Original Research Article

MICROBIAL SPOILERS OF ORANGE FRUITS FROM VARIOUS POINTS OF FOOD SUPPLY CHAIN IN OWERRI, NIGERIA.

Comment [NBA1]: So this should include collecting samples from the farm, shipping cargos, boxing facilities and the market too ?

ABSTRACT

The effect of room temperature storage on the microbial contents of oranges marketed in Owerri was investigated. The major cause of loss in perishable produce example of orange fruit stored at room temperature after harvest include certain pathogenic fungi (*Aspergilusniger*, *Rhizopus sp. Trichophytonsp*, *Fusiform* and *Canditatropicalis*)and Bacteria (*Staphylococcus aureus*, *Erwiniasp*and *Bacillus cereus*). A total of 24 orange fruit samples 6 each were purchased randomly fresh and strong from 4 different markets and were monitored and processed for a period of 11 days on sabourauddextoose agar, Nutrient agar and MacConkey broth by poor plate method and multiple dilution method for most probable number of coliform. The result of the study showed that Relief market's orange fruit sample has the highest number of bacteria 20% (174) bacteria counts and 74.4% (646) yeasts counts 5.5% (48) mould counts) with *Bacillus cereus* persisting followed by Ekemegbu markets orange fruit sample with 21.5% (127) bacteria count, 71.5% (423) yeast counts and 7.1% (42) mould counts with *Bacillus cereus* still persisting, then Ekonuwa 24.4% (120) bacteria counts, 64.8% (319) yeast counts and 10.8% (53) mould count and lastly Akwakuma markets orange fruit sample with 8.1% (53) bacteria counts 82.6% (541) yeast counts and 9.3% (61) mould count *Bacillus cereus* while the other bacteria organisms such as *Staphylococcus aureus and Erwinia*sp disappear with (*Candida tropicallis)* increasing in number virtually in all the market's orange fruits sample, and this is due to comparatively lower pH in orange juice as the juice so show a noticeable decline towards acidity during spoilage. *Bacillus* was present till the last day of observation (11th day) because they can survive, grow and sporulate despite changes in water activity, pH and temperature.

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Keywords: Citrus, food contaminants, fungi, bacteria, food microbes

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INTRODUCTION

Orange trees are widely grown in tropical and subtropical climates for its sweet fruit, which can be eaten fresh or processed to obtain juice, and for its fragment peel (Citrus sinensis information). They have been the most cultivated tree fruit in the world since 1987. Sweet oranges account for approximately 70% of the Citrus production (organism's citrus genome). In 2010, 68.3 million tons of the citrus (orange) were grown world-wide, particularly in Brazil and in the United States where California had the highest production of oranges in 2010 and Florida states which produced the most of popular kids' food [1].

*Citrus sinensis*Osbeckis specifically, the sweet orange of the citrus species in the family *Rutaceae Citrus sinensis*Osbeck. The fruit of the *Citrus sinensis* is called sweet orange to distinguish it from that of the *Citrus auantium* the bitter orange. The orange is a hybrid possibly between Pomelo (*Citrus Maxima*) and Mandarin (*Citrus reticulate*), cultivated since ancient times [2]. Probably originated in South East Asia, orange was already cultivated in China as far back as 2500 B.C. Between the late 15^{th} century and the beginning of 16^{th} century, Italian and Portuguese merchants brought oranges trees in the Mediterranean areas [1]. The Spanish introduced the sweet orange to the American continent in the mid-1500s. All citrus trees belong to the single genus citrus and remain almost entirely interceptive. This means that there is only one super species that includes grape fruits, lemon, limes, oranges and various others types and hybrids. The fruits of any citrus tree are considered hesperidia (a kind of modified berry). This is because it is fleshy and soft and has numerous seeds, derive from a single ovary wall [3].

Although the sweet orange present different sizes and shapes varying from spherical to oblong. It generally has 10 segments (carpsels) inside, contains up to 6 seeds (or pips) and a porous white

tissue-called pith or more properly, mesocarp or albedo lines its rind. When unripe the fruit is green. The grainy irregular rind of the ripe fruit can range from bright orange to yellow-orange, but frequently retains green patches or, under warm climate conditions, remains entirely green. Like all other citrus fruits, the sweet orange is non climacteric. The *citrus sinensis* is subdivided into four classes with distinct characteristics: common oranges, blood or pigmented oranges, navel oranges and acidless oranges [4, 5].

