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Studies on The Nutrient Quality and Mycoflora of Citrus Paradisi Sold in Port Harcourt Rivers State Nigeria

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Abstract: *Studies on the nutrient quality and fungal pathogen of Citrus paradisi (grapefruit)were carried out in the Department of Food Science and Technology Laboratory, Rivers State University. Moisture, ash, fibre, lipid, carbohydrate and protein were parameters assessed. Higher values of moisture (85.4 ± 0.011%), fibre (1.5 ± 0.001%) and protein (2.7 ± 0.021%) were recorded for the healthy samples while the spoilt fruit samples had higher content of ash, lipid and carbohydrate. Mineral and vitamin analyses revealed the presence of calcium, iron, magnesium, potassium, phosphorus and vitamin C. However, higher values of magnesium (7.5 ± 0.013mg\100g), phosphorus (12.5 ± 0.023%) and vitamin C (165 ± 0.014mg) were recorded for the healthy samples while calcium and sodium were higher for the spoilt samples. Iron and potassium had equal values (0.4 ± 0.011mg\100g) and (9. 9±0.022mg\100g) for both healthy and spoilt samples of C. Paradisi respectively. Phytochemical investigation showed the availability of tannin, saponin, oxalate and cyanogenic glycoside at various concentrations. Fungi isolated were : Aspergillus niger, A. tamarii, Fusarium oxysporum and Sclerotium rolfsii. While A. niger had the highest incidence of 40%, A. tamarii and S. rolfsii had equal incidences of 25%. The least incidence (10%) was recorded for F. oxysporum. Generally, the isolates were all able to cause soft rot when inoculated into healthy samples of C. paradisi.*

Key words: *Nutrient quality, Mycoflora and Citrus paradisi*

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I. Introduction

Citrus paradisi commonly known as grapefruit belongs to the Rutaceae family. It is uniquely distinguished from other citrus species because of the sour taste of its friut (Morton, 1987). The plant is said to have originated from Barbados and is cultivated both in the subtropics and tropics (Forsyth, 2003; Li et al., 2010). The tree attains a height range of 10 to 15m with dense leaves and thorns that are supple (PROSEA, 2016). Its leaves are 7.5 to 15cm long and are bigger than those of C. Sinensis(oranges). The leaves are dark green and the petals of its flower are conspicuously white. Also, the difference in the colour of C. paradisi fruit has lead to various classified varieties namely duncan, marsh, foster, oroblanco, redblush, paradise navel, sweetie and star ruby (Morton, 1987).

C. paradisi and its relatives are good sources of nutrients especially vitamins and minerals. Findings from early researches have shown that the grape fruit and its processed juice contains several proximate components including moisture, ash, protein, fat and carbohydrate (Kolawole et al., 2017; Fellers et al., 1990). However, the research of Kolawole et al., (2017) also showed that the hand squeezed C. paradisi juice had higer proximate contents than those extracted by blender and juice extractor.

Mineral and vitamin components of C. paradisi have also been assessed by early researchers. Their findings include the presence of calcium, phosphorus, iron, sodium, potassium, magnesium, zinc, Vitamins A, B, C and E (Morton, 1987; Kolawole et al., 2017; Benyahia et al., 2015). Nevertheless, the research of Morton, (1987) showed that the pulp of C. paradisi fruit had higher values for proximate, mineral and vitamin contents than the extracted juices.

Phytochemicals such as flavonoid, sterols, glycosides, pectin, phenols, alkaloids, tannins, phytates, saponins and oxalates were reported by Gupta et al., (2011)and Lucia et al., (2016).

Nothwithstanding, this fruit and its products are affected by several spoilage organisms, of which when consumed could cause several diseases (Kimball, 1991). Fungi have also been implicated in the spoilage of C. paradisi fruit juice. However, there is dearth of information on the spoilage fungi of the fruit. The research of Embaby et al., (2015) showed that Botryodiplodia theobromae, Penicillium digitatum, P. italicum and Rhizopus stolonifer were responsible for the spoilage of grape fruit juice.Aspergillus flavus, A. niger and Fusarium oxysporium have been also isolated from rotted and fresh Citrus spp (Adegokeet al., 2014). Drusch and Ragab, (2003) also isolated A. flavus and A. parasiticus from C. paradisi fruit juice and fruit peel. The findings of Oviasogie et al., (2015) revealed Rhizopus spp, Penicillium spp, Aspergillus spp, Mucor spp, Candida spp,

Alternaria spp and S. cerevisiae to be responsible for the spoilage of Citrus species. These organisms have been reported by early researchers to produce mycotoxins that are dangerous to the human health when consumed (Fabio et al., 2018; Embaby et al., 2015; Drusch and Ragab, 2003).

