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Characterization and Determination of pH, Alcohol, and Temperature Tolerance of Microorganisms Recovered From Vegetable Samples

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ABSTRACT

This study aims to investigate the spectrum of bacteria and yeasts present in soaked vegetables and their resilience to varying pH levels, alcohol, and temperatures. Four samples were collected from Ago-Iwoye market, and initial isolation and identification were conducted using streaking techniques and biochemical tests. Standard procedures were employed to assess bacteria and yeast tolerance to different pH levels, alcohol concentrations, and temperature ranges. The biochemical tests identified microorganisms, including *Lactobacillus* spp., *Lactobacillus* spp., *Enterococcus* spp., *Leuconostoc* spp., *Escherichia coli*, and *Saccharomyces cerevisiae*. Bacterial and yeast isolates demonstrated viability at both 10°C and 45°C, while no visible growth occurred at 60°C, indicating the respective heat resistance of these microorganisms commonly found in vegetable samples. The evaluation of microbial tolerance to varying pH conditions is pivotal for exploring adaptability in food processing and digestion, particularly in fermentation processes. The research concludes the microbial diversity present, distinguishing between beneficial and potentially harmful strains.

Keywords: *Lactobacillus, Saccharomyces*, Alcohol, pH, Temperature, Ago-Iwoye

INTRODUCTION

Food safety is a paramount concern in today's globalized and diverse culinary landscape (Osafo et al., 2022). As a staple in numerous cuisines, vegetables are subjected to various preparatory practices, among which soaking is prevalent (Bationo et al., 2023). Vegetables, being primary components of a balanced diet, are consumed in various forms worldwide, with many cultures incorporating soaking as a culinary technique (Amoah et al., 2023). Despite its widespread use, the potential microbial risks associated with soaked vegetables remain underexplored (Blikra et al., 2021). Bacterial and yeast contamination in food items, especially vegetables, can lead to severe health implications (Balali et al., 2020). The inherent characteristics of vegetables, often consumed raw or minimally processed,

amplify the risk of ingesting harmful microorganisms (Fadiji et al., 2023).

The prevalence of bacteria and yeasts in food matrices, particularly soaked vegetable samples, poses a critical concern for food safety (Chang et al., 2023). Soaking vegetables is a common practice in various culinary traditions, yet it introduces an environment conducive to microbial growth (Alegbeleye et al., 2022). Bacteria and yeasts are ubiquitous in nature and can contaminate vegetables at any stage of production, from farm to table (Erhirhie et al., 2020). The soaking process may serve as a reservoir for these microorganisms, potentially leading to the proliferation of pathogenic strains (Mahdi et al., 2022). Given that vegetables are often consumed raw or with minimal processing, any microbial contaminants present in soaked vegetables can pose a direct risk to human health (Azande et al., 2020).

Beyond mere identification, showing into the assessment of microbial tolerance to three key factors: pH, temperature, and alcohol. These factors play pivotal roles in the survival and proliferation of bacteria and yeasts. The assessment of microbial tolerance to pH, temperature, and alcohol becomes imperative in predicting their survival and potential pathogenicity (Bassey et al., 2021). Fluctuations in pH levels, temperature variations during processing and storage, and the presence of alcohol-based compounds either naturally occurring or introduced during cleaning procedures can significantly impact microbial communities (Das and Mishra, 2023). The adaptability of these microorganisms to such environmental conditions is crucial for anticipating and managing potential risks associated with different stages of food production and consumption.

By elucidating the specific conditions under which bacteria and yeasts thrive or perish in soaked vegetables, the research can inform strategies to minimize microbial contamination and enhance food safety in both household and commercial settings. Therefore, the aim of this study is to examine the range of bacteria and yeasts found in soaked vegetables and their tolerance to pH, alcohol and temperature at different range.

MATERIALS AND METHODS

Study area

The study area where this research was conducted is Ago-Iwoye, situated in Ogun, Nigeria. It is a part of the Ijebu Kingdom, depicted in Figure 1 through satellite imagery. Found within the Ijebu North Local Governmental Area, the primary town encompasses seven contiguous districts: Ibipe, recognized as the prominent settlement, along with Isamuro, Idode, Odosinusi, Igan, Imosu, and Imere. The primary campus of Olabisi Onabanjo University is positioned 7 km west of the city. In 1963, the town's population stood at 14,718; however, by 2013, it was approximated to have surged to around 190,000, with 40,000 of this population being university students (Hashimi, 2020).

