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UV-C Inactivation Doses for Pathogenic and Spoilage Bacteria and Fungi Isolated from Selected Tropical Fruits and Vegetables

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ABSTRACT

Food-borne microbes from sweet orange, okra and pepper were homogenized and combined based on fruit type. The homogenized mixtures of the isolates were subjected to Ultraviolet radiation using a fabricated irradiation system to determine the total viable colony count post-irradiation. This was used in calculating the decimal reduction doses for the crops, which were found to be 2.589 Jm^{-2}, 2.556 Jm^{-2} and 4.133 Jm^{-2}, for Sweet Orange, Okra, and Pepper, respectively. These doses will be helpful in the sterilization and shelf-life extension of the selected fruits and vegetables.

INTRODUCTION

Despite increasing efforts at food production geared towards food security, Nigeria's ranking on the Global Food Security Index (GFSI) has been on a steady decline with Nigeria ranked 43rd out of 52 countries in Africa and a score of 47.1 per cent (Sachs et al., 2019).

Fruits and vegetables are fundamental constituents of the human diet, offering a rich source of essential vitamins and nutrients vital for maintaining optimal health and bodily functions. However, the seasonal nature of these crops coupled with their susceptibility to spoilage, due to microbial contamination, poses a significant challenge to food preservation and safety. Addressing this issue is important, not only to reduce food waste but also to ensure the availability of safe and nutritious produce for consumers.

Orange, okra, and pepper are significant economic plants in the global and local food supply chain. Africa and Nigeria witnessed increased orange production from 2.73 and 1.5 million tonnes in 1971 to 9.76 and 3.98 million tonnes, respectively in 2020 growing at an average annual rate of 2.76% and 2.13%, respectively (Obayelu et al., 2022). World bell pepper production has been reported to be 38.0 million tonnes (Tiamiyu et al., 2023) while global okra production has been reported as 10.8 million tonnes (Knoema, 2022). In addition to the diminished food output posed by the loss of these crops, there is a concurrent threat of reduced economic power of farmers with a consequent decline in their living standards. Olayemi *et al*. (2011) reported high vegetable and fruit losses of up to 33% in some parts of Rivers State.

In recent years, ultraviolet (UV) radiation has emerged as a highly promising method for sterilization and microbial inactivation in the food industry. This environmentally friendly and chemical-free approach holds great potential as a viable alternative to traditional chemical preservatives. Its application in food safety, particularly concerning fruits and vegetables, is an area of increasing interest.

This study is on reducing fruit and vegetable spoilage using locally available materials to develop an ultraviolet irradiation system which can be adopted by farmers. It is aimed at identifying microorganisms responsible for the spoilage of oranges, okra, and peppers, and determining UV radiation doses for the effective decimal reduction of microbial populations in stored fruits and vegetables.

UV Interaction and Measurement

As UV light propagates through a medium, some of it is attenuated and absorbed by the medium. This process results in energy deposition along the propagation path. Beer's law describes the attenuative interaction of ultraviolet radiation with matter. Equation 1 is a mathematical statement of Beer's law.

$$I(x) = I_0\, e^{-\mu x} \quad (1)$$

Where:

I_0 is the peak UV intensity at the source.

$I(x)$ the intensity at a distance x, from the source also known as the UV irradiance.

μ is the attenuation coefficient which is dependent on the attenuating medium.

The absorbed energy is responsible for microbial inactivation through successive interactions that affect their structure. UV absorption by DNA molecules in the germicidal range results in the breakdown of DNA strands. In this process, the adenine bond with Thymine is broken and there is subsequent pairing between thymine and nucleotides on opposite strands of DNA giving rise to pyrimidine dimers (Koutchma, 2019). This structural change prevents cell replication and consequently renders the organism inactive and unable to cause harm. Beyond the damage to microbial DNA, UV to a lesser extent, denatures proteins. This is due to the cross-linking of amino acids at the carbon-carbon double bonds by UV radiation (Lado & Yousef, 2002). The resulting denaturation of proteins contributes to membrane depolarization and abnormal ionic flow.