Other citrus species also known as oranges are bitter orange *(citrus aurantium),* the bergamot orange (*citrus bargain*) trifoliate orange (*poncinus trifoliate*) and the mandarin orange (*citrus reticulate).*The common orange, also called "white", "round" or "bond" oranges constitute about 2/3 of all the orange production. The majority of their crop is used mostly for juice extraction for home fruit production oranges [4].

The naval oranges: They are characterized by the growth of a second fruit at the apex, which protrudes slightly and resembles a human naval. They are primarily grown for human consumption for various reasons. Their thicker skin make them easier to peel, they are less juicy and their bitterness is as result of the high concentration of limonin and which renders them less suitable for juice [4].

The blood oranges: they are a natural mutation of the *Citrussinensis*although today the majority of them are hybrids. High concentration of anthocyacin gives the rind, flesh and juice of the fruit their characteristics dark colors. The blood orange, with its distinct color and flavor is generally considered the most delicious juice orange and has found a niche as an ingredient variation intraditional serille marmalade [4].

The acid-less oranges: They are early season fruit with very low levels of acid and rather insipid. They are also called “sweet” orange in the U.S.A with similar names in other countries. Orange fruit have serious challenges to their existence which may be changes in climatic condition, pest, inadequate rain fall and microbial spoiler example bacteria (*Erwiniacarotora, Bacillus* spp *and sometimes Clostridium),* and majorly certain pathogenic fungi: *Aspergilusniger, Candida tropicalis*, species of P*enicillum, Rhizopus* and *Geotricihumcandidum*), viruses and nematodes play a minor role in post-harvest losses; rodents and insect are also generally of lesser importance in contrast to the significant damage they cause in food grains.The lack of acid, which protects orange juice against spoilage in the other groups, renders them generally unfit for processing, so that they primarily eaten. They remain profitable in areas of local consumption, but rapid spoilage renders them unsuitable for export to major centre [4].

Microbial spoilers such as the bacteria (*Erwiniacarotovora, Bacilus*sppand sometimes *Clostridium* and majority certain pathogenic fungi such as *Aspergilusniger, Candida tropicalis, Peniciliumcitinum, Rhizopus*sp,*Georichumcandidum*cause orange fruit spoilage following contamination through diverse ways which include poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation render orange fruit unattractive for consumption. Therefore, the knowledge of microbial spoilers of orange fruit is imperative and therefore goes along way to note the potential spoilers of orange fruit sold in Owerri and thus proffer preventive measures. An unfavourable experience by individual consumers may lead to loss of confidence in the quality of orange fruit sold in Owerri and therefore lead to a changein sources and subsequent economic loss to orange fruit producers and sellers.Bacteria species such as *Erwiniacarotovora, Bacilius*, sometimes *Clostridium* and major certain pathogenic fungi like *Aspergillum niger, Candida tropicalis,*

*Penicilliumcitrinum,Rhizopus and Geotriciumcandidum*cause spoilage of orange fruit, thereby making them unattractive to consumers.

2.0. MATERIALS AND METHODS

Sample Collection

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The samples used in this study were orange fruits total to 24 orange fruits samples were collected for the study, six each were randomly purchased from the four different markets mention in each market three sellers were randomly selected from whom oranges were purchased. They were differently packaged according to the different markets/sellers and sent to the microbiology laboratory for analysis.

Sample Processing

A total of eight oranges were processed on the day of purchase, two each from the four markets. The orange were stored at room temperature for eleven days (1 week and 3 days) starting from the first day of purchased.The orange juice was aseptically extracted out into a sterilized beaker differently according to the market form where they were purchased. Four Petri dishes each was used for both Sabouraud dextrose agar and nutrient agar each two Petri dishes each containing different m/s of the oranges juice. Prior to dispensing 0.2mLs of chloramphenicol solution was added into the Sabouraud agar inhibit bacterial growth and allowing only fungal growth. MacConkey broth was used to carry out multiple tube method for the estimation of most probable number of coli form present in the orange fruit juice. The orange fruit juice was mixed

and aseptically inoculated into already sterilized MacConkey broth of different strength (single strength and double strength) in test tubes containing Durhams tube as follows:

1n test tube x 50mls of broth + 50mls of orange fruit juice (double strength).

5 test tubes x 10mls of broth + 10mls of orange fruit juice (double strength).

5 test tubes x 10mls of broth + 1mls of orange juice (single strength).