Collection of vegetable samples

The following vegetables namely; *Brassica oleracea* (cabbage), *Daucus carota* (carrot), *Telfairia occidental* (Fluted pumpkins), *Amaranthus cruentus* (African spinach) leaves were collected from vegetable retailers in Ago-iwoye, Ogun State, Nigeria. Vegetables were kept in sterile ziplock bag and transported to the laboratory.

Preparation of soaking solution

All glass wares were thoroughly washed with detergent solution rinsed with clean water and sterilised using a hot air oven at 160ºC for at least one hour. Sterile distilled water was then poured into the glass ware. Vegetables were then minced/chopped into the glass wares containing the sterile water, this was left for over 24hours to soak.

Media preparations
All media used (Nutrient agar, Mac Conkey agar and Potato Dextrose agar) were prepared according to manufacturer's instructions and then autoclaved at 121ºc for 45 minutes.

Microbial recovery
Microorganisms were recovered from the prepared soaked vegetable sample through inoculation into selective media using the streak plate method.

Isolation of bacteria and yeast isolates
Nutrient agar and Potato dextrose agar were used to isolate organisms found to have grown on the inoculation plate. These was done repeatedly for a period of 3 days to obtain a pure sample culture.

Cellular morphology of bacteria and yeast isolates
Cells were observed with Gram staining under a microscope (oil immersion, 100x). Shape of cells (cocci or bacilli) and arrangement of cells (scattered, bunches, and chain) along with the Gram reaction (pink or purple) were observed by Falodun *et al.* (2018) and Onajobi *et al.* (2020).

Gram staining
A smear of the pure isolate was made on a clean grease free slide. The smear was air - dried by waving it around for a while. The smear was heat-fixed by passing it over a Bunsen burner flame. The smear was stained with crystal violet reagent for one minute. This was rinsed under slowly running tap for 5 seconds. The slide was treated with lugol iodine and allowed for 60 seconds. This was washed off under slowly running tap. Alcohol reagent was used to decolorize the primary stain, until no more dye runs off from smear. The smear was counter-stained with safranin for 30 seconds. Stained slide was slowly rinsed under running water. The slide was allowed to air- dry. The slide was observed using the oil immersion lens of the microscope. Gram positive cells stained purple/violet while gram negative cells stain pink or red (Falodun *et al.,* 2018; Onajobi *et al.,* 2023).

Lactophenol blue staining
A yeast smear was prepared using a sterile loop or inoculating needle, a small amount of the yeast culture was transferred onto a clean microscope slide. The yeast sample was evenly spread across the slide to create a thin smear. The yeast smear was allowed to air dry completely at room temperature. Heat fixation was not needed, as yeast cells are heat sensitive. A few drops of Lactophenol cotton blue solution was applied directly onto the yeast smear. Ensuring the smear is fully covered with the staining solution. A clean coverslip over was Gently placed on the stained yeast smear taking care not to introduce air bubbles. The slide was allowed to sit undisturbed for a few minutes to allow the Lactophenol cotton blue solution to permeate the yeast cells. The prepared slide was then placed on a microscope stage. Starting with a lower magnification objective (10X or 20X) to locate yeast cell and gradually increasing the magnification (e.g. 40x or 100x oil immersion) to observe yeast cell morphology and structures (Falodun *et al.,* 2018).

Biochemical characterization of the bacterial Isolates
The following biochemical tests; coagulase test, oxidase test, catalase test, indole test, urease test, citrate test and methyl red test were done to confirm and identify the bacteria isolates according to Falodun *et al.* (2018) and Onajobi *et al.* (2023).

pH Tolerance Assessment of bacterial and yeast isolates
Agar dilution method was used in carrying out pH tolerance assessment for the pure

isolate culture. A suitable agar medium was prepared and measured for pH range. Hydrochloric acid was added to reduce the pH to the desired acidic range and Sodium Hydroxide was used to increase the pH of the medium to the desired basic range, the media was autoclaved at the appropriate temperature (usually 121°C). The pure culture isolate was then inoculated into each agar plate containing different range of pH solution. After incubation, observation of growth of organism in the different pH conditions was observed (CLSI, 2017).