The UV energy is quantified to a dosage, D $\left(\frac{mW.s}{cm^2}\right)$ by multiplying the UV irradiance by the exposure time, $t\ (s)$ (Dyksen et al., 1998).

$$Dosage\ = I(x)\left(\frac{mW}{cm^2}\right) \times t\ (s) \qquad (2)$$

MATERIALS AND METHODS

Irradiator circuit

A schematic diagram of the control circuit for the irradiator is shown in Figure 1. The IC 555 is configured as a standard monostable multivibrator and is supplied power through a stable non-transformer power supply circuit, which consists of components C3, C4, a 0.33uF capacitor, and the 12V Zener diode.

Upon connecting the circuit to the AC mains, the 12V DC voltage supplied to the monostable immediately triggers the circuit. This trigger is achieved by utilizing a 1uF capacitor connected to pin 2 of the IC. This capacitor briefly grounds pin 2 of the IC, activating its output pin 3 with a positive supply.

The presence of a positive supply at pin 3 then activates the triac and, consequently, the UV lamp. The monostable circuit commences counting, and the duration of the ON state is determined by the values of the timing capacitor, C_1 and the timing resistor, R_2. During this time, both pin 3 and the triac remain in the ON state. Once the predetermined time elapses, pin 3 returns to zero, turning off the triac and subsequently the UV lamp. The relationship between C_1 and R_1 is given by:

$$T = 1.1 \times R_2 \times C_1 \qquad (3)$$

Where:

T is the time duration of the output in seconds.

R_2 is the resistance in Ohms.

C_1 is the capacitance in Farads.

To further enhance safety and control, a reed relay is incorporated into the circuit, which is connected in series with the positive input supply line. This reed relay is closely associated with the door mechanism of the UV box cabinet. When the cabinet door is open, the magnet remains distant from the reed relay, keeping its contacts open and preventing power to the monostable. However, when the door is closed, the magnet is drawn near the reed relay, causing its contacts to close and supply power to the monostable. Consequently, the monostable is enabled, allowing the timer and the UV lamp to carry out their functions.

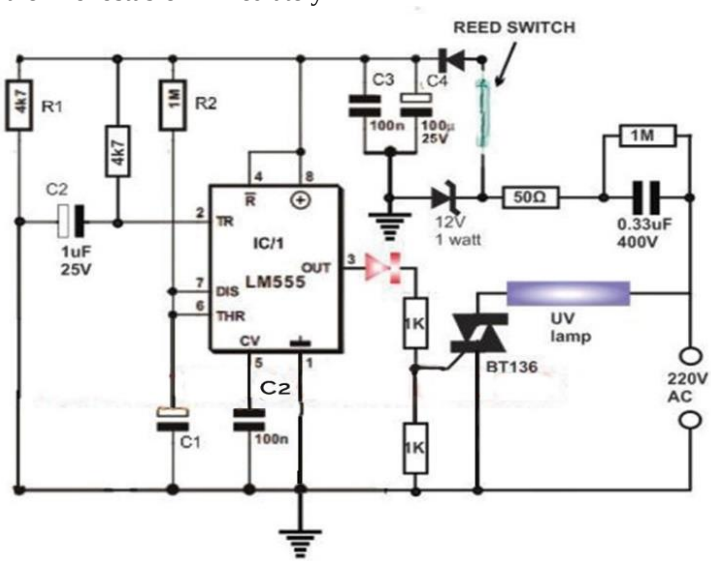

Figure 1: UV Irradiator Circuit Diagram (Source: Swagatam Innovations, 2021)

Construction and determination of UV distribution

The irradiation chamber was designed to be of 50 cm x 45 cm x 30 cm Three irradiation points at 15 cm, 30 cm, and 45 cm from the light source were made. This will enable modification of doses by variation of distance from the light source according to the inverse square law. The walls of the chamber were coated with reflective material to enhance UV distribution within the

irradiator chamber and minimize external scattering of UV light.

A UV radiometer, Linshang (LS 125), was used to assess the UV irradiance ($mWcm^{-2}$) and energy (mJ/cm^2). To do this, the UV probe was placed at various points within the chamber and several measurements were carried out. The average UV intensity at the points was obtained from the measurements.