The content of each test tube was mixed and all incubated at 37°C for 24hours with tubes loosely capped. All tubes showing aid (colour change to yellow) and gas (bubble in the durhams tubes) were regarded as presumptive positive. The negative tubes were re-incubated for further 24hours. Using probability tables, the most probable number of presumptive coliform bacilli per 105mls of the oranges fruit juice was determined.Colonies from mixed growth in nutrient and sabouraud agar were purified by sub-culturing on nutrient and sabouraud agar plates and incubated at 37^{o}C overnight.

Isolation and Identification of organisms

Plates were observed for characteristics bacteria colonies of the basis of shape, colour, pigmentation, consistency, elevation etc. typical colonies were subculture into a new agar plate to obtain pure culture of the required organism and then transferred into a slant of nutrient agar incubated for 24 hours for further identification by Gram staining technique, Catalase test, The coagulase test (bound coagulase), Indole test Germ tube test

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3.0. RESULTS AND ANALYSIS

A total of 24 orange fruit sample were randomly purchased from different markets in Owerri municipal (Ekemegbu, Akwakuma, Ekeonuwa and relief markets) were observed and processed for the total period of 11 days aim of determing microbial loads and ascertain any change in the microbial content as well as the most probable number of coliform count which will be reflected at the appendix isolated from orange fruit samples purchased form the different markets as well as the specific isolates on storage at room. Using the data collected and standardized for bacteria attack or orange fruits form Ekemegbu market, the graph of the log10 of the mean of the total count against time (t) suggest a function graph with two cures suggesting a function to the 3^{rd} degree in almost all the sample markets (Akwakuma Market, Ekeonunwa market and Relief market) in Owerri Municipal implying that TC is directly proportional to time ($TC \propto t$)

$TC = at^3 + bt^2 + ct + dt^0$. The above function will be established when the constants a, b, c, and d are obtained. Where t = time (0 days, 4 days, 7 days and 11 days) until the last day of observation.

Using Ekemegbu market's pattern of spoilage of orange fruit sample to establish my observations which is almost similar in all other markets pattern of spoilage of orange fruit sample.

Table 1: Specific bacteria isolate count

	ISOLATE	TOTAL COUNT	PERCENTAGE
EKEMEGBU	*Bacillus* sp	46	36%
	Staphylococcus aureus	44	35%

	Erwinia sp	37	29%
AKWAKUMA	*Bacillus* sp	23	40%
	Staphylococcus aureus	21	39%
	Erwinia sp	16	30%
EKEONUNWA	*Bacillus* sp	44	37%
	Staphylococcus aureus	42	35%
	Erwinia sp	34	28%
RELIEF	*Bacillus* sp	62	35%
	Staphylococcus aureus	60	35%
	Erwinia sp	52	30%

Table 2: Specific fungal organisms isolated from each of the markets, their counts and the percentage

	ISOLATE	TOTAL COUNT	PERCENTAGE
EKEMEGBU	*Candida tropicalis*	423	92.8%
	Apergilusniger	13	31%
	Rhizopus	12	29%
	Trichophyton sp	8	19%
	Fusiform	9	21%
AKWAKUMA	*Candida tropicalis*	541	89.9%
	Apergilusniger	18	30%
	Rhizopus	17	28%

	Trichophyton sp	16	26%
	Fusiform	10	16%
EKEONUNWA	*Candida tropicalis*	319	85.6%
	Apergilusniger	16	30%
	Rhizopus	14	26%
	Trichophyton sp	13	25%
	Fusiform	10	19%
RELIEF	*Candida tropicalis*	640	93.0%
	Apergilusniger	14	29%
	Rhizopus	13	27%
	Trichophyton sp	11	13%
	Fusiform	10	21%