Alcohol Tolerance Assessment of bacterial and yeast isolates

Agar dilution method was used in carrying out alcohol tolerance assessment for the pure isolate culture. A suitable agar medium measured according to manufacturer's instruction and the medium solvent usually distilled water was measured at different alcohol concentration by percentage range (50%, 20%, 30%) through the addition of alcohol (ethanol) into the measured distilled water, the media was autoclaved at the appropriate temperature (usually 121°C). The pure culture isolate were then inoculated into each agar plate containing different range of pH solution. After incubation, observation of growth of organism in the different pH conditions was observed (CLSI, 2017).

Temperature Tolerance Assessment of bacterial and Yeast Isolates

A suitable agar growth medium such as nutrient agar and Potato dextrose agar was prepared in multiple plate and were inoculated with pure culture isolate. These was Incubated at different temperature ranges. After incubation for about 18-24 hours, observation of the growth pattern of the isolate was observed (CLSI, 2017).

RESULTS AND DISCUSSION

Results

The organism's growth pattern and morphology when introduced to nutrient agar. It outlines the morphological traits of each isolate derived from cabbage (CBG), carrot (CRT), Fluted pumpkin (FPK), and African spinach (SPH) as illustrated table 1 below. Microorganisms showcase a diverse range of morphological characteristics crucial for identification and classification. Cultivating them on various media allows for the observation of growth patterns, colony features, and responses to specific nutrients. The table delves into the morphological aspects of bacterial isolates cultured on nutrient agar, revealing diverse colony forms—round, irregular, filamentous, or spreading. This diversity mirrors the varied growth habits inherent in different bacterial species.

The growth pattern and morphology of the organism inoculated into Mac Conkey Agar. Table 2 shows the morphological characteristics of each isolate on the agar plate gotten from the four vegetable samples; cabbage (CBG), carrot (CRT), Fluted pumpkin (FPK), and African spinach (SPH). The table explores the morphological features of bacteria isolates when cultured on MacConkey Agar (MAC) media.

The growth pattern and morphology of the organism inoculated into Potato Dextrose Agar. Table 3 below shows the morphological characteristics of each isolate on the agar plate gotten from the four vegetable samples; cabbage (CBG), carrot (CRT), Fluted pumpkin (FPK), and African spinach (SPH). The table explores the morphological features of yeast isolates when cultured on Potato Dextrose Agar (PDA) media. Yeast colonies on PDA can appear as creamy, may develop a distinct margin or elevation, produce a characteristic powdery appearance, exhibit various colors, from white to cream, and even shades of pink or

yellow and pigmentation can be species-specific.

The biochemical characterization of each of the isolated organisms. These organisms were isolated from their first culture plate into a suitable growth media Nutrient Agar (NA), Potato Dextrose Agar (PDA) to obtain a pure culture colony which was then used to perform different biochemical test as revealed in table 4. This is done for microbial identification, classification and understanding of the metabolic capabilities of microorganisms which helps distinguish between different species and strains by evaluating their ability to ferment specific sugars, producing acids and gases, Identify the presence of cytochrome c oxidase, Detects the presence of catalase enzyme which breaks down hydrogen peroxide into water and oxygen, Detects the ability of bacteria to produce indole from tryptophan metabolism.

The possible organisms recovered during microbial recovery based on their biochemical reactions as depicted in table 5.

The pH tolerance assessment result for the bacterial isolate as shown in table 6 below, this shows the ability of isolated organism to grow in different pH range media (such as pH 1-3, pH 4-6, pH 7, pH 8-10 and pH 11-12). In similitude, the pH tolerance assessment result for the yeasts isolates as illustrated in table 7, this shows the ability of isolated organism to grow in different pH range media (such as pH 1-2, pH 3-4, pH 5-6, pH 7-8, pH 9-10 and pH 11-12).

The alcohol tolerance assessment result for bacteria and yeasts isolate as presented in table 8. This shows the ability of isolated organism to grow in different percentage of alcohol (ethanol) in a suitable growth media (such as 10%, 30%, 50%, 70%, 100%). Only isolates CBG_2PD, CRT_2PD, SPH_2PD and FPK_2PD are found to be detected at 10% of alcohol concentration while the rest isolates were not detected at 10%, 30%, 50%, 70%, 100% alcohol concentration.

The evaluation of bacterial and yeast isolates' response to varying temperatures was conducted after an incubation period at different temperature ranges, specifically at 10°C, 45°C, and 60°C, as presented in Table 9 below. The isolates exhibited robust growth within the temperature range of 45°C, displayed moderate growth at 10°C, and demonstrated no growth at 60°C.

