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Research Article

Phytochemical and microbial study of pepper (*Capsicum annuum* L.) obtained from three different markets in Benin city, Nigeria

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Abstract: Pepper (*Capsicum annuum*) is a widely cultivated vegetable plant. A comparative study of the phytochemical and microbial composition of peppers obtained from different markets is necessary to ensure their safety and quality, as pepper is a spice that is consumed worldwide, and its quality and safety are of utmost importance. This study was conducted to evaluate the phytochemical component and microbial load of fresh and spoilt pepper collected from three different major markets in Benin-city, Edo state, Nigeria. Using standard methods, the prepared samples were analyzed for fungi composition. The same samples were also analyzed for phytochemical constituents and data collected were subjected to descriptive analysis. The results obtained show the composition of alkaloids, flavonoids, tannins, saponin, and polyphenols. There were significant differences in the microbial constituents between spoilt and fresh pepper obtained from the three markets. The phytochemicals analyzed show an increase in the fresh pepper samples when compared to the spoilt ones. For the fresh pepper, alkaloids have a range of 11.10–15.20%, the tannins show a range of 2.09–4.22% and the flavonoids range from 7.06-23.10g from the three markets. The microbial occurrence in fresh peppers for Uselu and Oba markets is 100%. The results of this study confirm that the environmental and physical conditions surrounding the packaging of *C. annuum* can affect its phytochemical composition and microbial load. *C. annuum* is a rich source of active phytochemical constituents, which play a key role in disease treatment and prevention and also effective against various ailments.

1. INTRODUCTION

Ecologically speaking, *Capsicum* species are perennial shrubs in tropical areas and are commonly grown as annual and herbaceous plants in temperate areas. Pepper (*Capsicum* spp.) is a major Solanaceae crop planted for culinary usage as a vegetable and condiment, and a source of food colorants and secondary metabolites (Olawale *et al*., 2015; Zohoungbogbo *et al*., 2024b). A significant source of revenue for farmers, particularly smallholder farmers in Asia and Africa, pepper is a high-value commodity. Whether they are fresh, dried, or processed, peppers are a staple in all African local markets. The total production of green chillies and peppers in Africa is estimated to be 2.88 million tonnes from 363,937 hectares of land (Zohoungbogbo *et al*., 2024b); in West Africa, the total production is 754,260 tonnes from

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108,452 hectares, with Nigeria contributing 500,000 tonnes from 60,000 hectares (Olawale *et al*., 2015; Zohoungbogbo *et al*., 2024b).

The genus *Capsicum* includes 43 species, among which five are domesticated and cultivated, only four main varieties are grown in Nigeria: Bird peppers-atawere (*Capsicum frutescens*), Cayenne pepper or red pepper-Sombo (*C. frutescens*), Hot pepper-atarodo (*Capsicum chinense*), Sweet pepper-tatase (*Capsicum annuum*) (Olawale *et al*., 2015; Tambuwal *et al*., 2018; Zohoungbogbo *et al*., 2024b). There are two main species of pepper found in markets in Benin; *Capsicum annuum* and *C. frutescens*. Culturally, pepper (*Capsicum annuum* L.) holds significance due to its pungency and intense flavor, representing purification and cleansing. They are excellent sources of many essential nutrients and contain high amount of antioxidant and anti-inflammatory phytonutrients which include alkaloids, tannins, polyphenols, capsaicinoids, flavonoids, carotenoids, potassium, magnesium, fiber, and vitamins are among the phytochemicals present in pepper (Olatunji & Afolayan, 2018; Peregrine *et al*., 2021; Sadiq *et al*., 2020; Tambuwal *et al*., 2018).

Sweet pepper (*Capsicum annuum*) in Benin markets and other markets around Nigeria are susceptible to spoilage due to microorganisms, soft skin, chemical compounds, shelf life, and post-harvesting management methods (Nnenna and Alabo, 2021). Fungal species such as *Alternaria alternate*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Alternaria*, *Mucor* and *Botrytis cinerea* often infect sweet pepper (*Capsicum annuum*), causing diseases before and after harvest (Garuba *et al*., 2022; Nnenna & Alabo, 2021). This puts the income of small and large pepper growers around the world at risk. These fungi produce many spores and are found almost everywhere sweet pepper is grown. They attack the fruits at all stages, from harvest to consumption, taking advantage of any damage or bruising on the fruit wall (Garuba *et al*., 2022). They can also make mycotoxins, which are poisonous chemicals that are made by filamentous fungi. Several studies have found a high frequency of mycotoxigenic fungal strains in *Capsicum* fruits. Moreover, it has been documented that climatic factors in tropical or subtropical countries, including elevated temperatures, humidity, and precipitation, lead to a significant fungal burden and mycotoxin contamination in pepper samples (Garuba *et al*., 2022).