Isolation and identification of microbes

To identify spoilage-causing microorganisms, spoilt samples of the crops were collected from the Railway market in Makurdi. The samples were put in plastic bags and transported to the laboratory. The samples were surface sterilized using 70% ethanol after which rotten pieces were cut and inoculated onto culture media. Potato Dextrose Agar (PDA) was used for fungal isolation and Nutrient Agar (NA) for bacterial isolation. Isolated microbes were assigned unique codes and successively sub-cultured onto fresh plates until pure cultures were obtained. Colony morphology, staining and microscopic examination were used to identify the isolates while additional tests were used for further identification of the bacterial isolates. A standard identification guide was also consulted for all isolates.

Pathogenicity test

To ascertain the pathogenicity of isolated microbes, fresh samples of fruits of the same species from which the isolates were obtained were obtained from the Railway market. Healthy crops were selected, and surface sterilized with 70% ethanol. A sterile cork borer was used to bore holes in the fruit samples. The microorganisms were introduced into the fruits and the holes were sealed. The inoculated fruits were stored and observed for spoilage after four days. The crop samples were cut and observed to ensure that spoilage was caused by inoculated isolates after which the length of the crop affected by spoilage pathogens was measured.

Determination of decimal reduction dose

To determine the log 10 decimal reduction dose (D_{10}), isolated organisms were homogenized and serially diluted. 1 ml of homogenized solution of each of the microbes was taken from 10^{-3} of its solution and combined based on fruits from which they were isolated. 1 ml of the mixed solutions from each fruit was put in four petri dishes and exposed for 60 seconds and 90 seconds. All irradiation exposures for this study were carried out at the 15 cm irradiation point. The exposed microbes were mixed with molten media and incubated at 35^0C. The viable colony count was determined and used to determine the decimal reduction dose according to the equation:

$$D_{10} = \frac{t_2 - t_1}{\log X_2 - \log X_1} \quad (4)$$

Where:

X_1 and X_2 are corresponding viable counts after treatment times t_1 and t_2 respectively.

RESULTS AND DISCUSSION

The results from the study are presented in Table 1.

Table 1: UV energy distribution for 20 seconds of exposure

S/No.	Distance (cm)	Irradiance ($mWcm^{-2}$)	Dose (mJ/cm^2)
1	15	2.6580 ± 0.0755	54.8465 ± 2.1014
2	30	1.0796 ± 0.0339	21.4448 ± 0.9501
3	45	0.6112 ± 0.0113	13.1410 ± 0.8284

The measured irradiance represents the intensity of ultraviolet (UV) radiation observed at these specific points. It is worth noting that there is an inverse relationship between irradiance and the distance from the source. As the distance from the UV source increases, the observed irradiance values decrease. However, it is essential to recognize that this relationship does not precisely adhere to the inverse square relationship, as this relationship assumes that the UV source acts as a point source emitting radiation from a single, infinitely small point in space and that the radiation is both homogeneous and isotropic.

The size of the UV lamp is significantly larger in comparison to the distance between the source and the irradiation point. This contradicts the assumption of a point source in the inverse square relationship between irradiance and distance from the source.

The incident energy from the radiation source is directly proportional to the irradiance (intensity) and the exposure time, as illustrated by Equation 2. The dose values at the 15 cm, 30 cm, and 45 cm exposure points are 54.8465 ± 2.1014, 21.4448 ± 0.9501, and 13.1410 ± 0.8284 mJ/cm^2, respectively.

Table 2: Coding for isolated microbes

CODE	Type	Colony description
Organisms isolated from pepper		
FY	Yeast	Filamentous yeast with pale milky colouration. Irregularly shaped, Elevation- raised, margins – undulate
B_3	Bacteria	Brown Filamentous irregularly shaped colonies with entire margins, serrated edges, and dry appearance.
DWM	Mould	White cottony mould fills the plate in about 48 hours and dirty brown spores begin to form around the edges of the plate from day 4.
GM	Mould	Initial growth as a cottony white mould. Yellow and green spores begin to form on the third day and mature on the fifth day.
BM	Mould	Initial growth as a white featherlike mould turning black. Black tiny sporangia appear on day 2 and reach maturity on day 3.
B_1	Bacteria	Brown irregularly shaped colonies begin as punctiform colonies but spreading to cover the plate. margin -entire, elevation- raised
Organisms isolated from okra		
Yeast,	Yeast	Large dry surface yeast. Irregularly shaped, Elevation- raised, margins – undulate
BLM	Mould	Initiates as tiny white colonies. Bluish-green sporangia begin to form from 24 hours and mature in 48 hours with dark green colouration which turns black with ageing
GM	Mould	Colonies initiated as tiny white and feather-like, yellow and green spores begin to form after 48 hours and mature in about 4 days.
B_1	Bacteria	Brown irregularly shaped colonies beginning as punctiform colonies but spreading to cover the plate. margin -entire, elevation- raised
Organisms isolated from orange		
CWMPI	Mould	Cottony white mould with pink colouration in media. Matures on day 4 and white colour turns grey with ageing
LWDC	Yeast	Large dry surface yeast. Irregularly shaped, Elevation- raised, margins – undulate
B_2	Bacteria	Milky brown dry colonies with undulated edges
B_1	Bacteria	Brown irregularly shaped colonies beginning as punctiform colonies but spreading to cover the plate. margin -entire, elevation- raised