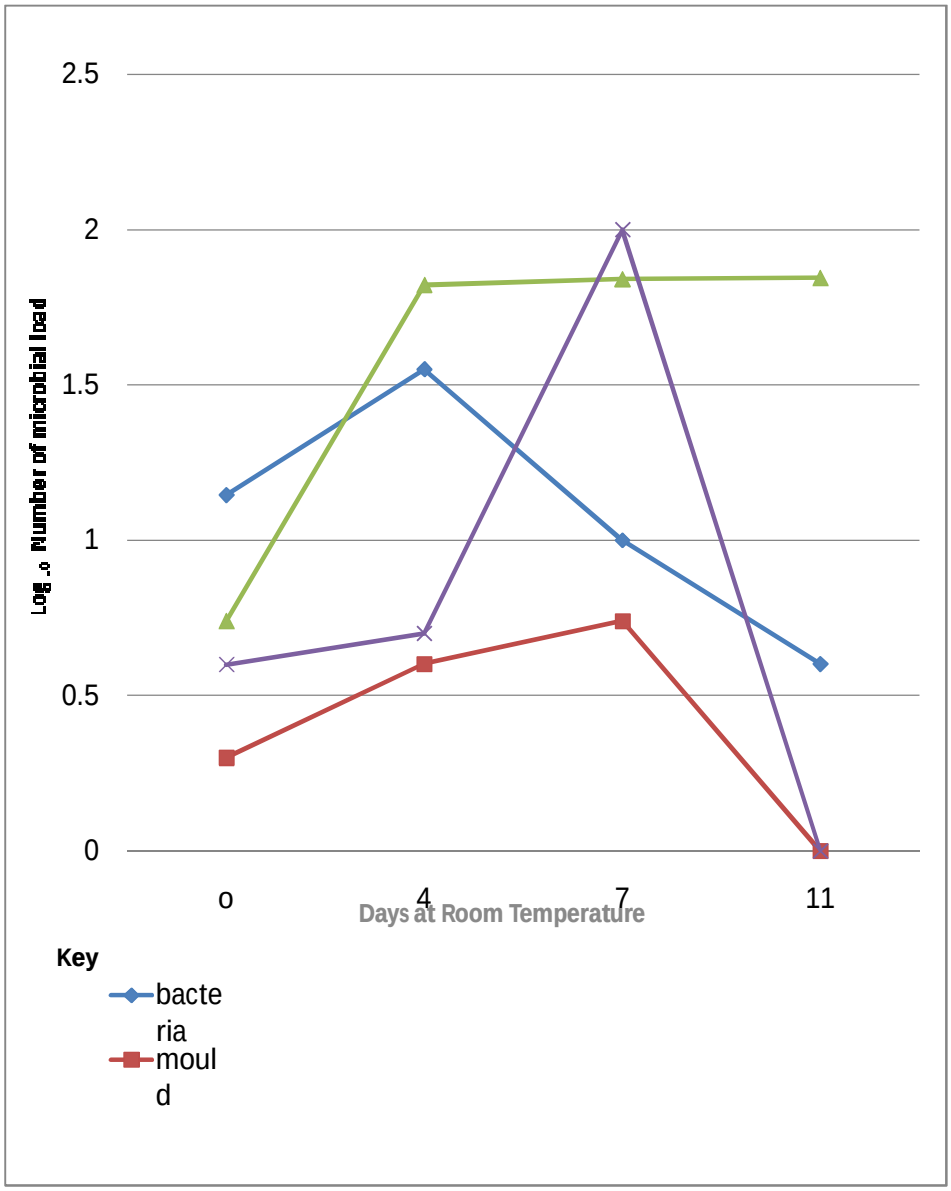

Figure 1: Graphical representation of log10 microbial load of orange from Ekemegbu Market at Room temperature for 11 days.Coliform MPN x10

