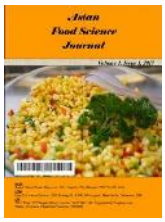

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# Useful Volatile Organic Biomarkers for Discriminating Spoilt Onion Cultivars in Sokoto

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author KBA performed mycological analysis. Authors ADI and AAF designed the study and wrote the protocol. Author ADI wrote the first draft of the manuscript. Authors AJM, SAA, MHU, SIM and KBA prepared subsequent versions of the manuscript and managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Microorganisms degrade food components in order to access nutrient available in such food and in return produce organic compounds from the breakdown of the food components. These organic compounds hold potential to be exploited as biomarkers to detect the presence of such microorganisms. This study was conducted to evaluate fungi associated with volatile organic compounds production in spoilt onion cultivars. A total of 54 onion bulbs which include 45 healthy and 9 spoilt onion bulbs belonging to three cultivars were used for this study. All laboratory investigations were carried out by standard methods. Four fungal genera were identified and these include *Aspergillus*, *Fusarium*, *Mucor* and *Candida*. The GC-MS analysis revealed the presence of twenty six (26) volatile compounds in spoilt white onion bulb, (9) in yellowish-brown and (8) in the purple variety, respectively. White onion bulb had the following dominant compound; nonane (11.97%), decane (11.96%), octane (9.3%) of which ten was unknown. The compounds sulphurous acid, 2-ethylhexylisohexyl ester (18.60%), phenol 2,6-bis(1,1-dimethylethyl)-4-methyl (7.90%) were dominant in yellowish-brown cultivar including five (5) unknown, while the

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predominant compounds in the spoilt purple onion variety were, phthalic acid, di-(1-hexen-5-yl)ester (69.64%) and phenol-2,6-bis (1,1-dimethylethyl)-4-methyl, of which five were unknown. This study suggests that these unique volatile organic compounds could provide the bedrock for restraining postharvest losses and the volatile organic compounds could also form baseline knowledge for discriminating diseases associated with onion.

*Keywords: Aspergillus; Fusarium; spoilt onion; volatile compounds.*

## 1. INTROD UCTION

Onion (*Alluim cepa*) is one of the major commercial farm product planted by the farmers in Sokoto metropolis. The farm produce is usually transported to other parts of the country like the South and East where the demand for the product is high. Onion has a principal effect in preventing heart diseases and other ailments. The pungent nature of onion is due to volatile oil known as allyl-propyl-disulphide. Many diseases affects onion during post-harvest and these diseases include black mould, blue mould, neck rot, brown rot, soft rot, and smudge among which black mould and blue mould are the predominant ones which restricts the availability of onion to domestic and international trade. The introduction of spoilage microorganisms to crop can be by the seed itself, in the field during crop growth, during harvesting, post harvesting handling, or during product storage and distribution. Early measures aimed at intervening on crop spoilage during growth and harvest through the use of 'good agricultural practices' (GAP) will naturally reduce yield loss due to spoilage [1].

Many researchers have extensively use volatiles produced by rotten fruits and vegetables as biomarkers in discriminating diseases in order to reduce losses in storage [2,3]. Despite this, the use of GC retention time and peak area data alone is not enough to discriminate diseases; further confirmation based on compound detection is needed for higher quality assurance.

The production of volatile metabolites from diseased carrot, potato and onions have been analysed using GC-MS [2,3,4]. This research was conducted; to determine the fungal pathogens associated with onion spoilage and to identify volatile compounds associated with spoilt onion bulbs.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

Sokoto state is located in the extreme northwest of Nigeria, near the confluence of Sokoto River and Rima River. In 2006, it has a population of 427,760. Sokoto is a modern day capital of Sokoto state and also a Sahel savannah. It has the GPS coordinates of latitude 13.005873°C and longitude of 5.247552°C.

### 2.2 Sample Size and Sample Collection

Fifty four (54) spoilt onion bulbs belonging to three cultivars (white, yellowish brown and purple) were collected from meat and vegetable market within Sokoto metropolis into clean polythene bags and transported to the laboratory for analysis.