Basedon these findings, this research aims to assess the nutrient constiuents and associated spoilage fungi of C. paradisi fruits sold in Port Harcourt, Rivers State of Nigeria

II. Materials And Methods

Sample Collection

Samples of healthy fruits of C. paradisi and partially rotted fruits were bought from the Fruit Garden Market at D. Line Diobu, Port Harcourt, Rivers State and brought to the Department of Food Science and Technology Laboratory for analyses.

Determination of nutrient composition of spoilt and healthy fruits of C. Paradisi

Proximate composition, mineral and phytochemical analyses were carried out according to AOAC (2012) standard methods of analysis.

Mycological studies

Preparation of mycological medium

Sterilization of conical flask, slides, Petri dishes and all the equipment needed for the experiment was carried out in the laboratory. The glass wares were sterilized in the oven at 120^{o}C for an hour after washing with soap, while other equipment were surface sterilized with 70% ethanol to reduce microbial contamination (Agrios, 2005). Inoculating loops and scalpels were sterilized by dipping for 20 seconds in 70% ethanol and heated to red hot. The mycological medium used was Sabouraud dextrose agar (SDA) prepared in a conical flask using the standard method.. The conical flask containing the mycological medium was autoclaved at 121^{o} C and pressure of 1.1kg cm-3 for 15 minutes. The molten agar was allowed to cool to about 40 ^{o} C and dispensed into Petri dishes at 15mls per plate and allowed to cool and solidify.

Isolation of fungi from partially rotted C. paradisi fruits.

One gram of each sample showing visible signs of spoilage by moulds was cut from the healthy portions of the fruits up to the points where rot had established and inoculated onto Sabouraud dextrose agar in Petri dishes in triplicates. Ampicillin was overlaid on the agar to hinder the growth of bacteria. The inoculated plates were incubated for 5 days at ambient temperature 25^{o} C ± 3^{o} C (Baudoni, 1988, Chuku, 2009, Samson et al, 1981). The entire set up was observed for 7 days to ensure full grown organisms. Pure culture of isolates were obtained after a series subculturing of the isolates on fresh SDA**.**

Identification of fungi from c. paradisi

Microscopic examination of fungal isolates was carried out by the needle mount method (Cheesebrough, 2000). The fungal spores were properly teased apart to ensure proper visibility. The evenly spread spores were stained with cotton blue in lactophenol and examined microscopically using both low and high power objectives. The fungi were identified based on their spore and colonial morphology, mycelia structure and other associated structures using the keys of (Samson et al, 1981 and Olds, 1983).

Pathogenicity studies

Pathogenicity studies was carried out on healthy C. paradisi to check if the fungi isolated from the rotted fruits were capable of causing spoilage on healthy fruits samples. The methods of Agrios(2005), and Trigiano, (2004) were basically followed. The fungal isolates were introduced into healthy fruits and observed for seven days. The set up was monitored regularly for growth.

Determination of nutrient components of healthy fruits of C. paradisi

Samples from healthy C. Paradisi**fruits** were analysed for macro nutrient composition using the methods of AOAC (2012) .

III. Result And Discussion

Table 1: Proximate composition of healthy and spoilt C. paradisi fruits.

Parameters (%)	Healthy	Spoilt
Moisture	85.4 ± 0.011	80.50± 0.014
Protein	2.70±0.0.021	2.40±0.006
Lipid	2.50± 0.012	3.15± 0.013
Ash	1.20±0.021	1.40±0.025
Fibre	1.50 ± 0.001	1.25±0.004
Carbohydrate	6.60± 0.013	11.30± 0.013

Table 2: Minerals and vitamin composition of healthy and spoilt C. paradisi fruits.