A comparative study of the phytochemical and microbial composition of peppers obtained from different markets is necessary to ensure their safety and quality, as pepper is a spice that is consumed worldwide, and its quality and safety are of utmost importance. However, peppers sold in markets may contain a variety of phytochemicals and microorganisms that can affect human health (Garuba *et al*., 2022; Peregrine *et al*., 2021; Sadiq *et al*., 2020). This study was therefore conducted to evaluate the phytoconstituents and microbial load of fresh and spoilt pepper collected from three different markets in Benin-city, Edo state, Nigeria.

2. METHOD

2.1. Collection of Specimens

The materials used for this study included ripened healthy and spoiled pepper fruits collected from three (3) major open markets, New Benin, Oba and Uselu in Benin City, South-southern Nigeria, within February (period of transition from dry to rainy), 2024 (Figure 1). The healthy pepper fruits were undamaged, fresh and firm; the spoilt ones were noticeably damaged. They were separately

2.2. Microbial Analysis

2.2.1. *Preparation of potato dextrose agar*

A conical flask containing 39g of dehydrated potato dextrose agar (PDA) was filled with 500mL of water. The conical flask was then heated. After heating, the flask was topped up to 1 litre. The medium was then sterilized by autoclaving at 15psi (121℃) for 15 minutes. Chloramphenicol at 10g per 200mL of the medium was added to subdue the growth of bacteria.

Inoculation and transfer of culture were carried out on a sterile inoculating bench after wiping with methylated spirit (Okhuoya *et al.,* 2012).

(a) (b)

Figure 1. (a) Fresh Peppers and (b) Spoilt Peppers 0.5 x packaged, labeled and carefully transported to the laboratory for the analyses.

2.2.2. *Isolation of fungi from fresh and spoilt pepper (Capsicum annuum)*

The pour plate method of inoculation was used in the isolation of the microorganisms associated with the samples. One mL each of the serial dilution prepared samples was pipette with the aid of a syringe and was transferred into the corresponding labeled Petri dishes. 9mL of molten prepared nutrient agar (NA) and potato dextrose agar (PDA) was poured into the Petri dishes, respectively. The Petri dishes were inoculated at room temperature for 24 hours (Garuba *et al*., 2022; Nnenna & Alabo, 2021;).

2.2.3. *Determination of microbial load*

The microbial load of the samples was determined by counting the colony-forming units with the eyes after 24 hours for bacteria and after 72 hours for fungi. The microbial load/mL was then determined by the formula stated below.

$$\text{Count/mL} = \frac{\text{No of colonies on plate}}{\text{Amount plated}} \times \frac{1}{dilution\ factor}$$

2.2.4. *Determination of percentage occurrence*

This was done using the formula stated below

$$\%\ \text{occurrence} = \frac{\text{No of a particular isolate on plate}}{\text{Total number isolates on same}} \times \frac{100}{1}$$

2.3. Phytochemical Analysis of Pepper

The pepper sample (*Capsicum annuum*) was subjected to a series of chemical tests to determine the presence or absence of various phytochemical components in accordance with conventional protocols. The alkaloid, flavonoids, tannin, saponin and total polyphenols contents were determined using the methods described by Pushpa *et al*. (2018), Tambuwal *et al*. (2018) and Saranya *et al*. (2021).

2.4 Data Analysis

Data collected were subjected to descriptive analysis using the 2013 version of Microsoft Excel Software from the Microsoft Corporation. They were expressed as mean and percentage composition.

3. RESULTS

The microbial study results show the fungi associated with the fresh and *Capsicum annuum* samples obtained from three different markets in Benin city, after 72 hours (Table 1). Two fungal species and yeast cells were isolated from the fresh and spoilt fruits of *Capsicum annuum*

in this study. The macroscopic descriptions are shown in Figures 2 and 3, while their morphological characteristics of the isolates are summarized and presented in Table 1. Based on these, the fungal isolates obtained tentatively from fresh and spoilt samples of Pepper (*C. annuum* L) were identified as *Aspergillus niger*, *Penicillum chrysogenum* and Yeast cells.

Table 1. Fungi associated with Fresh and spoilt *Capsicum annuum* after 72 hours.

Market	Sample	Microbial Description	Fungi Isolated	Occurrence (%)
New Benin	A	Black mycelia growth with yellow edges	*Aspergillus niger*	20
		White spotted growth	Yeast cells	80
	B	Black mycelia growth with yellow edges	*Aspergillus niger*	31.6
		White spotted growth	Yeast cells	68.4
Uselu	A	Black mycelia growth with yellow edges	*Aspergillus niger*	40.9
		Grey mycelia growth	*Penicillium chrysogenum*	18.2
		White spotted growth	Yeast cells	39.9
	B	White spotted growth	Yeast cells	100
Oba	A	Black mycelia growth with yellow edges	*Aspergillus niger*	5
		Grey mycelia growth	*Penicillum chrysogenum*	5
		White spotted growth	Yeast cells	90
	B	White spotted growth	Yeast cells	100

Note: Sample A stands for fresh pepper and Sample B stands for spoilt pepper.

