger* was the least active with a rot diameter of 25mm. Bali et al [22] reported that the black mold, *Aspergillus niger* caused the post harvest spoilage of sweet orange and acid lime in the field.

This result agreed with the reports of Baiyewu et al [16] and Chukwuka et al [17]. The occurrence of these fungi in the sweet orange fruits was as a result of contamination during harvesting, transportation, storage and handling.

These fungi isolated from the sweet orange fruits have been reported to produce toxins [23]. These moulds have also been reported to produce secondary metabolites such as aflatoxins which have been associated with cancer of the liver, aflatoxicosis and acute hepatitis in humans especially in the developing world [16]. These fungi have also been reported to be pathogenic and could cause diseases [24]. *Aspergillus* spp are known to produce several toxic metabolites such as malformins, naphthopyrones and ochratoxins which pose a serious health hazard to human and

animal health [25,26].

In Nigeria, sweet oranges are usually transported to areas of high demand in open vehicles with no preservation facilities. In the markets, they are usually displayed in open trays, baskets, pans, and tables thereby exposing them to contamination by microorganisms including the filamentous fungi. It is therefore pertinent that adequate storage facilities must be put in place for these important fruits. Good hygiene must also be observed during their handling and processing. In addition, orange fruits with any symptom of spoilage must be properly disposed off and not be sold and consumed by the public because of their adverse effects on health.

5. Conclusions

This work showed that the filamentous fungi isolated from the sweet orange fruits examined were associated with their spoilage as shown by the varying degree of symptoms they produced when inoculated into the healthy fruits. These fungi are known to be toxigenic or pathogenic to health, therefore their presence in sweet oranges must be controlled. This should be achieved through proper washing of the harvested fruits, disinfection of transit containers, proper handling of the fruits to avoid injuries, adequate hygienic practices by the handlers, provision of good storage facilities and the use of safe food grade fungicides.

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