Parameter mg/100g	Healthy	Spoilt
Calcium	9.2± 0.025	9.4± 0..023
Iron	0.4± 0.011	0.4± 0.011
Magnesium	7.5± 0.013	7.0± 0.013
Potassium	9.9± 0.022	9.9± 0.022
Phosphorus	12.5± 0.023	12.0± 0.013
Sodium	3.4± 0.015	3.5± 0.021
Vitamin C	165±0.014	150±0.011

Table 3: Phytochemical composition (%) of C. paradisi

Parameter	Percentage
Tannin	0.06 ± 0.004
Saponin	0.12± 0.001
Oxalates	0.20± 0.012
Cyanogenic glycoside	3.1± 0.014

Table 4: Fungi isolates and percentage incidence

Isolates	Percentage incidence (%)
Aspergillus niger	40
Fusarium oxysporum	10
Aspergillus tamarii	25
Sclerotium rolfsii	25

The result of proximate composition presented in Table 1. shows that C. paradisi had higher value of moisture (85.4 ±0.011%) for the healthy fruit samples compared to the 80.5±0.014% recorded for the spoilt fruits. Higher value of ash (1.40 ± 0.025) was recorded for the bad samples while the healthy samples had a lower value of 1.20 ±0.021. Fibre values of 1.50 ±0.001 and 1.25±0.004 were recorded for the healthy and spoilt fruit samples of C. paradisi respectively. Lipid and carbohydrate had higher values for the spoilt samples and lower values for the healthy samples. There was a 71.2% increase in carbohydrate content with spoilage.Nevertheless, higher values of protein (2.70±0.021) was recorded for the healthy fruit samples while a lower value of of 2.40±0.006 was obtained for the spoilt samples representing a 12.0% decrease. The values of parameters assessed in this study conform with those reported by Fellers et al., 1990). However, the ash and protein values of this study are higher than those reported by Kolawole et al., (2017).They reported moisture cvontent of 90 – 91% for C. paradisi fruit juice. The findings of Morton, (1987) for protein, fibre and ash disagreed with those of this study as lower values for these parameters were reported. Meanwhile, the moisture and carbohydrate values obtained in this study are lower than those reported by Morto (1987).

Table 2. reveals the mineral and vitamin composition of C. paradisi. Higher values of calcium (9.40 ±0.023) and sodium (3.50 ± 0.021) were recorded for the spoilt fruit samples comprared to their equivalents in the healthy samples. The healthy fruit samples of C. paradisi had higher values of magnesium, phosphorus and vitamin C. However, equal values of iron and potassium were recorded for both healthy and spoilt grape fruits. Findings fromearlier research had implicated all the parameters assessed in the current study to be present in C. paradisi (Benyahia et al., 2015). The report of kolawole et al., (2017) also supports the presence of mineral and vitamin found in this current study as they were all recorded to be present in grape fruit juice. The calcium value reported in this study is line with the 9.2 to 32.0mg reported earlier by Morton, (1987).

The phytochemical composition of C. paradisi presented in Table 3. indicate the presence of tannin, saponin, oxalate and cyanogenic glycoside in appreciable amounts. The phytochemicals reported in this study were also reported in earlier studies and are important in pharmaceutical industries as they play a vital role in the human health (Gupta et al., 2011; Lucia et al., 2016).

Table 4. reveals the fungi genera associated with the spoilage of C. paradisi fruit. These are Aspergillus niger, A. tamarii, Fusarium Oxysporum and Sclerotium rolfsii.and they all proved to be pathogenic and caused spoilage when inoculated into healthy fruit samples of C. paradisi. Highest percentage incidence of 40% was recorded for A. niger. This was followed by 25% incidence recorded for both A. tamarii and S. rolfsii. F. oxysporum had the least incidence of 10%. The fungal isolates of this study concurs with those reported earlier. These fungal isolates and their relatives were implicated to cause spoilage of fruit juice extracted from C. paradisi (Embaby et al., 2015). This was supported by the studies of Drusch and Ragab, (2003) as they also isolated A. flavus and A. parasiticus from C. paradisi fruit juice and fruit peel. Also, earlier research has shown that these isolates were reponsible for the spoilage of other Citrus species fruits (Oviasogie et al., 2015). Onuorah et al., (2015) also isolated F. oxysporum and A. niger from C. sinensis which is in agreement with the isolates of this study. More so, the pathogenic potential of Aspergillus spp and Fusarium spp were also reported earlier by Bukar et al., (2009) as they were able to cause spoilage of C. sinensis. These organisms have the

potential to produce pathologies in man when consumed along with fruits as they produce mycotoxins which are implicated in certain diseases (Adegoke et al., 2014).

IV. Conclusion

The fruits of C. paradisi contain essential food nutrients and phytochemicals that would boost human health if consumed appropriately. Fungi cause spoilage in fruits, resulting in loss of income of the vendors as a result of increase in quantity of fruits wasted. The fungal species associated with spoilage of C.paradisi are also capable of causing several diseases in man especially through the consumption of contaminated fruits. It is therefore important to ensure appropriate hygenic measures during pre and post production stages to ensure that healthy fruits are sold to consumers.

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