Discussion

The study revealed the recovery of bacteria and yeasts from soaked vegetable samples and assesses their tolerance to pH, alcohol, and temperature. This approach provides a comprehensive understanding of microbial dynamics in commonly consumed food items. The research highlights the diversity of microorganisms, distinguishing both beneficial and potentially harmful strains. Evaluating microbial tolerance to varying pH conditions is crucial for understanding adaptability in food processing and digestion, especially in fermentation processes where pH fluctuations impact flavors and textures (Sato-Takabe & Hamasaki, 2018).

In the current study, based on the biochemical test reaction, microorganism was identified as *Lactobacillus brevis, Lactobacillus plantarum, Enterococcus feacalis, Leuconostoc mesenteriodes, Escherichia coli, Saccharomyces cerevisiae* and this is in accordance with some of the reports of Zavišić *et al.* (2020) which shows the history of lactic acid bacteria application in fermented foods due to their beneficial influence on nutritional, organoleptic, and shelf-life characteristics. Observations on the relative heat strength of bacteria and yeasts in vegetable samples align with previous findings, underscoring the strain-dependent temperature preferences of *Saccharomyces cerevisiae.*

In the current investigation, isolates CBG_2PD, CRT_2PD, SPH_2PD, and FPK_2PD

were detected at a concentration of 10% alcohol. Conversely, the remaining isolates did not register detection at alcohol concentrations of 10%, 30%, 50%, 70%, and 100%. This study points into the diverse capacities and resilience of bacteria and yeasts to thrive in the presence of ethanol (alcohol). This aligns with the findings of Adams *et al.* (2015), who underscored well-established variations in the ethanol tolerance among different strains of *Saccharomyces cerevisiae*, highlighting the strain-specific nature of ethanol tolerance within this yeast species. The research sheds light on the genetic and physiological aspects governing ethanol tolerance in *Saccharomyces cerevisiae*.

He *et al*. (2020) investigated the role of alcohols, including ethanol, on the growth, lipid composition, and membrane fluidity of various organisms, including bacteria. The study aimed to determine whether unique lipid profiles confer resilience to ethanol stress, providing insights into the physiological adaptations of bacteria to ethanol. Furthermore, Cubas-Cano *et al.* (2020) highlighted the abundance of lactic acid bacteria (LAB) in the bioethanol process, possibly due to their tolerance to ethanol, low pH, and high temperature. This emphasizes the importance of ethanol tolerance in the context of bioethanol production and the potential role of LAB in ethanol-containing environments.

It was observed that bacterial and yeast isolates thrived at 10°C and 45°C and both had died and had no visible growth at 60°C in this study showing the relative heat strength of bacteria and yeasts that are commonly found in vegetable samples and this correlates with the findings of Nakamura *et al.* (2014). This supports the growth properties of *Saccharomyces cerevisiae* at high temperatures differ according to the strain used, but the optimum temperature of proliferation is around 30 °C (Nakamura *et al.,* 2014). The impact of temperature on bacterial growth has also been investigated in specific contexts. The high temperature accelerates the growth of aerobic anoxygenic phototrophic bacteria in seawater, indicating a positive correlation between bacterial abundance and growth rate with increasing temperature (Sato-Takabe & Hamasaki, 2018).

Moreover, Adams *et al.* (2015) demonstrated that temperature controlled the cellular response to added nutrients, with higher temperatures increasing the speed at which bacterial activity increased (Adams *et al.,* 2015). Additionally, the study highlights the impact of temperature on bacterial growth, with higher temperatures influencing the speed of bacterial activity in response to added nutrients.

CONCLUSIONS

The research provides essential data on temperature resilience, guiding the selection of strains appropriate for specific applications, thereby ensuring adherence to food safety standards and risk reduction. By aligning the identified microorganisms with the historical use of lactic acid bacteria (LAB) in fermented foods, the study shows positive impact on nutritional and sensory attributes. Additionally, it elucidates the strain-specific ethanol tolerance in *Saccharomyces cerevisiae* and examines how alcohols, especially ethanol, influence growth, lipid composition, and membrane fluidity across various organisms. This research offers significant contributions to understanding the complex interplay between microorganisms and food, encompassing areas like food safety, fermentation, preservation, and microbial ecology.