(a) (b)

Figure 2. (a) and (b) shows the macroscopic description of the fungi isolated from fresh pepper obtained from three markets.

(a) (b)

Figure 3. (a) and (b) shows the macroscopic description of the fungi isolated from fresh pepper obtained from three markets.

3.2. Phytochemical Composition of Fresh and Spoilt Pepper Samples

Result shows significant differences in terms of phytochemical properties. The results showed that the percentage of phytoconstituent is higher in fresh pepper from all three markets than spoilt pepper. The highest phytochemical was observed in Polyphenols while the lowest phytochemical was shown in Saponin. The percentage of alkaloid content range from 5.09 to 15.20 while that of tannin and saponin range from 1.00 to 4.22 and 1.01 to 3.11. Flavonoids content ranging from 7.6 to 23.1g for fresh pepper samples but the spoilt peppers for the same phytochemical shows a range from 5.2 to 13.2g. Polyphenol content ranges from 11.21 to 32.11 mg/L (Table 2).

Table 2. Phytochemical constituent of fresh and spoilt pepper samples.

Phytochemicals	New Benin Market		Uselu Market		Oba Market	
	Fresh	Spoilt	Fresh	Spoilt	Fresh	Spoilt
Alkaloids (%)	12.10	9.10	11.10	5.09	15.20	5.20
Tannins (%)	2.09	1.20	3.01	1.00	4.22	3.62
Saponin (%)	3.11	1.01	2.90	1.10	1.20	1.20
Flavonoids (g)	7.60	6.10	12.20	5.20	23.10	13.20
Polyphenols (mg/L)	23.22	12.12	32.11	17.51	21.11	11.21

4. DISCUSSION and CONCLUSION

Pepper is one of the most cultivated crops with many varieties. It is a source of nutrients and functions as spices and antioxidants in both traditional and modern medicine (Sadiq *et al*., 2020). Microorganisms which are normally widespread have been demonstrated in the fresh and spoilt samples of pepper (*C. annuum*) purchased from three different markets (New Benin, Oba and Uselu market) in Benin City, Edo State. The fungal isolates obtained from the pepper (*C. annuum*) samples were morphologically identified as *Aspergillus niger*, *Penicillum chrysogenum* and Yeast cells (Otusanya, 2022). As moisture is one of the physical factors that enhances the growth of microorganisms, the frequency of occurrence is higher in fresh pepper samples than spoilt pepper samples. This result was in agreement with an earlier study Ojo *et al.* (2016), on microbial assessment and proximate composition of pepper (*Capsicum annuum*) and tomato (*Solanum Lycopersicum*) displayed for sales.

Aspergillus and *Penicillum* species presence in pepper can make them produce mycotoxigenic substances that cause disease in humans, animals and plants (Costa *et al.,* 2019; Frimpong *et al*., 2019). *A. niger* and *Penicillum chrysogenum* are the only species of potentially ochratoxigenic fungi that were isolated from *Capsicum* that is able to produce Ochratoxin A (OTA) in culture media. OTA is the second most common mycotoxin (after aflatoxins) that was found in pepper samples, and its immunosuppressive, nephrotoxic, and carcinogenic potential made it a global health concern when it was discovered that OTA contamination of a variety of food commodities (Costa *et al.,* 2020; Costa *et al.,* 2019; Frimpong *et al*., 2019). According to the International Agency for Research on Cancer (IARC), this mycotoxin is classified as possibly carcinogenic to humans. Although it was not confirmed in this study, OTA contamination of commercialised *Capsicum* pepper samples has been documented in the past (Frimpong *et al*., 2019). There is a prevalence of Yeast cells colonizing the majority of the spoilt pepper. This is as a result of the nature of the fruit (fresh and spoilt), which can affect the microbial composition of the pepper (Costa *et al.,* 2019; Garuba *et al*., 2022; Okonko *et al*., 2017). The presence of these organisms are a direct reflection of the poor sanitary conditions of the cultivation water, harvesting, transportation, improper handling operations at market and storage of the produce (Costa *et al.,* 2019; Garuba *et al*., 2022; Ojo *et al.,* 2016). The fungi isolated and morphologically identified in this study have previously been isolated from fruits and vegetables of pepper (*C. annuum*) in other studies, both within Nigeria and elsewhere (Costa *et al.,* 2019; Ojo *et al.,* 2016). This study is in agreement with the submission of Nnenna

and Alabo (2021), who conducted a microbial assessment and proximate composition of both fresh and spoilt pepper (*Capsicum annuum*) and Tomato (*Solanum lycopersicum*) displayed for sale in Rivers State, Nigeria.

