STORAGE FUNGI OCCURRENCE IN ROSELLE (*Hibiscus sabdariffa*) CALYX AND EFFECTS ON IT’S COMPOSITION

**ABSTRACT**

Roselle *(Hibiscus sabdariffa*) is a tropical plant grown mainly for its vibrant red calyces used in making herbal teas and beverages rich in antioxidants and vitamin C. However, global production of roselle is greatly hampered by severe fungi attack especially at post-harvest stage. This study therefore, investigated the incidence, pathogenicity, and effects of various fungal species on the quality of *Hibiscus sabdariffa* (zobo) calyx. Samples of infected roselle calyx were collected from Ubani and Ndoro markets located in Umuahia and Ikwuano LGA, respectively in Abia State. Fungi isolation from the infected samples was done using standard procedures. Proximate, mineral and vitamin composition of fungi inoculated roselle samples were done using AOAC procedures. Experiments were laid out in CRD in triplicates. Data were analysed using ANOVA at α0.05. Fungi species including *Aspergillus* (*A. flavus*, *A. niger*), *Rhizopus* sp., and *Fusarium* sp. were isolated from zobo samples. Among these, *A. niger* exhibited the highest incidence (51.39 %), followed by *Fusarium* sp (28.01%), *A. flavus* (17.13 %), and *Rhizopus* sp. (3.47 %). Pathogenicity tests revealed that *A. niger* and *Rhizopus* caused 100 % rot in the zobo samples, while *A. flavus* and *Fusarium* sp caused 90 % rot. Zobo samples inoculated with sterile water (control) had significantly higher minerals: than those inoculated with fungi. The control samples exhibited the highest concentrations of iron (0.0046 mg/100g), Β-carotene (0.0058 mg/100g), K (1.5250 mg/100g), Ca (0.2600 mg/100g) and vitamin A (4.2800 mg/100g), Vitamin C (2.3550 mg/100g), while fungal-inoculated samples had reduced nutrient levels iron (0.0300-0.0415 mg/100g), Β-carotene (0.0035-0.0051 mg/100g), K (0.9650-1.1300 mg/100g), Ca (0.1400-0.2200 mg/100g), C (1.1650-1.1800 mg/100g) and Vit A (2.4750-3.1400 mg/100g). The proximate composition of zobo calyx was influenced by fungal contamination. Control had the higher ash (1.76 %) and crude fibre (2.68 %) contents than the fungi inoculated samples. Control had the least moisture (12.27 %) content compared to the fungi inoculated roselle (12.68-14.06 %). This study highlights the negative impact of fungal contamination on the nutritional and proximate composition of zobo calyx, emphasizing the need for their control.

Key words: Hibiscus calyx, proximate composition, phytochemical, fungi rots

**1.0 Introduction**

*Hibiscus sabdariffa* commonly known as or rosellee is a member of malvaceae family; it is also known as hibiscus. It is a tropical plant recognized for its vibrant red calyces and deep green leaves. The calyces are used in many culinary and medicinal applications, more often to make a tart and refreshing tea (Cid-Ortega and Guerrero-Beltrán, 2015). It is very rich in antioxidants and vitamin C. *Hibiscus sabdariffa* is associated with numerous health benefits, including cardiovascular support and anti-inflammatory properties. *Hibiscus* has more than three hundred species distributed in tropical and subtropical regions around the world which are used as ornamental plants (Mahadevan and Kamboj, 2009; Singh *et al.*, 2017).

*Hibiscus sabdariffa* is traced to West Africa, particularly Sudan and Senegal (Mhazo and Chivandi, 2017). From there, it spread throughout tropical regions in Africa, including Nigeria, Ghana, and Ethiopia. The plant was later introduced to India and other parts of Asia, where it is also widely cultivated. Today, *Hibiscus sabdariffa* can be found in many tropical and subtropical regions, including Central and South America, the Caribbean, the Middle East, and Southeast Asia. The crop has been widely cultivated and intentionally spread to different parts of the world due to its economic importance. The plant is adaptable to different climates and can be grown in both tropical and subtropical regions (Mhazo and Chivandi, 2017). *Hibiscus sabdariffa* has a relatively short life cycle, allowing it to produce multiple harvests in a year (Haron *et al.,* 2016).

