

Potential Pathogenic Organisms Associated With Spoilt Tomatoes Sold In Ikeji-Arakeji, Nigeria

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Abstract: The present study evaluated the potential pathogenic bacteria and fungi which are associated with spoilt tomatoes in Ikeji-Arakeji, Southwestern Nigeria. Tomatoes are widely used in cooking of stew and preparation of other delicacies in Nigeria. Sometimes tomatoes are eaten without processing and if contaminated, the organisms are ingested directly into the body. Spoilt tomatoes, due to low cost of purchase are sometimes preferred by consumers. This act is more rampant among food vendors whose aim is to make maximum profits. Questionnaires were administered randomly to food sellers and inhabitants of Ikeji-Arakeji and from the sampled population, it was observed that about 48% of food sellers and 30% of domestic consumers preferred to purchase spoilt tomatoes due to the low cost and the questionnaire also reflected that most consumers are unaware of the health implications of spoilt tomatoes. Spoilt tomato samples were purchased from tomato vendors in Ikeji-Arakeji and instantly taken to the laboratory for microbial analysis. The bacteria species isolated from the sampled spoilt tomatoes were: *Bacillus* sp., *Escherichia* sp., *Klebsiella* sp., *Pseudomonas* sp., *Salmonella* sp. and *Staphylococcus* sp., while the fungi isolated were: *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Mucor* sp., and *Rhizopus* sp. These organisms are potential pathogens which if consumed could pose a serious health implication on consumers. Awareness should be made on the health implications of spoilt tomatoes, consumption of spoilt tomatoes should be discouraged and tomato vendors should be educated and advised to adhere to hygiene practices to prevent food-borne illness associated with spoil tomatoes.

Keywords: Ikeji-Arakeji, Microorganisms, Nutrient, Pathogenic, Questionnaires, Tomatoes.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum*), is a plant that grows annually and is cultivated in many parts of the world. Tomato is eaten raw as salad, used in garnishing various cooked foods [1] and also generally used in cooking stew in Nigeria [2].

Tomatoes seed are nutritious and contain carbohydrates, fats, organic acids, water, minerals, vitamins and pigments. Ripe tomato fruits has been reported to contain carbohydrates (4.3%), protein (1%, water (94%), fat (0.1%), 0.6% fibre and vitamins. The nutrients present in tomatoes seeds are sufficient enough to support and encourage the growth of microorganisms such as fungi and bacteria that produce enzymes which degrade the nutrients present in the seeds. The water content in tomatoes seed makes them more susceptible to spoilage by microorganisms and makes the preservation and transportation more difficult. Recently, there has been high incidence of diseases in tomato fruits and this has been a cause for global concern. Rigorous researches have been undertaken to understand the measures which can be taken to effect some essential control [3]. Some studies have reported outbreaks of food borne diseases associated with tomatoes [7] [8].

Food-borne pathogens has been reported to be accountable for vast numbers of the illnesses and death in developing countries [6]. There are a some reports of studies on microorganisms associated with spoilage of tomato fruits in Nigeria[1] [4] [5]. Similar research information on tomato in Ikeji-Arakeji are not available.

Due to the high cost of fresh tomatoes seed, food sellers and domestic consumers has resulted into the patronage of spoilt tomatoes locally called 'Esa' by the Yoruba people. Most of these consumers are ignorant of the health implications of the spoilt tomatoes and they see the purchase of spoilt tomatoes as an advantage because of the less cost.

2. METHODOLOGY

Prior to the study, questionnaires were administered randomly to food sellers and domestic tomatoes consumers in Ikeji-Arakeji on the choice of tomatoes seed (fresh or spoilt), the rationale behind their choice as well as the health implications associated with the consumption of spoilt tomatoes. Also Tomato sellers were also questioned on the hygiene of the containers where the tomatoes were displayed for sales, the time it takes to sell each batch of tomatoes, where left over tomatoes were kept for preservations. Spoilt tomatoes samples were purchased from the local market in Ikeji-Arakeji, Southwestern Nigeria. Tomatoes samples were collected in triplicates at each sampling period and immediately transported to the laboratory for microbial analysis. The spoilt tomatoes purchased were homogenized with sterile Maximum Recovery Diluent after pounding in a sterile mortal using a sterile pestle. 1 ml of the solution derived was taken and serial dilutions were carried out.