The phytochemical analysis indicated that alkaloid, tannins, saponin, flavonoids and total polyphenols were found in both fresh and spoilt pepper samples gotten from the three different markets (New Benin, Oba and Uselu market) in Benin City, Edo State (Pushpa *et al*., 2018; Saranya *et al*., 2021; Swamy, 2023; Tambuwal *et al*., 2018). The phytochemical analysis shows a number of distinct compositions among the fresh and spoilt pepper samples. Fresh pepper sample contains more phytoconstituents than spoilt pepper samples. This is in agreement with the submission of Tambuwal *et al*. (2018), who did a comparative evaluation of the phytochemical, proximate and mineral quality of Nigerian *Capsicum annuum.*

Alkaloids and saponins possess antibiotic, poisonous, and anti-feeding characteristics. The functional category of alkaloid constituents, termed Capsaicinoids, constitutes the primary component of *Capsicum* spp. Generally, *Capsicum annuum* was used to relieve odontalgia. The fruits of chili or red pepper are useful to stimulate abdominal activities and increase blood circulation (Peregrine *et al*., 2021; Swamy, 2023). Saponins, found in plants and plant-based products, are crucial for human and animal nutrition. They are often used as dietary supplements for patients with diabetes or other disorders. Saponin is used in folk medicine worldwide and its extract is used in treating libido disorders, infertility, and cardiac diseases (Sharma *et al*., 2023).

Tannins in products provide relief from various ailments, such as diabetes, by enhancing glucose uptake and lowering blood sugar levels. They also aid in wound healing by precipitating wound protein, preventing bleeding, and aiding faster healing. Condensed tannins are effective against allergies like asthma and mite allergens (Sharma *et al*., 2019). Tannins have biological properties like anti-inflammatory, anti-cancerous, anti-allergic, anthelmintic, and antiviral properties against viruses like enteric and herpes simplex. They are used in medicine for antihemorrhoidal, antidiarrheal, and hemostatic treatment. Tannin also acts as a precipitating agent and has beneficial effects on vascular health (Sharma *et al*., 2019).

Plants contain flavonoids, which are beneficial chemicals. Previous research suggested that eating foods containing these chemicals may lessen the risk of cancer and cardiovascular disease (Sricharoen *et al*., 2017). Flavonoids featuring flavones as their fundamental structure, are present in flowers, fruits and stems of plants and are recognized for their antioxidant, anticancer, and anti-inflammatory properties. Due to the antioxidant properties of flavonoids, it inhibits the activity of several enzymes implicated in the genesis of cancer, including prostaglandin synthase, lipoxygenase, and cyclooxygenase (Batiha *et al*., 2020).

According to the findings Saidu and Garba (2011), pepper with a high phenolic content contains dietary antioxidants and adds flavour to food, which suggests that it could be used as a value-added ingredient to stabilise food against lipid peroxidation events. Phenolic compounds possess several physiological effects, including free radical scavenging, antibacterial, anticancer, and antioxidant properties (Jang *et al*., 2024). Phenolic compounds, also known as polyphenols, are defined as molecules that have at least one aromatic ring attached to one or more hydroxyl groups. The main polyphenols found in peppers are flavonoids, tannin phenolic acids, and their derivatives. These phytochemical components from pepper species are known for their pharmacological, neurological, analgesic, anti-obesity, cardio-protective, and dietetic properties. Many of these functional features are largely attributed to the antioxidant activity of tannin, capsaicinoids, and polyphenols, which are essential for scavenging the free radicals that cause oxidative damage. Antioxidant compounds also stop lipids, like cholesterol, from oxidising (García-González and Silvar, 2020).

This study has shown that pepper fruits are sensitive to environmental and physical damages as well as microbial infestation. In order to avoid fungal contamination in the postharvest stage, the pepper can be dried or stored in a healthy area, since most of the fungi isolated are

contaminants rather than originating from the native plant. *Capsicum annuum* is a rich source of active phytochemical constituents, which play a key role in disease treatment and prevention and also effective against various ailments. This study confirms that the environmental and physical conditions surrounding the packaging of *Capsicum annuum* can affect its phytochemical composition and microbial load.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Authors are expected to present author contributions statement to their manuscript such as; **Chime Anthonia:** Investigation, Resources, Visualization, Software, Formal Analysis, and Writing-original draft. **Olalekan Ogunoye:** Methodology, Supervision, and Validation. Authors may edit this part based on their case.

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