The production of roselle is subject to certain constraints that can affect its yield and quality. Like any agricultural crop, roselle is susceptible to various pests and diseases that can impact its production. Common pests include aphids, caterpillars, and spider mites, while diseases such as powdery mildew, anthracnose, and bacterial blight can affect the plants. Appropriate pest and disease management strategies, including the use of insecticides, fungicides, and cultural practices, are necessary to mitigate these issues (Ansari *et* al., 2013; Mether *et al.,* 2019; Shrestha and Kuwar, 2016).

*Hibiscus sabdariffa* or rosellee is medicinal plant with a worldwide fame. Roselle, having various medically important compounds called phytochemicals, is well known for its nutritional and medicinal properties. Seeds, leaves, fruits and roots of the plant are used as food and herbal medicine. Roselle is commonly used in the production of herbal teas and beverages. Its dried calyxes, which are the fleshy structures enclosing the seeds, are used to make a tart and refreshing tea that is rich in antioxidants. Also, roselle can be used to make various food products, such as jams, jellies, syrups, and sauces. It is often incorporated into dishes to add a tangy flavor and vibrant colour. Moreso, roselle has been used in traditional medicine for centuries due to its various health benefits. It is believed to have antioxidant and anti-inflammatory properties, and it is often used to treat high blood pressure, cholesterol, and digestive issues.

The numerous importance of roselle cannot be over emphasized. The global production of roselle for its uses is greatly hampered by severe fungi attack especially at post-harvest stage, affecting the quality and quantity of roselle for use. Roselle is majorly cultivated for its nutritional value, yet fungi infection may contribute to the breakdown of nutrients in roselle, leading to a loss of nutritional value during storage (Eslaminejad and Maziah, 2011). This justifies the need to carry out a scientific research to identify the various pathogenic fungi hampering the production, availability and nutritional value of roselle. Therefore, the objectives of this study are: to isolate and identify fungi pathogens causing rot spoilage of stored roselle calyx and determine the effect of identified fungi on the nutrient composition of roselle.

 **2.0 MATERIALS AND METHODS**

2.1 **Experimental Location**

The experiment was carried out in the laboratory of the Department of Plant Health Management, College of Crop and Soil Sciences and fishery laboratory in Michael Okpara University of Agriculture, Umudike, Abia state

**2.2 Sources of Roselle Samples**

The healthy and the diseased stored roselle calyx used in this study were purchased from two different markets Ubani and Ndoro located in Umuahia and Ikwuano LGA respectively, Abia state. Five samples of infected roselle calyx were purchased from different traders in both markets (n=10). The diseased roselle calyx were first purchased and used for isolation and identification of pathogens while the healthy calyx was used for pathogenicity test. Each sample zobo from a trader was collected in a separate sterile and labelled polythene bags and taken to the laboratory for studies.

**2.3 Preparation of Culture Medium**

Thirty-nine grams (39 g) of Potato Dextrose Agar (PDA) per litre of distilled water was mixed thoroughly and carefully dispensed into four sterile 1000ml conical flasks and autoclaved at 121°C/151b pressure for 15 minutes and was allowed to cool. The prepared PDA was allowed to cool down to 40°C and 0.05 ml of lactic acid added to prevent bacterial contamination before dispensing (15ml) into sterile Petri-dishes and allowed to solidify before use (Obani and Ikotun, 2014).

**2.4 Isolation and identification of the fungal pathogens from zobo/roselle calyx**

Some roselle calyx were selected, cut into pieces of 3mm and surface sterilized with 1% hypochloride and washed in three changes of sterile distilled water to remove surface contaminants. The cut pieces were then plated in Petri dishes containing solidified PDA. Pieces of the cut calyx 5 were plated per Petri dish. The plating was done in a closed and sterile inoculation chamber. After plating, the Petri dishes were incubated for 5 days at room temperature. Then they were examined under microscope (40x) to identify the fungi growth on the roselle calyx tissues for the period of incubation. Fungi were identified based on the colony characteristics of the fungal pathogens recorded. Observations were recorded on colony colour, structure, shape, size, pigment and structure of mycelium, its branching, presence of Conidiophores, sclerotia, shape were compared with literatures (Barnett and Hunter, 1999; Bakr and Rahman, 2001, Alexopoulos *et al*., 2002; Obani and Ikotun 2014). The fungi species incidence was determined using the formula:

$$Percentage incidence = \frac{No of observation in which a species appeared}{Total no. of observations}X\frac{100}{1}$$

(Amadioha, 2004; Obani *et al*., 2021).