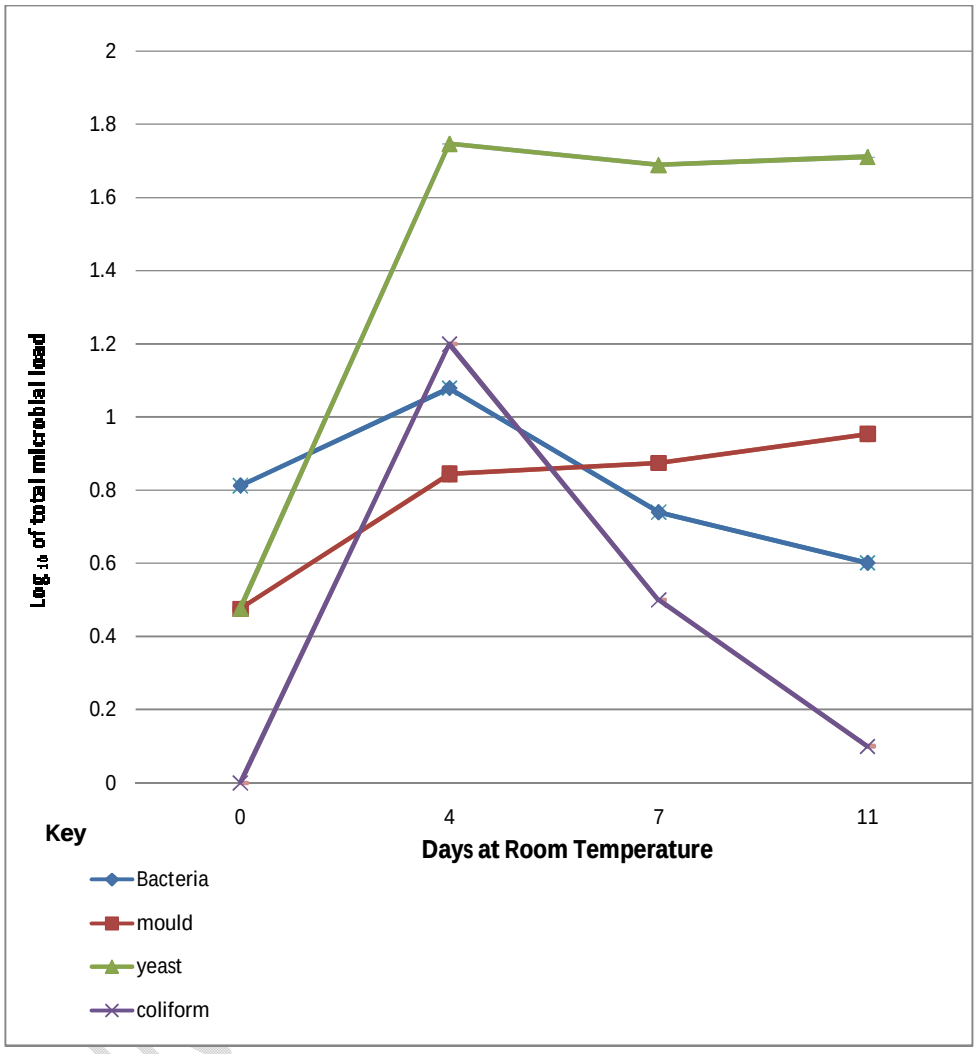

Figure 2: Graphical representation of log10 microbial load 0f Orange from Akwakuma Market at Room temperature for 11 day. Coliform MPN x10