### 2.3 Isolation and Identification of Fungi

To isolate fungal pathogens responsible for spoilage of the different onion cultivars, the bulbs were stripped of their outer scales, surface sterilized with (1%) sodium hypochlorite, rinsed with distilled water before blotted with dry sterile filter paper [5]. Sabouraud dextrose agar plates were inoculated with a small portion of the rotted lesions using sterile forceps. The inoculated plates were incubated upright at room temperature for five days and all observed colonies were sub-cultured to obtain a pure culture [6].

Fungal colonies were studied using Lactophenol cotton blue mount as described by Oyeleke and Manga [7]. The colour, growth rate, texture, pigmentation and morphology of each sample were examined macroscopically.

### 2.4 Inoculum Preparation

This was done as described by Negi and Banerjee [8]. Twenty five millilitre (25 ml) of sterile distilled water was added to a 5 day- old slant and scrapped aseptically with an inoculating loop. 0.5 ml ($1.3 \times 10^7$ cells/ml) of the suspension was used as inoculum for subsequent pathogenicity test.

### 2.5 Pathogenicity Test

This was done as described by Kutama et al. [9]. Forty five healthy and matured onion fruits were

sterilized with 1% sodium Hypochlorite and rinsed with distilled water. One side of each replicates was aseptically punctured using a sterile scalpel beyond its epidermal layer. The identified isolates were inoculated into the punctured portion and aseptically sealed with Vaseline. All samples were incubated at room temperature for 21 days and observed weekly for spoilage symptoms.

### 2.6 Extraction of Volatile Compounds

Volatile compounds were extracted using general purpose solvent as described by Ibrahim et al. [10]. Two gram of inoculated onion (for the three varieties) was weighed into different bottles, saturated with 20 ml of diethyl ether and allowed to stand at room temperature overnight. Each bottle was filtered using Whatman filter paper and the filtrate was collected in a sterile bottle, closed tightly before the GC-MS analysis.

### 2.7 Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis was performed using GC-MS-QP2010 plus (Shimadzu, Japan), at 60°C for 5 min in the oven and finally at 10°C/min to 280°C (held for 10 min). Chromatography separations were performed using DB-WAX analytical column 30 m, 0.25 mm (J&W scientific, Folsom C.A) with helium as carrier gas at a constant flow rate of 0.8 ml/Min.

### 2.8 Identification and Quantification of Volatile Compounds

The chromatography peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library (Hewlett-Packard co., Palo Alto, CA). Approximate quantification of volatile compounds was estimated according to methods of Wanakhachornkrai and Lertsiri [11], by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). The results were presented as the peak area normalized (%).

## 3. RESULTS

Table 1 shows the isolated fungal organisms and their percentage of occurrence in spoilt onion cultivars. Several fungal organisms were responsible for the spoilage of the three varieties of onions used in this study. These organisms include *Aspergillus niger*, *Mucor hiemalis* and *Candida tropicalis* from the purple variety, *A. niger* from the yellowish-brown variety and *A. niger, Fusarium oxysporum* and *Mucor* spp from the white variety of onions.

The results of the pathogenicity test showed that onion bulbs inoculated with *A. niger* present the most severe symptom after three weeks (3) of inoculation, followed by bulbs inoculated with *Mucor hiemalis* and those inoculated with *Fusarium oxysporum*, while those inoculated with *Candida tropicalis* have the least symptoms of spoilage (Figs. 1-4).

The results of the GC-MS analysis of the spoilt onion cultivars obtained from meat and fish markets (Sokoto) are presented in Table 2. From the results, twenty two (22) organic compounds were determined in spoilt white onion bulb of which 6 were unknown, 25 compounds were determined in spoilt purple onion bulb with 5 unknown, while 34 organic compounds were also determined in spoilt yellowish-brown bulb, including 10 unknown.

Table 3 showed the results of the volatile compounds obtained from onion cultivars inoculated with pathogenic fungi. From the results, twenty-six (26) organic compounds were determined in spoilt white onion bulb, 8 from spoilt purple onion bulb and 9 from the yellowish-brown variety.