Pour plate method was used to enumerate the heterotrophic microorganism present in the spoilt tomatoes samples. Nutrient Agar was used in the bacteria enumeration while Sabouraud Dextrose Agar was used to enumerate the fungi. The bacteria plates were incubated at 37⁰C for 24 hours while the fungal plates were incubated at 27⁰C for 72 hours. After incubation, the plates were observed for growth and the colonial characteristics which include the shape, size, colour, elevation, surface, opacity, edge were observed and recorded.

2.1 ISOLATION OF PURE COLONIES:

Colonies on the plates were picked carefully from the mixed colonies with the aid of sterilized inoculating loop and transferred on freshly sterilized Nutrient Agar and Sabouraud agar plates. This procedure was repeated until pure colonies were observed. Cultural and microscopic examinations were carried out to identify the fungi while gram staining and biochemical tests were carried out to identify the bacteria present in the samples.

2.2 FUNGI IDENTIFICATION:

To identify the fungal isolates, wet mount was prepared on a clean slide from a 72hrs pure culture by adding a drop of cotton blue lactophenol. The slides was covered with a cover slip and viewed under the microscope using the 40x objective lens.

2.3 BACTERIA IDENTIFICATION:

2.3.1 GRAM STAINING:

Twenty-four hours old isolates were emulsified with sterile distilled water on clean grease free slides. The smears were heat fixed and stained with crystal violet solution for 1 minutes before rinsing and flooding with Gram's iodine solution which was also left for 1 minute. The smears were washed with 95 % alcohol for 5 seconds and rinsed with running tap water. The smears were finally counter stained with safranin for 1 minute and rinsed under running tap water. The slides were blotted dry and examined under oil-immersion objective. Gram-positive cells appeared purple while Gram-negative cells appeared pink. Cell shapes were also observed.

2.3.2 SPORE STAINING:

Five days old culture of the test organisms were emulsified and heat fixed on grease free slides. The slides were stained with malachite green solution and steamed for 5-10 minutes ensuring that the stains did not dry out. The malachite green stains was carefully rinsed under tap water. The slides were then counter stained with Safranin solution for 15 seconds and washed with water. The slides were blotted dry and examined under oil-immersion objective lens. Bacterial endospores stained green while the vegetative cells stained red.

2.3.3 CATALASE TEST:

Twenty-four hours old isolates were emulsified with drop of 3 % hydrogen peroxide (H₂O₂) on clean microscope slides. The slides were observed for formation of gas bubbles which indicated catalase positive reaction and the absence of gas bubbles were also observed which as well indicated a catalase negative reaction. A drop of hydrogen peroxide was also placed on the slide as a negative control.

2.3.4 CITRATE UTILIZATION TEST:

Sterile citrate media were inoculated with 24 hours old isolates of the test organism using a straight inoculating wire. The inoculated media were incubated at 35^oC for 24 hours together with an un-inoculated medium which served as control. A change in colour of the media from green to blue indicated positive reactions showing that the organisms were able to utilize citrate as a sole carbon source while no colour change indicated negative reactions and which implies that the organisms was unable to utilize citrate as a sole carbon source.

2.3.5 STARCH HYDROLYSIS:

The test isolates were streaked once across the center of the surface of sterile starch agar plate and incubated at 35^oC for 24hours. The plates were then flooded with 5.0ml of Lugols iodine solution. Positive reactions indicating hydrolysis of starch appeared as a clear zone around the line of streak and while negative reactions indicating unhydrolysed starch formed a blue-black colouration with the iodine.