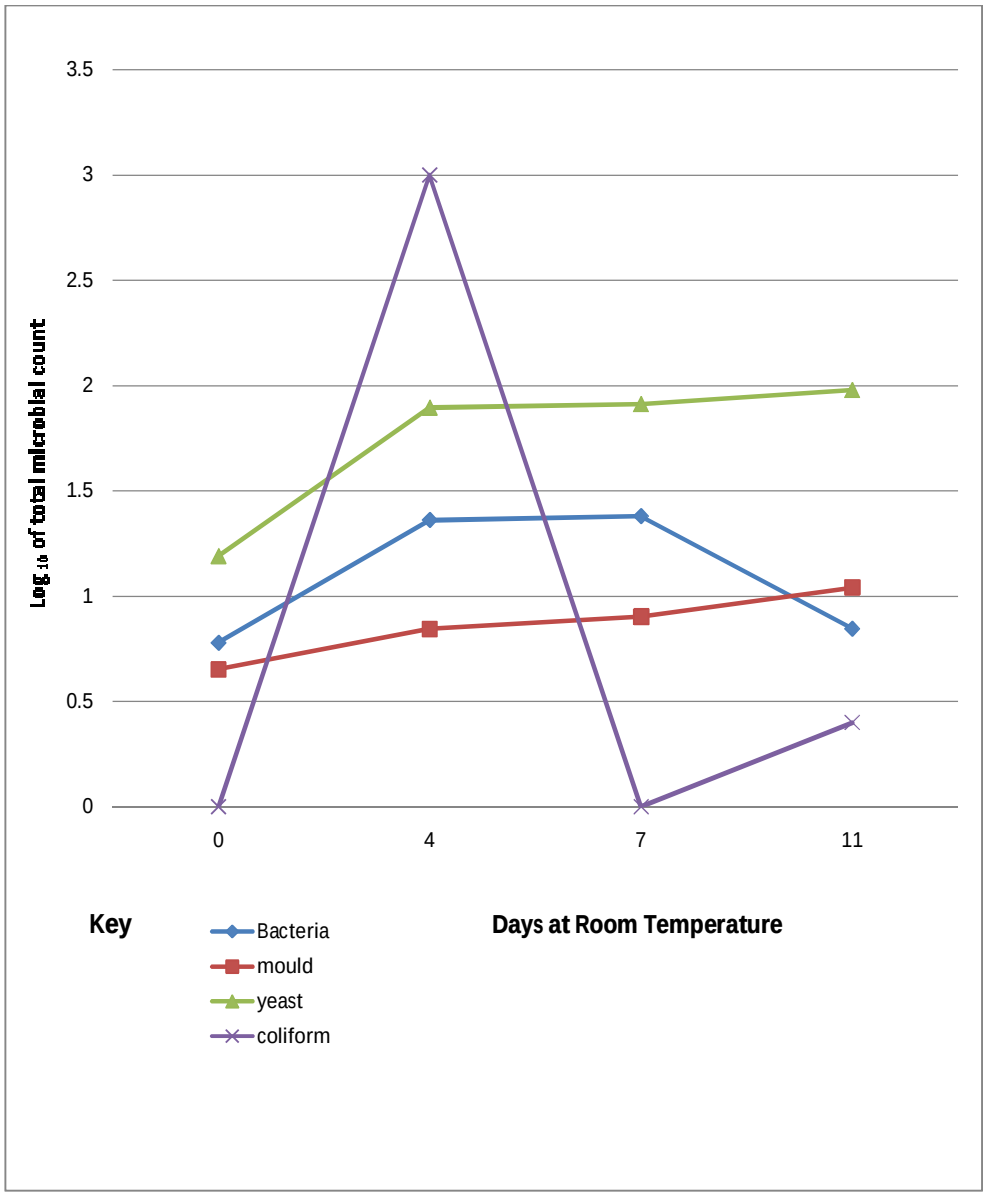

Figure 3: Graphical representation of microbial count of orange from Ekeonunwa Market at room temperature for 11 days.Coliform MPN x10