The volatiles; phenol-2,6-bis(1,1-dimethyl)-4-methyl and sulfurous acid, 2-ethylhexyl isohexyl esters occurred both in yellowish-brown and purple variety at varying degree of concentration.

## 4. DISCUSSION

Many diseases affects onion during post-harvest and these diseases include black mould, blue mould, neck rot, brown rot, soft rot, and smudge among which black mould and blue mould are the predominant ones which restricts the availability of onion to domestic and international trade. This study reveals that spoilt onion cultivars in Sokoto are associated with pathogenic fungal organisms, which might be attributed to factors such as exposure of the onion bulbs to soil, post harvesting operations, personal hygiene of food handlers and air. The fungal organisms identified with onion spoilage are *Aspergillus niger*, *Fusarium oxysporum*, *Mucor hiemalis* and *Candida tropicalis.* Among these organisms, only *Fusarium* and *Aspergillus*

*sp*. have been implicated in the spoilage of onion bulbs (Kuchareck, 2004).

The result of the infectivity test showed that onion bulbs inoculated with *A. niger* manifested the highest severity of spoilage followed by *Mucor hiemalis* (in both white and purple variety) and lastly those inoculated with *Fusarium oxysporum*. This is in correspond to [12,13], where in their separate work reported that *A. niger* and *Fusarium oxysporum* are the major fungal pathogens associated with spoilage of onion bulbs. The ability of these organisms to infect onion bulbs may suggest that they have the prerequisite enzymes that can degrade the various polymers such as hemicelluloses, lignin, polygalacturonic acid and proteins [14,15].

The results of the GC-MS analysis showed that the number and relative amount of volatile compounds produced varied among the spoilt onion varieties. Forty three volatile compounds were detected in high abundance in the inoculated onion bulbs. Of the 43 compounds, 20 were disease discriminatory, including 16 that were unique to white onion bulb. Very few of the compounds were detected in high abundance; sulfurous acid, 2-ethylhexyl isohexyl ester, phthalic acid di-(1-hexen-5-yl) ester, phenol 2,6-bis (1,1-dimethylethyl)-4-methyl, 1,6-methano (10) annulene and 6-methyl tetralin. Not all of these compounds were produced by the pathogens. Low amounts of sulfur compounds are found in spoilt onion bulbs compared to healthy bulbs [16]. The low amounts of

**Table 1. Fungi species associated onion cultivars obtained with Sokoto**

| Isolate | Colony Description | Microscopy | Probable Identity |
|---|---|---|---|
| P.O.1-i | Black colonies with edges and raised surface. Powdery in nature. The hyphae diffused into the media | Conidiospores terminate in vesicles and conida in chains | *Aspergillus niger* |
| P.O.1-ii | White cottony colonies | Ascospores yellowish. Ovale ellipsoidal with hyaline wall smooth | *Candida tropicalis* |
| P.O.2-i | Grey and cottony with aerial growth | Aerial hyphae bearing sporangiospores | *Mucor hiemalis* |
| Y.B.1-i | Black large, powdery colonies | Conidiospores terminate in vesicles | *Aspergillus niger* |
| Y.B.2 | Black large, powdery colonies | Conidiospores terminate in vesicles | *Aspergillus niger* |
| W.O.1-i | Black large, powdery colonies | Conidiospores terminate in vesicles | *Aspergillus niger* |
| W.O.1-ii | Grey and cottony with aerial growth | Aerial hyphae bearing sporangiospores | *Mucor* spp. |
| W.O.2 | Whitish mycelium spore | Conidiophores with septa | *Fusarium oxysporum* |

*Key:-P.O – Purple onions, Y.B – Yellowish-brown onion, W.O – White Onion*
*From the table, the predominate organism in all the 3 onion cultivars is the Aspergillus niger*

**Fig. 1. Pathogenicity test of bulbs inculated with *Fussarium oxysporum***

sulfur compound detected in our work may be attributed to the fact that spoilage microorganism tend to degrade these sulfur compounds during spoilage of the onion bulbs and thereby contributing to the biogeochemical sulfur cycle. This corresponds to the work of [17] who also reported that sulphur compounds are available in wild *Allium* species.