2.3.6 OXIDASE TEST:

Filter papers were moistened with a few drops of 1% tetramethyl-p-phenylenediaminendihydrochloride. With the aid of a sterile wire loop, each test organism was picked and smeared on a filter paper. Oxidase production is indicated by the appearance of a purple colour within 5 to 10 seconds. Absence of purple colour indicated a Oxidase negative result.

2.3.7 SULPHIDE INDOLE MOTILITY TEST:

Sulphide Indole Motility medium was used two carry out this biochemical test. The Sulphide Indole Motility medium after preparation was dispensed into bijou bottles and sterilized at 121^oc for 15 minutes and allowed to set. With the aid of a sterile inoculating wire, sterile media were carefully stab-inoculated with a 24 hours old isolate and incubated at 37^oC for 24 hours. Motile organisms deviated from the line of stab while non-motile organisms grew along the line of stab. A stabbed but un-inoculated medium which served as control was also made. Observation of black colouration in the test tubes indicated sulphide positive reactions while the absence of black colouration indicated sulphide negative reactions.

About 0.5ml of Kovac's indole reagent was added to each test tube of inoculated Sulphide Indole Motility agar and the test tubes were shaken gently and allowed to stand for two minutes. A pink colouration which separates out in alcohol layer indicated indole production i.e. indole positive reactions, while no colour change indicated indole negative reaction.

2.3.8 SUGAR FERMENTATION AND HYDROGEN SULPHIDE PRODUCTION TEST:

Triple Sugar Iron agar (TSI) containing lactose, glucose and sucrose was used to carry out this biochemical test. The agar was prepared according to the manufacturer's instruction and sterilized at 121^oC for 15 min. Using a sterile inoculating wire, the medium were stabbed-inoculated with 24 hours old isolate and incubated at optimum temperature. The agar was observed for color change and gas production. The tubes where the organism produced hydrogen sulphide showed a black colouration while no colour change indicated a negative result.

2.3.9 MANNITOL FERMENTATION:

Phenol red mannitol broth was used to carry out the biochemical tests. The medium were prepared according to the manufacturer's instructions and dispensed into test tubes. The tubes were sterilized at 121^oC for 15 minutes. Each tube was inoculated with the test isolate and was incubated at 37^oC for 24 hours. Colour change from red to yellow indicated a positive reaction while negative test shows no colour change.

2.3.10 METHYL RED AND VOGES PROSKAUER TEST (MRVP):

Sterile MRVP medium (glucose phosphate broth) was prepared and dispensed into test tubes which were inoculated with 0.1ml of 24 hours old nutrient broth culture of the test organisms and incubated for 5 days at 35^oC. The content of each

test tube was divided into two portions and labeled MR and VP respectively. Five drops of methyl red solution was added to the test tubes labeled MR and examined for a colour change. A red colour formation in the MR tubes indicated a positive reaction while yellow coloration indicated a negative reaction. To the VP tubes, 0.5ml of 6% naphthol solution was added to each tube and 0.5ml of 16% potassium hydroxide (KOH) solution was also thereafter. The tubes were then shaken and left for 5 minutes. Observation of red coloration indicated a positive reaction (i.e. acetoin production), no change in coloration indicated negative reaction.

2.3.11 UREASE TEST:

Urease basal medium was prepared and sterilized at 121 °C for 15 minutes and cooled to about 45 °C. Urea solution was added to the basal medium to give a final concentration of 2 % urea. The solution derived was dispensed into test tubes. The tubes, while the contents were still warm were slanted and the content was left to cool and set in a slanting position. The test organisms were streaked on the slants and also stabbed into the agar with the aid of sterile inoculating loop and wire loop respectively. The slants were incubated at 37 °C for 24 hours and observed after 24 hours. The production of urease was indicated by the colour change from yellow to pink and represented a positive reaction while no colour change indicated negative results.