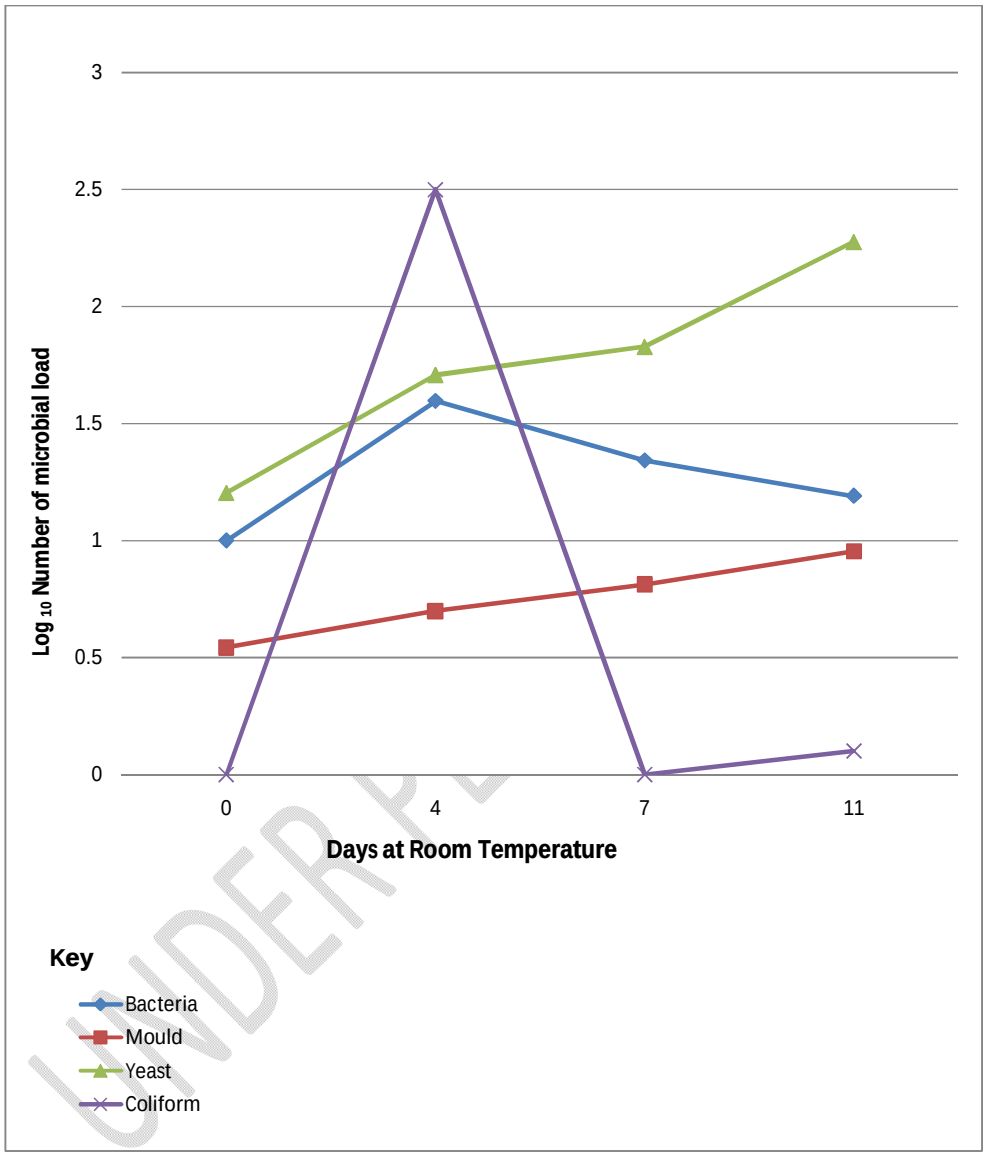

Figure 4: Graphical representation of log_{10} microbial load of orange from Relief Market at room temperature for 11 days. Coliform MPN x10

Day

4.0. DISCUSSION

The roots of the function (when TC = 0) gives t = 1.13 days and 9.21 days implying that the bacteria attack of oranges lasts for about 10.34 days, the sample oranges were attacked about 1.13 days before the observation within which period the growth showed a total mean count of 14 (i.e. for t =0, tc =14) and the TC reached peak after 4 days of the attack. Then a gradual slowdown of the attack which leads to almost a total disappearance of bacterial after about 7 days from the peak time.This might be due to some chemical changes within this period in the orange which militated against further survival and growth of bacteria within the orange or it might be due to the effective life span of bacteria within the resultants more acidic medium of the orange fruit.

It was also observed that from the four different markets in Owerri municipal, relief market has the highest number of viable bacteria count with 174 bacteria counts followed by Ekemegbu market with 127 bacteria count then Ekeonuwa market with 53 bacteria counts. Most strikingly *Baciliuss*p was present in all the markets orange fruit samples with the highest number compared to the other microorganisms such as *Staphylococcus aureus* and *Erwinia*sp and was persistent from the 4^{th} day until the last day of observation 11^{th} day alongside *Candida tropicalis* which outnumbered the bacteria and mould counts. This may be due to increase in acid content of the groups which increased yeast count (*Candida tropicalis*) over bacteria. This is in line with the reports of Mandoza*et al*., [6], Suaad and Eman, [7], and Correa de souza*et al.*, [8], who stated in their work that orange fruit juice showed the most significant increase in total yeast count over time perhaps due to continued increase in acidity (5.03-4-39), over the period of storage as yeast thrive well in acidic medium.

The presence of these microorganisms in orange fruit pose serious health challenges to consumers especially those orange fruit stored for a long number of days. Such health challenges are; food poisoning with symptoms of vomiting and diarrhea within or up to 24 hours of intake, pneumonia, broncho-pneumonia and wound infections although these conditions are not common.

Conclusion

The results of this study also suggest that suing the bacteria attack incidence, the duration of orange, its validity, viability and mode of storage can be ascertained.Secondly, that orange fruit can be a good and quick source of raw material for bacteria culture in laboratories and industries especially as the study has indicated its pressure and maximum yield period in orange fruit.Conclusively also, knowing the serious health challenges posed by the consumption of orange fruit stored for a long time, orange fruit that are yellow, that is, properly ripped should not be stored for long, in fact, should be taken right away more especially those plucked immediately from the orange tree, and for those that are not yellow outwardly, but are ripped should not be stored for a long time to avoid taking unhealthy orange fruit which may look healthy outwardly, but harbour microorganisms inside. Thus getting infected in place of vitamin C, for which purpose one takes it.Orange fruits that are not preserved by using certain techniques such as waxing, refrigerating, etc., should not be stored for more than 1 week from the day of harvest and for those purchased from the market they should not be stored up to 1 week, especially those that are usually yellow in colour. This is to ensure that the purpose of consumption is maximally achieved.

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