In a study with tomatoes, the compound 1,2-dimethyl benzene was found to be unique to fruits inoculated with *F. oxysporum*, while the compounds 1,2-dimethylbenzene, Nonane, 1,2,3-trimethyl benzene, Tetralin, tetracyclo[3,3,1(2,8)0(4,6,)-non-2-ene, tricycle[5,2,10,(sup2,6)decane, 4-phynyl(but-3-ene-1-yne) and 1,8-dimethylnaphthalene were unique to *A. flavus* [18]. The variation in the presence of *A.niger* rather than *A. flavus* could be as a result of mixed infections especially in the same lesion, whereby *A. niger* was more dominant and suppresses the growth of *A. flavus*. Fungi emit cocktails of dozens to hundreds of unique volatile compounds that fall into many chemical classes including alcohols, aldehydes, acids, ethers, esters, ketones, hydrocarbons, terpenes and sulfur compounds [19].

**Fig. 2. Pathogenicity test bulbs inoculated with *Mucor hiemalis***

**Fig. 3. Pathogenicity tests of bulbs inoculated with *Aspergillus niger***

**Fig. 4. Pathogenicity tests of bulbs inoculated with *Candida tropicalis***

Volatiles have been used to discriminating diseases associated with spoilage of carrot [20]. However, little or no information has been cited in literature regarding the changes in the volatile metabolite profile of onion infected by fungi. In this study, forty three compounds were unique to one or more of the inoculated *fungal spp*. These compounds could be used in different combinations to discriminate disease associated with onion.

The purple and yellowish-brown onion variety inoculated with pathogenic fungi showed a marked decrease in the abundance of volatile compounds compared to spoilt onion bulbs obtained from the market. This phenomenom could be attributed to the fact that some of the metabolites like indane, octane, Nonane, indane-2, 3-dihydro, benzene, P-xylene, hexadecane, hexadecanoic acid could have been utilized by some of the pathogenic fungi to serve as source of hydrogen and electron acceptors. Octane (9.32%), Nonane (11.97%), 1, 2, 3-trimethyl benzene (7.73%) and decane (9.27%) were in abundance to a specific onion variety after inoculation, their abundance varied greatly among the other onion cultivars. These compounds could be employed in the quantitative discrimination of diseases associated with spoilt onion cultivars.

**Table 2. The volatile compound profile of spoilt onion cultivars**

| $RT^{-1}$ (min) | Volatile Compounds | Area normalized (%) | | |
|---|---|---|---|---|
| | | White | Yellow | Red |
| 3.43 | 2-methyl heptanes | - | 2.07 | - |
| 3.99 | Octane | - | 6.4 | 5.87 |
| 3.99 | Oktagen | 8.33 | - | - |
| 4.70 | 1-Ethyl-3-methyl cyclopentane | - | - | 1.5 |
| 4.70 | Unknown | - | 2.62 | |
| 5.43 | P-xylene | 3.58 | - | - |
| 5.43 | P-xylene(1,4-Dimethyl benzene) | - | - | 2.89 |
| 5.62 | hexane-2,3,4- Trimethyl | 3.07 | - | - |
| 5.63 | P-xylene | - | 3.04 | - |
| 6.72 | Nonane | 12.77 | 9.29 | 7.61 |
| 7.59 | Unknown | - | - | 1.71 |
| 7.60 | Unknown | 2.17 | 2.78 | - |
| 7.88 | Unknown | 3.10 | 2.99 | - |
| 8.34 | 1-ethyl-4-methyl benzene | 3.57 | - | - |
| 8.34 | 2-methyl-3-methylene-1-hepten-5yne | - | - | 2.30 |
| 8.34 | benzene-1-ethyl-3-methl | - | 3.67 | - |
| 8.91 | Unknown | - | - | 3.03 |
| 9.14 | Unknown | - | 3.58 | - |
| 9.44 | Unknown | 5.83 | - | 4.87 |
| 9.45 | Unknown | - | 6.54 | - |
| 10.14 | Decane | 13.82 | - | 8.48 |
| 10.15 | Decane | - | 10.31 | - |
| 10.36 | Cumene (2-phenyl propane) | - | 2.43 | - |
| 10.70 | Indane | - | - | 2.32 |
| 10.70 | Indane-2,3-dihydro | - | 3.32 | - |
| 10.71 | Indane | - | 3.50 | - |
| 10.95 | Unknown | - | 1.81 | 1.80 |
| 11.42 | 1-methyl-3-propylbenzene | - | 1.88 | - |
| 12.52 | 1,4-dimethyl-2-ethylbenzene | - | - | 1.37 |
| 12.53 | 1,4-dimethyl-2-ethylbenzene | - | 2.00 | - |
| 12.9 | Octahydro-4,7-methanoindene | 4.08 | - | - |
| 12.9 | tricyclo[5.2.1.0(Sup2,6)] decane | - | 2.75 | 2.42 |
| 13.5 | Hendecane | 3.91 | - | - |
| 13.5 | Undecane | - | 2.56 | - |
| 13.57 | Undecane | - | - | 0.70 |