Table 3: Biochemical tests on bacterial isolates

Test	Microorganisms		
	B_1	**B_2**	**B_3**
Gram Staining	+	-	-
Catalase	-	+	-
Oxidase	NA	-	-
Citrate Utilization	NA	+	+
Gas Production	NA	-	-
H_2S Production	NA	-	+
Motility	NA	+	+

Key: + = positive reaction
- = negative reaction
NA= not applicable

The coding for isolates is provided in Table 2. Fungal codes were assigned based on prominent observable colony characteristics on plates, while bacterial colonies exhibiting similar characteristics were grouped and identified as B_1, B_2, and B_3.

To identify B_2 and B_3, colony characteristics, Gram staining reactions, and biochemical test results, in conjunction with a standard identification guide for bacterial isolates, were utilized. B_1 was confirmed as *Streptococcus*, B_2 was confirmed as *Proteus*, and B_3 was identified as *Erwinia carotovora*. The results of the biochemical tests carried out are presented in Table 3.

The colony characteristics, along with the microscopic examination of a few hyphae strands in lactophenol cotton blue and reference to a fungal identification guide, confirmed the identification of coded fungal isolates. The mould isolates were recognized as *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Penicillium roseopurpureum, and Fusarium solani.* The yeast isolates were determined to be

Zygosaccharomyces bailii and *Zygosaccharomyces rouxii*.

From pepper, the following isolates were identified: *Erwinia carotovora, Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger,* and *Streptococcus spp*. From okra, the isolates included *Zygosaccharomyces bailii, Penicillium roseopurpureum, Aspergillus flavus, and Streptococcus spp*. From orange, the isolates were *Streptococcus spp., Proteus,* and *Fusarium solani, along with Zygosaccharomyces bailii.*

These findings align with the existing data regarding the isolation of pathogenic microbes found in the available literature. Tafinta et al. (2014) conducted a study where they identified *Rhizopus stolonifer, Aspergillus flavus*, and *Aspergillus niger* in sweet orange specimens from Sokoto, Nigeria. Another research by (Adesemoye et al., 2011) highlighted *Fusarium solani* as a pathogen causing dry rot in oranges. Additionally, *Zygosaccharomyces* yeast species have been recognized as common pathogens affecting fruit juices, particularly orange (Ashurst et al., 2017). *Zygosaccharomyces bailii* and related species exhibit a particular preference for fructose, metabolizing it faster than glucose when found in fruits (Sá-Correia et al., 2014). These yeasts are notorious for causing spoilage through fermentation in various products such as fruit juices, concentrates, syrups, sauces, alcoholic beverages, honey, jams, and confectionery items (Petruzzi et al., 2017).

In relation to pepper spoilage, *Erwinia carotovora, Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger,* and *Streptococcus spp*. are commonly reported as spoilage microorganisms (Balamurugan & Kumar, 2023; Iqbal et al., 2011). *Erwinia carotovora* and *Rhizopus stolonifer* have been reported to cause soft rot in bell peppers (Balamurugan & Kumar, 2023; Hadas et al., 2001), while *Aspergillus* species have been associated with soft rot in peppers (Edobor et al., 2017). Moreover, Penicillium and Aspergillus species are responsible for spoilage in okra (Abubakar et al., 2019).