3. RESULTS

The administered questionnaires reflected that 48% of the sampled food vendors and 30% of the domestic consumers preferred the use of spoilt tomatoes because they are cheap. It was also observed from their responses that the purchasers were ignorant of the health effect of the spoilt tomatoes and they assumed no health complications could be associated with the spoilt tomatoes due to ignorance. The vendors also revealed that due to low patronage, the duration of time it takes to sell each batch of tomatoes are long.

The bacteria species isolated from spoilt tomatoes were: *Bacillus species*, *Escherichia species*, *Klebsiella species*, *Pseudomonas species*, *Salmonella species* and *Staphylococcus species* while the fungi isolated from the spoilt tomatoes include *Aspergillus sp.*, *Fusarium sp.*, *Mucor sp.*, *Penicillium sp.*, and *Rhizopus sp.*

4. DISCUSSION

The bacteria detected from the cultural, morphological and biochemical tests carried out on isolates recovered from spoilt tomatoes were *Bacillus sp.*, *Escherichia sp.*, *Klebsiella sp.*, *Pseudomonas sp.*, *Salmonella sp.*, and *Staphylococcus sp.* All the organisms recovered which are mentioned above are potential pathogens which has been associated with some infections. *Escherichia sp.* whose strain has been reported to be the leading causes of human food born infections worldwide and fatal complications such as hemolytic uremic syndrome that ends in renal failure (Saeed *et al.*, 2013). The presence of *Escherichia species* and *Salmonella species* in the tomatoes samples could be as a result of application of organic fertilizers that still contained pathogens, the use of faecal contaminated water for irrigation and washing of harvested tomatoes could be a source of introduction of these organisms to the tomatoes. Contamination could also be by handling tomatoes with faecally contaminated hands. *Staphylococcus species* which could be introduced to tomatoes by poor handling after harvesting

Bacillus species which was isolated from spoilt tomatoes during this research was also observed in the research carried out by [1] [9] [11]. *Bacillus species* [11], *Staphylococcus sp.*, *Salmonella sp.* [13] and *Klebsiella sp.* [14] has been reported to be pathogenic when ingested by humans.

Proteus species was absent from all the samples used in the course of this research and this is not in accordance with the work done by [1].

The fungi species (*Mucor sp.*, *Aspergillus sp.*, *Penicillium sp.*, *Cladosporum sp.*, *Fusarium sp.* and *Rhizopus sp.*) isolated from the spoilt tomatoes also have being reported to be pathogenic.

Mucor sp. are reported to be pathogenic when ingested [15] while *Aspergillus sp.*, *Penicillium sp.*, *Fusarium sp.* and *Rhizopus sp.* [16] has been reported be toxigenic fungi which could cause hazard to the health of humans if ingested.

5. CONCLUSION AND RECOMMENDATION.

There should be awareness of the adverse effect of spoiled tomatoes to the health of humans. Though spoiled tomatoes are cheap but they are very dangerous to the human health. Spoiled tomato contains potential pathogens and these pathogens if ingested could cause infections or death to humans. Tomato vendors should be advised to stock a limited amount of tomatoes due to the fact that tomatoes are highly perishable foods because of the high moisture content which predispose them to microbial proliferation. Organic fertilizers which are used for planting tomatoes should also be screened for faecal pollutants to prevent contamination of tomatoes fruits by contaminated organic fertilizers. Water from streams, rivers or lakes that are used for irrigation of tomato plants should as well be free from pathogens. Raw consumption of tomatoes should be discouraged, tomatoes should be subjected to heat especially when stew is being prepared, the tomatoes should be properly boiled. Vendors should ensure proper washing of their hands when handling tomatoes especially after visiting the toilet to avoid fecal contamination of tomatoes. The bowls used to display and store tomatoes should also be washed regularly since it could be a reservoir for microorganisms. This study has revealed that contaminated tomatoes could be potentially pathogenic, hence, proper hygiene by tomato vendors and non-consumption of spoiled tomatoes will go a long way in preventing Food-borne illnesses associated with Tomatoes.

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