**2.5 Pure Culture of Isolated Fungi**

This was done by getting the identified organism isolated from diseased roselle calyx to grow all alone in the medium without any contamination. An inoculation needle was sterilized and used to pick the fungi from the pieces of calyx tissues that were earlier plated and transferred into the Petri dishes containing PDA medium. They were incubated for 7 days at room temperature. Any plate that was found contaminated by any other organism was discarded and the fungus sub cultured until axenic cultures were obtained.

**2.6 Pathogenicity Study of the Isolated Fungi**

The pathogenicity test of the isolates was carried out using the modifications of Amadioha and Uchendu (2003). The purpose of this study was to know if the fungi isolated from the diseased looking roselle calyx can cause disease in the healthy looking roselle calyx when they were inoculated. Clean roselle calyx were selected and surface sterilized and put in Petri dishes lined with moist sterilized cotton wool. The spores of pure fungal cultures were harvested with sterile water, final inoculum size was adjusted to a concentration of 1.0x10 spore/ml by microscopic enumeration with a hemocytometer and used to inoculate healthy zobo calyces. The control was inoculated with sterile water without any fungi, these served as control. All the inoculated calyx were incubated for 10 days at room temperature and observed for rot development. The experiment was laid out in complete randomized design with three (3) replicates. After colonization by fungi, re-isolation was made from inoculated zobo samples which showed symptoms of rot on a fresh plate containing PDA and incubated again for 7 days at room temperature to confirm pathogenicity. The culture was compared with the original isolate. The isolates that caused rot were identified as pathogenic organisms causing the rot of zobo calyx. Disease severity to determine the extent of rot development in each roselle calyx was determined using a scale of 0-5 : 0 -no infection, 1-20 % slight infection, 21-40 % moderate infection, 41-60 % severe infection, 61-80 % highly infected and 81-100 % complete rot.

**2.10 Proximate analysis**

Moisture content was determined by drying fresh sample to constant weight in a hot air circulating oven at 100oC. Proximate compositions which included percentage moisture, fat, crude protein, fibre and ash were determined according to the standard methods of the AOAC (1984, 2005, 2010).

The total percentage carbohydrate content was determined by the difference method as reported by Onyeike *et al,* (1995). This method involved adding the total values of crude protein, crude fat, crude fibre, moisture and ash constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate constituent of the sample.

**2.11**  **Statistical Analysis**

Data on disease incidence and proximate composition collected were analyzed using Statistical Package and Service solutions (SPSS) version 2023 analysis of Variance (ANOVA) and means were separated using least significant difference (LSD) at 5 % probability level.

**3.1 Results**

**3.1.1 Incidence of fungi species in zobo calyx.**

The incidence of different fungi isolated from zobo calyx is shown in Figure 1. *Aspergillus* species (*A. flavus, A. niger)* and *Rhizopus* sp and *Fusarium* sp were isolated and identified from zobo samples (Plate 1). *Aspergillus niger* had the highest percentage incidence of (51.39%), followed *Fusarium* sp (28.01%),then *A. flavus (*17.13%), while *Rhizopus sp* (3.47%) recorded the least incidence.

**3.1.2 Pathogenicity of fungi isolated from zobo samples**

Table 1 shows the pathogenicity of various fungi isolated from zobo samples. *Rhizopus* and *A. niger* recorded 100.0% colonization of of zobo samples followed by *A. flavus* and *F. solani* which both recorded 90% colonization of the samples while control had no fungi (Plate 2).

**3.1.3. Effects of Different Fungi on Mineral and Vitamin Content**

The effect of different fungi on the mineral and vitamin content of Zobo calyx is presented in Table 2. Zobo samples inoculated with sterile water significantly (P≤0.005) had the highest mg/100g of vitamins /minerals except for calcium. For iron the control (zobo not inoculated with fungi) had the highest iron content (0.0046mg/100g) followed by *A. flavus* (0.004mg/ 100g) while *Fusarium* had the least. The control was significant higher than the inoculated samples.

Figure 1: Percentage incidence of fungal species isolated from zobo calyx.