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Table 1. Colonial morphology of bacterial isolates on Nutrient Agar

SAMPLE	SIZE	COLOUR	TEXTURE	SHAPE	ELEVATION	MARGIN
CBG	Tiny	Whitish - creamy	Mucoidy	Round	Convex	Entire
CRT	Tiny	Creamy	Slimy	Irregular	Raised	Undulate
FPK	Tiny	Yellowish	Dry	Irregular	Flat	Curled
SPH	Big	Creamy	Slimy	Circular	Convex	Entire

Keys: CBG - Cabbage culture; CRT - Carrot culture; FPK - Fluted pumpkin culture SPH - African spinach culture

Table 2. Morphology of isolates on Mac Conkey Agar

Sample	Size	Colour	Texture	Shape	Elevation	Margin
CBG	Medium	Pinkish (LF)	Oily	Circular	Pulvinate	Entire
CRT_2McY	Medium	Pinkish (LF)	Smooth	Irregular	Pulvinate	Undulate
CRT_2McP	Medium	Pinkish (LF)	Oily	Circular	Raised	Entire
FPK_2McF	Tiny	Pinkish (LF)	Dry	Circular	Flat	Entire
FPK_2McC	Medium	Pinkish (LF)	Dry	Circular	Crateriform	Entire
SPH	Tiny	Pinkish (LF)	Dry	Filamentous	Convex	Undulate

Keys: CBG - Cabbage; CRT_2McY - Carrot 2 Mac Yellow; CRT_2McP - Carrot 2 Mac Pink; FPK_2McF - Fluted Pumpkin Mac Flat; FPK_2McC - Fluted Pumpkin Mac Crateriform; SPH - African Spinach; LF - Lactose fermenter

Table 3. Morphology of yeast isolates on Potato Dextrose Agar (PDA)

SAMPLE I.D	SIZE	COLOUR	TEXTURE	SHAPE	ELEVATION	MARGIN	ODOUR
CBG	Large	Creamy-white	Moist	Oval	Convex	Entire	Buttermilk
CRT	Large	Creamy-white	Moist	Oval	Convex	Entire	Buttermilk
FPK	Large	Creamy-white	Moist	Oval	Convex	Entire	Buttermilk
SPH	Large	Creamy-white	Moist	Oval	Convex	Entire	Buttermilk

Table 4. Biochemical Characterization of bacterial and Yeasts isolates

Organism I.D	GRAM RXN	LACTOSE	GLUCOSE	H2S	GAS	CATALASE	CITRATE	MOTILITY	INDOLE	OXIDASE
CBG_2Mc	+B	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
CBG_2Nt	+B	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
CRT_2McY	+C	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
CRT_2McP	+C	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve
CRT_2Nt	+B	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
FPK_2Nt	+C	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
FPK_2McF	+C	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
FPK_2McC	+C	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
SPH_2Mc	-B	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve	-ve
SPH_2Nt	+C	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
CRT_2PD	+Y	-ve	+ve	-ve	+ve	+ve	NA	NA	-ve	-ve
CBG_2PD	+Y	-ve	+ve	-ve	+ve	+ve	NA	NA	-ve	-ve
SPH_2PD	+Y	-ve	+ve	-ve	+ve	+ve	NA	NA	-ve	-ve
FPK_2PD	+Y	-ve	+ve	-ve	+ve	+ve	NA	NA	-ve	-ve

Keys: -ve - Negative; +ve - Positive; NA- Not Applicable; +B- Gram positive Bacilli; -B- Gram Negative Bacilli; +C- Gram Positive Cocci; -C - Gram Negative Cocci; +Y- Positive Yeast cell

Table 5. Suspected organism based on the biochemical analysis

ORGANISM CODE	SUSPECTED ORGANISM
CBG_2Mc	*Lactobacillus* spp.
CBG_2Nt	*Lactobacillus* spp.
CRT_2Nt	*Lactobacillus spp.*
CRT_2McY	*Enterococcus feacalis*
CRT_2McP	*Enterococcus feacalis*
FPK_2Nt	*Leuconostoc* spp.
FPK_2McF	*Leuconostoc* spp.
FPK_2McC	*Leuconostoc* spp.
SPH_2Nt	*Leuconostoc* spp.
SPH_2Mc	*Escherichia coli*
CRT_2PD	*Saccharomyces cerevisiae*
CBG_2PD	*Saccharomyces cerevisiae*
FPK_2PD	*Saccharomyces cerevisiae*
SPH_2PD	*Saccharomyces cerevisiae*