Table 4: Pathogenicity length measurement for pepper

S/No.	Organism Code	Organism	Type	Length of Fruit Affected (cm)
1	B_3	*Erwinia corotovora*	Bacteria	1.0±0.7071
2	DWM	*Rhizopus stolonifer*	Mould	0.5±0.0000
3	GM	*Aspergillus flavus*	Mould	5.0±0.0000
4	BM	*Aspergillus niger*	Mould	5.6±0.7778
5	FY	*Zygosaccharomyces rouxii.*	Yeast	1.2±0.3536
6	B_1	*Streptococcus spp*	Bacteria	0.95±0.7778
7	All	-	Combination	2.00±0.0000

Table 5: Pathogenicity length measurement for okra

S/No.	Organism Code	Organism Name	Type	Length of Fruit Affected (cm)
1	B_1	*Streptococcus spp.*	Bacteria	5.62±1.5412
2	BLM	*Penicillium roseopurpureum*	Mould	4.20±1.0607
3	GM	*Aspergillus flavus*	Mould	2.50±0.7071
4	Yeast	*Zygosaccharomyces bailii*	Yeast	1.75±0.3536
5	ALL	-	Combination	6.25±1.7678

Table 6: Pathogenicity length measurement for orange

S/No.	Organism Code	Organism Name	Type	Length of Fruit Affected (cm)
1	B_1	*Streptococcus spp*	Bacteria	1.58±0.1061
2	B_2	*Proteus spp*	Bacteria	0.55±0.07
3	CWMPi	*Fusarium solani*	Mould	All bad
4	LWDC	*Zygosaccharomyces bailii*	Yeast	0.50±0.00
5	COMBINE	Combination	Combination	All bad

From Table 4, it is evident that *Aspergillus flavus* and A*spergillus niger* exhibit the highest level of spoilage in pepper. Other isolates also caused spoilage even though to a lesser extent. Notably, the spoilage effect is observed when all these microbes act together on the fruits. From Table 5, it is observed that the combined impact of the isolates results in the most significant damage to okra, particularly in terms of the length of the crop affected. *Streptococcus* is also notable for its substantial spoilage effect on okra. Furthermore,

Penicillium roseopurpureum, *Aspergillus flavus*, and *Zygosaccharomyces bailii* contribute to spoilage, although to a lesser extent. Table 4.3 reveals that *Fusarium solani* and the combined inoculation of isolates have the most substantial spoilage effect on oranges. When inoculated with the *Fusarium solani* isolate, the entire orange fruit deteriorated within two days, while the combined treatment resulted in complete spoilage after four days of inoculation. The other isolates also presented some level of damage to the crops.

The results presented in Tables 4 to 6 collectively underscore the pathogenic nature of both bacterial and fungal isolates when acting on crops, either individually or in combination.

Table 7: Viable colony count and decimal doses

S/No.	Fruit	Viable Colony Count for		Decimal Reduction Dose (D_{10})		Energy (J/m^{-2})
		60 seconds	90 seconds	Time (Seconds)	Time (Minutes)	
1	Pepper	128	124	1507	15.7	4.133
2	Orange	145	156	944	25.1	2.589
3	Okra	39	42	932	15.5	2.556

The viable colony counts post-irradiation for isolates in combination for 60 and 90 seconds of exposure are presented per crop type in Table 5. The viable counts were used in equation 5 to determine the decimal exposure time required to achieve the decimal reduction doses which are found to be 15.7 minutes, 25.1 minutes and 15.5 minutes for pepper orange and okra respectively. The UV-C energy for exposure is 2.589 Jm^{-2} for orange, 4.133 Jm^{-2} for pepper, and 2.556 Jm^{-2} for Okra. Doses within this range have been utilized for studies for sterilization of fruits and vegetables. Allende & Artés (2003) utilized 4.06 - 8.4 kJm^{-2} UVC to sterilize lettuce while Gündüz et al. (2015) achieved decimal reduction with 2.75 – 3.33 kJm^{-2} doses of UVC.

CONCLUSION

A successful isolation and identification of pathogenic microbes from various fruits and vegetables was achieved thus, laying a foundation for better understanding and management of food safety. The determination of 10 log UVC decimal reduction doses underscore the potential of UV-C irradiation as a valuable tool for microbial inactivation, thus offering enhanced food safety and extended shelf life. These findings contribute to the ongoing efforts to ensure safer and longer-lasting food products for consumers and the food industry.

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