Plate 1: Fungi isolates from dried zobo calyx of a). *Aspergillus niger* b). *Aspergillus flavus* c). *Fusarium solani* d). *Rhizopus oryzae*  isolated from zobo growth on potato dextrose ager after 7 days of incubation

**Table 1:** The pathogenicity of fungi isolated from zobo calyx.

|  |  |  |
| --- | --- | --- |
|  |  |  |
| Fungi | Percentage rot (%) | Severity Rating |
| *Rhizopus* sp. | 100 | 5 |
| *Aspergillus flavus* | 90 | 5 |
| *Aspergillus niger* | 100 | 5 |
| *Fusarium solani* | 90 | 5 |
| Control | 0 | 0 |



Plate .2: a). *Aspergillus flavus* b). *Aspergillus niger* growing on inoculated healthy zobo calyx growth on potato dextrose ager after 7 days of incubation

The control sample recorded the highest B-carotene content (0.058 mg/100g) followed by *A. niger* (0.0051 mg/100g) while *Fusarium* had the least value. The control was significantly (p≤0.005) higher than other samples. For calcium content, control recorded the highest (0.2600mg/100g) while *A. niger* had the least calcium content (0.1400mg/100g). Control had the highest potassium content (1.5250 mg/100g) while *Flavus* recorded the lowest content (0.9650 mg/100g). Vitamin A was found to be highest in control sample (4.2800 mg/100g) while *Fusarium* had the least value (2.4750 mg/100g). Vitamin C was highest in control sample (2.3550 mg/100g) followed by *A. flavus* (1.1650 mg/100g) and *A. niger* (1.1200 mg/100g).The control samples also recorded the highest Zinc content (3.5350mg/100g) followed by *A. niger* (1.8600mg/100g).

**Table 2:** Effect of different fungi on Mineral/vitamin (mg/100g) content of zobo calyx.

|  |  |
| --- | --- |
|  | Mineral/vitamin mg/100g |
|  | Iron | Β-carotene | Calcium | Potassium | Vitamin A | Vitamin C | Zinc |
| Control | 0.0046 | 0.0058 | 0.2600 | 1.5250 | 4.2800 | 2.3550 | 3.5350 |
| *A. Flavus* | 0.0415 | 0.0044 | 0.1850 | 0.9650 | 3.1400 | 1.1650 | 1.8050 |
| *A. Niger* | 0.0340 | 0.0051 | 0.1400 | 1.1300 | 2.7750 | 1.1200 | 1.8600 |
| *Fusarium* sp | 0.0300 | 0.0035 | 0.2200 | 1.0750 | 2.4750 | 1.1800 | 2.0500 |
| LSD (p≤0.05) | 0.00 | 0.00 | 0.02 | 0.04 | 0.06 | 0.05 | 0.04 |

**3.1.4 Effect of different fungi on proximate composition of zobo calyx**

Table 3 shows the effect of different fungi on proximate composition of zobo calyx. *Fusarium* sp recorded the highest fat percentage (0.52%) while Control had the highest crude fibre (2.68%) and ash (1.68%) contents. Crude protein was found to be highest in zobo sample inoculated with *Fusarium* sp(5.84%) followed by *A. flavus* (4.73%) and *A. niger* (4.58%),while control had the lowest crude protein content (3.53%). The highest moisture content (14.06%) was recorded in samples inoculated with *A. flavus,* while the un-inoculated zobo samples recorded the least moisture content which was significantly (P≤0.05) lower than that recorded in *A. flavus*  and A. *niger* inoculated samples(Table 3).

**Table 3:** Effect of different fungi on proximate composition of zobo calyx.

|  |  |
| --- | --- |
|   | Proximate composition % |
|  | Fat | Ash | Crude fibre | Crude protein | Moisture |
| *Aspergillus flavus* | 0.44 | 1.34 | 1.43 | 4.73 | 14.06 |
| *Aspergillus niger* | 0.45 | 1.59 | 1.42 | 4.58 | 13.18 |
| *Fusaruim* sp | 0.52 | 1.68 | 1.57 | 5.84 | 12.68 |
| Control | 0.36 | 1.76 | 2.68 | 3.53 | 12.27 |
| LSD (p≤0.05) | 0.05 | 0.04 | 0.04 | 0.04 | 0.49 |