Table 6. pH Tolerance Assessment for bacterial isolates

ISOLATE IDENTITY	pH RANGE				
	pH 1-3	**pH 4-6**	**pH 7**	**pH 8-10**	**pH 11-12**
CBG_2Mc	-	+	+	+	+
CRT_2McY	-	+	+	+	+
FPK_2Nt	-	+	+	+	+
FPK_2McF	-	+	+	+	-
CRT_2Nt	-	±	+	+	-
CBG_2Nt	-	-	+	+	-
CRT_2McP	-	+	+	+	±
SPH_2Mc	-	+	+	+	-
SPH_2Nt	-	+	+	+	+
FPK_2McC	-	+	+	+	±

Keys: - - Negative; + - Positive; ± - Partial Growth

Table 7. pH Tolerance Assessment for Yeasts isolates

ISOLATE IDENTITY	pH RANGE					
	pH 1-2	**pH 3-4**	**pH 5-6**	**pH 7-8**	**pH 9-10**	**pH 11-12**
CBG_2PD	-	+	+	+	+	-
CRT_2PD	-	+	+	+	+	-
SPH_2PD	-	+	+	+	+	-
FPK_2PD	-	+	+	+	+	-

Keys: - - Negative; + - Positive

Table 8. Alcohol Tolerance Assessment of bacterial and Yeast Isolates

ISOLATE IDENTITY	10%	30%	50%	70%
CBG_2Mc	ND	ND	ND	ND
CRT_2McY	ND	ND	ND	ND
FPK_2Nt	ND	ND	ND	ND
FPK_2McF	ND	ND	ND	ND
CRT_2Nt	ND	ND	ND	ND
CBG_2Nt	ND	ND	ND	ND
CRT_2McP	ND	ND	ND	ND
SPH_2Mc	ND	ND	ND	ND
SPH_2Nt	ND	ND	ND	ND
FPK_2McC	ND	ND	ND	ND
CBG_2PD	+	ND	ND	ND
CRT_2PD	+	ND	ND	ND
SPH_2PD	+	ND	ND	ND
FPK_2PD	+	ND	ND	ND

Keys: ND Not Detected; + Positive

Table 9. Temperature Tolerance Assessment of bacterial and Yeast Isolates

ISOLATE IDENTITY	10°C	45°C	60°C
CBG_2Mc	+	+	ND
CRT_2McY	+	+	+
FPK_2Nt	+	+	ND
FPK_2McF	+	+	ND
CRT_2Nt	+	+	ND
CBG_2Nt	+	+	ND
CRT_2McP	+	+	+
SPH_2Mc	+	+	ND
SPH_2Nt	+	+	ND
FPK_2McC	+	+	ND
CBG_2PD	+	+	ND
CRT_2PD	+	+	ND
SPH_2PD	+	+	ND
FPK_2PD	+	+	ND

Keys: ND Not Detected; + Positive

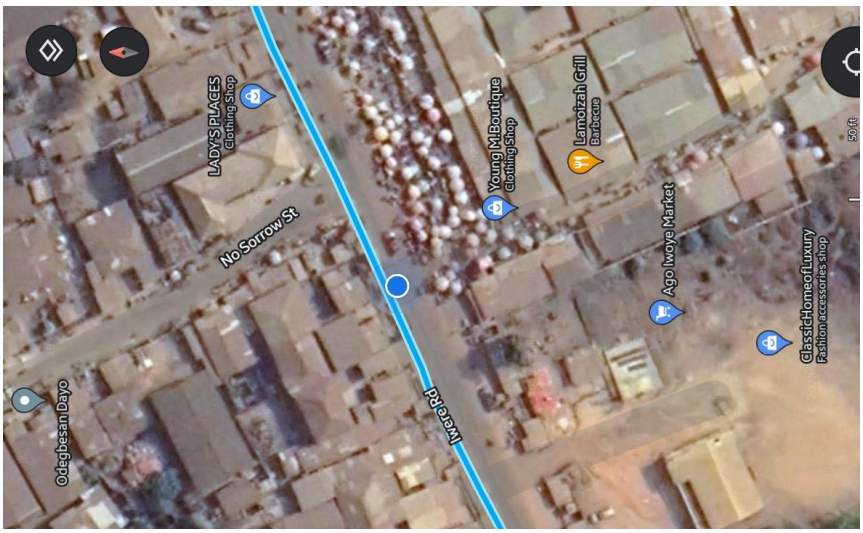

Figure 1. Satellite imagery of Ago-Iwoye market and sample location