| RT$^{-1}$ (min) | Volatile Compounds | Area normalized (%) | | |
|---|---|---|---|---|
| | | White | Yellow | Red |
| 14.78 | Tetralin | - | 2.28 | 2.01 |
| 14.78 | Benzocyclohexane | 3.18 | - | - |
| 15.36 | Azulene | 3.80 | 2.55 | 2.35 |
| 16.62 | Unknown | 1.26 | - | 0.79 |
| 16.62 | Dodecane | - | 0.87 | - |
| 17.95 | G-methano[10] annulene | 1.58 | - | - |
| 17.96 | 6-methyl Tetralin | - | 1.08 | 0.98 |
| 18.72 | Unknown | 13.19 | - | - |
| 18.73 | 1,6-methano[10] annulene | - | 8.79 | 8.06 |
| 19.15 | 1,6-methano[10] annulene | - | - | 6.17 |
| 19.51 | Unknown | 1.21 | 0.88 | - |
| 20.93 | (1-ethynyl-2-methyl-1-propenyl) benzene | - | - | 0.61 |
| 20.93 | Phenyl benzene(Lemonene) | - | 0.74 | - |
| 21.7 | Pent-1-yn-3-ene, 4-methyl-3-phenyl | - | 0.58 | - |
| 21.97 | 2,3-dimethyl naphthalene (Guajen) | 2.68 | - | - |
| 21.98 | Guajen | - | 1.90 | - |
| 22.31 | 2-Hexyl-5-methyl-3(2H)- furanone | - | - | 20.64 |
| 23.63 | Phenol-2,3,-bis (1,1-dimethyl)- 4-methylcarbonate | 1.05 | - | - |
| 23.72 | Unknown | - | 0.89 | - |
| 24.45 | Diethylphethalate | 2.91 | 1.68 | - |
| 24.95 | Hexadecane (cetane) | - | 1.15 | - |
| 25.16 | Unknown | 0.68 | - | - |
| 25.17 | 5-methyl-2-octyl-3(2H)- furanone | - | - | 5.39 |
| 26.81 | Unknown | - | 0.60 | - |
| 27.91 | dexadecanoic acid | - | 8.29 | - |
| 27.92 | Hexadecanoic acid | 0.91 | - | - |
| 27.93 | Hexadecanoic acid | - | - | 1.64 |
| 30.23 | Unknown | - | - | 1.14 |
| 32.91 | Unknown | - | 0.27 | - |