**3.2 Discussion**

The study identified four fungal species isolated from zobo calyx: *Aspergillus species* (*A. flavus and A. niger), Rhizopus* sp, and *Fusarium* sp*.* Notably, *Aspergillus niger* had the highest incidence of (51.39%), which indicates its predominance in zobo samples. This is a concern because *A. nige*r is known for producing mycotoxins that can pose health risks to zobo concumers. Incidence of *Fusarium* sp (28.01%) wasalso significantly present, while *Rhizopus* sp recorded (3.47%). This confirms the study by Akinyosoye and Akinyele (2000); Ogiehor and Nwafor (2004); Ezeigbo *et al.* (2015a) who reported these fungi to be associated with zobo beverage during storage. The presence of these fungi highlights the vulnerability of zobo calyx to fungal contamination, potentially affecting the quality and safety of the beverage made from it. The presence of these fungi may be linked to faecal, environmental and human contaminations. The result of this study highlights potential contamination issues in zobo production and consumption, emphasizing the need for strict quality control measures. Pathogenicity tests revealed that *Rhizopus* and *A. niger* caused 100% rot in zobo samples, suggesting their aggressive nature in degrading the zobo product. In agreement to this, Ezeigbo *et al*. (2015b) also reported the ability of these fungi to cause rot in vegetables like zobo calyx. *A. flavus* and *Fusarium solani* also showed high rot rates (90%), indicating that they are equally capable of spoilage. The control samples, free from fungal contamination, showed no signs of rot, emphasizing the importance of maintaining hygiene during the processing and storage of zobo (Elghany  *et al.,* 2022) and controlling of the identified fungi in zobo (Shuping and Eloff, 2017).

The effects of fungal inoculations on the mineral and vitamin content of zobo calyx were notable: The control sample had the highest iron concentration (0.0046 mg/100g), significantly higher than *A. flavus* (0.004 mg/100g) and *Fusarium,* indicating that fungal contamination negatively impacts iron levels. The control sample recorded the highest content (0.058 mg/100g), while *A. niger* and *Fusarium h*ad lower values, suggesting that fungal presence diminishes the nutrient profile. The control had the highest calcium (0.2600 mg/100g) and potassium (1.5250 mg/100g) levels, with *A. niger* showing the lowest calcium content. This suggests that fungal contamination adversely affects mineral retention. Eslaminejad and Maziah (2011) also reported that fungi pathogens affect the mineral and vitamin composition of zobo. The control sample also had the highest vitamin A (4.2800 mg/100g) and vitamin C (2.3550 mg/100g) levels which are comparable to that reported by Ilodibia *et al*. (2019). Lower concentrations in fungal-inoculated samples point to the detrimental impact of fungi on these vitamins. The control had the highest Zinc levels (3.5350 mg/100g), reinforcing that uncontaminated zobo is a better source of essential minerals (Ogiehor and Nwafor, 2004)

 Ekanem, (2018) reported high moisture and carbohydrate contents from laboratory prepared zobo drink with values of 82.4% and 8.54% respectively. In this study, *Fusarium* inoculated samples were found to have the highest fat content (0.52%), which might affect the overall flavor and texture of zobo. Conversely, the control exhibited the highest crude fiber content (2.68%), suggesting that untainted zobo retains better fiber levels, which are beneficial for digestive health. Zobo is a good source of nutrient which can provide energy and nourishment apart from its obvious medicinal value. Additionally, crude protein was highest in the zobo sample inoculated with fungi than the control indicating that some fungi can enhance protein levels, potentially offering some nutritional benefits despite the associated risks. *A. flavus* recorded the highest moisture content (14.06%), which could lead to a higher susceptibility to spoilage, further emphasizing the need for careful monitoring of moisture levels during storage. The microbes identified from this study has some similarity with the findings of other authors on zobo drinks sold in different locations in Nigeria including Kano (Bukar *et al*., 2010), Aba (Ezeigbo *et al*., 2015a, Zumbes *et al.,* 2014). This study showed ths storage fungi that could have negative impact on stored roselle calyx as well as the effects of fungal contamination on the nutritional and proximate composition of zobo calyx, emphasizing the need for their control to prevent loss of the quality of roselle and its products.

**Conclusion and Recommendations**

The study highlights the significant impact of fungal contamination on the nutritional quality and safety of zobo calyx. The control samples consistently exhibited superior levels of vitamins, minerals, and proximate contents compared to fungi-inoculated samples. The high pathogenicity of *Aspergillus* and *Rhizopus* further raises concerns about food safety and spoilage.

There is need to apply stringent hygiene and quality control measures during the harvesting, processing, and storage of zobo to minimize fungal contamination. Also, producers and consumers should be educated about the risks associated with fungal contamination and the importance of proper storage techniques. Further research should be conducted to explore effective methods for reducing fungal contamination of zobo and to assess the safety of consuming products contaminated with specific fungal species.

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