**Table 3. The volatile compound profile of three onion cultivars inoculated with different pathogenic fungi**

| RT$^{-1}$ (mm) | Compound | Peak area (%) | | |
|---|---|---|---|---|
| | | Purple | yellowish- brown | White |
| 3.43 | 2 methylheptane | - | - | 3.15 |
| 3.98 | Octane | - | - | 9.32 |
| 5.42 | O-xylene (1,2-Dimethylbenzene | - | - | 5.15 |
| 6.70 | Nonane | - | - | 11.97 |
| 7.58 | Unknown | - | - | 3.27 |
| 7.86 | Unknown | - | - | 3.34 |
| 8.32 | 2-Phenylpropane | - | - | 3.84 |
| 9.42 | 1,2,3 trimethylbenzene | - | - | 7.73 |
| 10.11 | Decane | - | - | 11.98 |
| 10.68 | tetracyclo [3,3,1(2,8)0(4,6)-non-2-ene | - | - | 3.21 |
| 11.31 | Toluene M-Propyl) | - | - | 0.75 |
| 11.39 | M-cymene | - | - | 1.40 |
| 12.50 | Tricyclo [5,2,1,0, (Sup 2,6) ] decane | - | - | 1.62 |
| 12.88 | Unknown | - | - | 3.09 |
| 13.48 | Tetralin | - | - | 2.87 |
| 14.75 | 4-Phyny (But 3-ene-1-yne | - | - | 2.31 |

| $RT^{-1}$ (mm) | Compound | Peak area (%) | | |
|---|---|---|---|---|
| | | Purple | yellowish- brown | White |
| 15.33 | 6-Methyl tetralin | - | - | 2.41 |
| 17.94 | 1,6-methano (10 )annulene | - | - | 0.91 |
| 18.67 | 1,8-Dimethylnapthalene | - | - | 9.27 |
| 21.95 | Unknown | - | - | 1.54 |
| 23.62 | Phenol 2,6-bis (1, 1-dimethylethyl)-4-methyl | 12.17 | 7.90 | - |
| 23.67 | Unknown | - | - | 1.15 |
| 24.44 | Unknown | - | - | 5.28 |
| 24.44 | Unknown | - | 42.06 | - |
| 24.44 | Phthalic acid, di-(1-hexen-5-yl) ester | 69.64 | - | - |
| 24.94 | Unknown | - | - | 0.99 |
| 26.03 | Unknown | 2.12 | - | - |
| 26.80 | Unknown | 1.92 | - | - |
| 26.80 | Unknown | - | - | 0.46 |
| 27.65 | Unknown | 4.43 | - | - |
| 27.91 | Unknown | 4.43 | - | - |
| 28.35 | Elaidic acid, methyl ester | - | 0.02 | - |
| 28.82 | Unknown | 2.6 | - | - |
| 30.12 | sulfurous acid, 2 ethylhexyl isohexyl ester | - | 18.60 | - |
| 30.39 | Unknown | - | 8.16 | - |
| 31.91 | Unknown | - | - | 0.68 |
| 31.95 | Unknown | - | - | 0.09 |
| 31.96 | Unknown | - | 6.88 | - |
| 32.72 | Unknown | - | 1.92 | - |
| 33.37 | Unknown | - | 1.57 | - |
| 34.34 | Sulfurous acid, 2-ethylhexyl isohexyl ester | 5.2 | 0.03 | - |

*Key: $RT^{-1}$ = Retention time*

The headspace analysis of onion bulb detected several sulphur compounds such propanol, dimethyl disulfide, methyl propyl disulfide [21-23] which were not detected in our research work. The difference may be due to the method of extraction, the extraction solvent used and column [18]. The data generated in this study could be used to develop neural network models to discriminate onion diseases [23].

## 5. CONCLUSION

The results of this research revealed that spoilt onion bulbs contains organisms such as *Aspergillus niger*, *Fusarium oxysporum*, *Mucor hiemalis* and *Candida tropicalis* thus rendering them unsafe for human consumption. Forty three volatile compounds were encountered in the three onion varieties, which suggest that those volatile metabolites could be exploited as biomarkers to discriminate disease/pathogen or toxigenic fungi, thereby preventing postharvest losses and preventing the risk of consuming mycotoxigenic fungi and or their metabolite after further validation. Spoilt onion bulbs regarded as waste could also serve as a substrate for biogeneration of volatile compounds.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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