Original Research Article

MICROBIAL SPOILERS OF ORANGE FRUITS FROM VARIOUS MARKETSIN OWERRI, NIGERIA.

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 producessuch as the orange fruit include certain pathogenic fungi (*Aspergilusniger, Rhizopus*sp.*Trichophyton*sp, *Fusiform* and *Candidatropicalis*)and Bacteria (*Staphylococcus aureus, Erwinia*spand *Bacillus cereus*). A total of 24 fresh orange fruit samples were randomly purchased from four (4) different markets in Owerri (six oranges from each). They were processed and inoculated ontosabourauddextoose agar, Nutrient agar and MacConkey agar by pour plate method. Multiple dilution method was also done for most probable number of coliforms. The result of the study showed that Relief market's orange fruit sample has the highest number of bacterial counts[20% (174)]. Its yeast and mould counts were 74.4% (646) and 5.5% (48), respectively. This was followed by Ekemegbu market's orange fruit samples with 21.5% (127) bacteria count, 71.5% (423) yeast count and 7.1% (42) mould count. Then Ekeonuwawith 24.4% (120) bacteria count, 64.8% (319) yeast count, and 10.8% (53) mould count. Following this was the Akwakuma market's orange fruit samples with 8.1% (53) bacteria count, 82.6% (541) yeast count, and 9.3% (61) mould count. While the other bacteria organisms such as *Staphylococcus aureus and Erwinia*spgradually disappeared, *Bacillus cereus* with *Candida tropicallis*increased in number in all the markets' orange fruit samples.This is due to the comparatively lower pH in orange juice since the juice usually show a noticeable decline towards acidity during spoilage. *Bacillus* was present till the last day of observation (11^{th} day) because they can survive, grow and sporulate despite changes in water activity, pH and temperature.

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

INTRODUCTION

Food losses are a major concern worldwide especially with an ever-growing world population and the fact that approximately one-third of all food produced for human consumption is either lost or wasted [**1]**. The reasons for this massive global food loss are diverse, but microbial spoilage, which affects organoleptic product quality (aspect, texture, taste, and aroma), plays a major role. Among spoilage microorganisms, fungi are a major issue at any stage of the food chain because of their ability to grow in different and even harsh environments [2]Literature datarevealed that about 20 to 30% of vegetables and fruits were lost every year due to post harvest diseasespoilage[3, 4]. Orange is susceptible to a large number of diseases and is known to be the cause of huge economic loss.Orange trees are widely grown in tropical and subtropical climates for its sweet fruit, which can be eaten fresh or processed to obtain juice. They have been the most cultivated tree fruit in the world since 1987. Sweet oranges account for approximately 70% of the Citrus production. In 2010, 68.3 million tons of citrus (orange) were grown world-wide, particularly in Brazil and in the United States [5].

*Citrussinensis*Osbeckin the family *Rutaceae Citrussinensis*Osbeck is actually the sweet orange. 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 [6]. All citrus trees belong to the single genus Citrus and remain almost entirely interceptive. This means that there is only one super specie 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, derived from a single ovary wall [7].

Although the sweet orange presents with different sizes and shapes, varying from spherical to oblong, it generally has 10 segments (carpsels) inside, and contains up to six seeds (or pips), as well as a porous white tissue-called pith or more properly, mesocarp or albedo lines its rind.The fruit is green when it is not ripe. 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. They are: common oranges, blood or pigmented oranges, navel oranges and acidless oranges [8, 9].

Microbial spoilers such as the bacteria, *Erwiniacarotovora, Bacillus*sppand sometimes *Clostridium*, and certain pathogenic fungi such as *Aspergilusniger, Candida tropicalis, Peniciliumcitinum, Rhizopus*sp,*Geotrichumcandidum*cause orange fruit spoilage. They do this through diverse ways that include poor handling practices, storage conditions, distribution, marketing practices and transportation. These in most casesmake orange fruit unhealthy for consumption. Due to this, the knowledge of the microbial spoilers of orange fruits sold in Owerri is imperative as it would aidthe adoption of the necessary preventive measures. This would also help mitigate economic loss due to the purchase of spoilt orange fruits.

2.0. MATERIALS AND METHODS

Sample Collection

A total of 24 orange fruit samples were collected for this study. From each of the four markets, six oranges were purchased. These samples were packaged separately according to their market source, appropriately labelled, and sent to the microbiology laboratory for analysis.

Sample Processing

The orange were stored at room temperature for eleven days till they were needed for processing. According to their market sources, the orange juices were aseptically extracted into different sterile beakers and properly labelled. For each of the samples, four petri dishes (two Sabourad dextrose agar and two nutrient agar) were used.Pour plate was used for this analysis. Prior to dispensing the orange juices, 0.2mLs of chloramphenicol solution was added into the Sabouraud agar inhibit bacterial growth and allowing only fungal growth. MacConkey broth was then used to carry out multiple tube method for the estimation of most probable number of coliforms 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:

1 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, the tubes loosely capped, and all incubated at 37°C for 24hours. All tubes showing acid (those that their colours changed to yellow) and gas (as indicated by bubbles in the durham's 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 10 mL 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 based on shape, colour, pigmentation, consistency, elevation, and others.Typical colonies were subcultureonto a new agar plate to obtain pure culture of the required organism and then transferred into a slant of nutrient agar and incubated for 24 hours for further identification. Gram reaction, Catalase, coagulase test (bound coagulase), Indole, andGerm tube tests were employed in the identification.

3.0. RESULTS AND ANALYSIS

Using the data collected and standardized for bacterial contamination of orange fruits from Ekemegbu market, the graph of the log10 of the mean of the total count against time (t) suggests a function graph with two curves suggesting a function to the 3rd degree in almost all the sample markets (Akwakuma Market, Ekeonunwa market and Relief market) in Owerri Municipal. This implies that TC is directly proportional to time ($TC \propto t$)

$TC = at^3 + bt^2 + ct + dt^0$. This 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.

The pattern of orange fruits spoilage is almost similar in all the markets.

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%

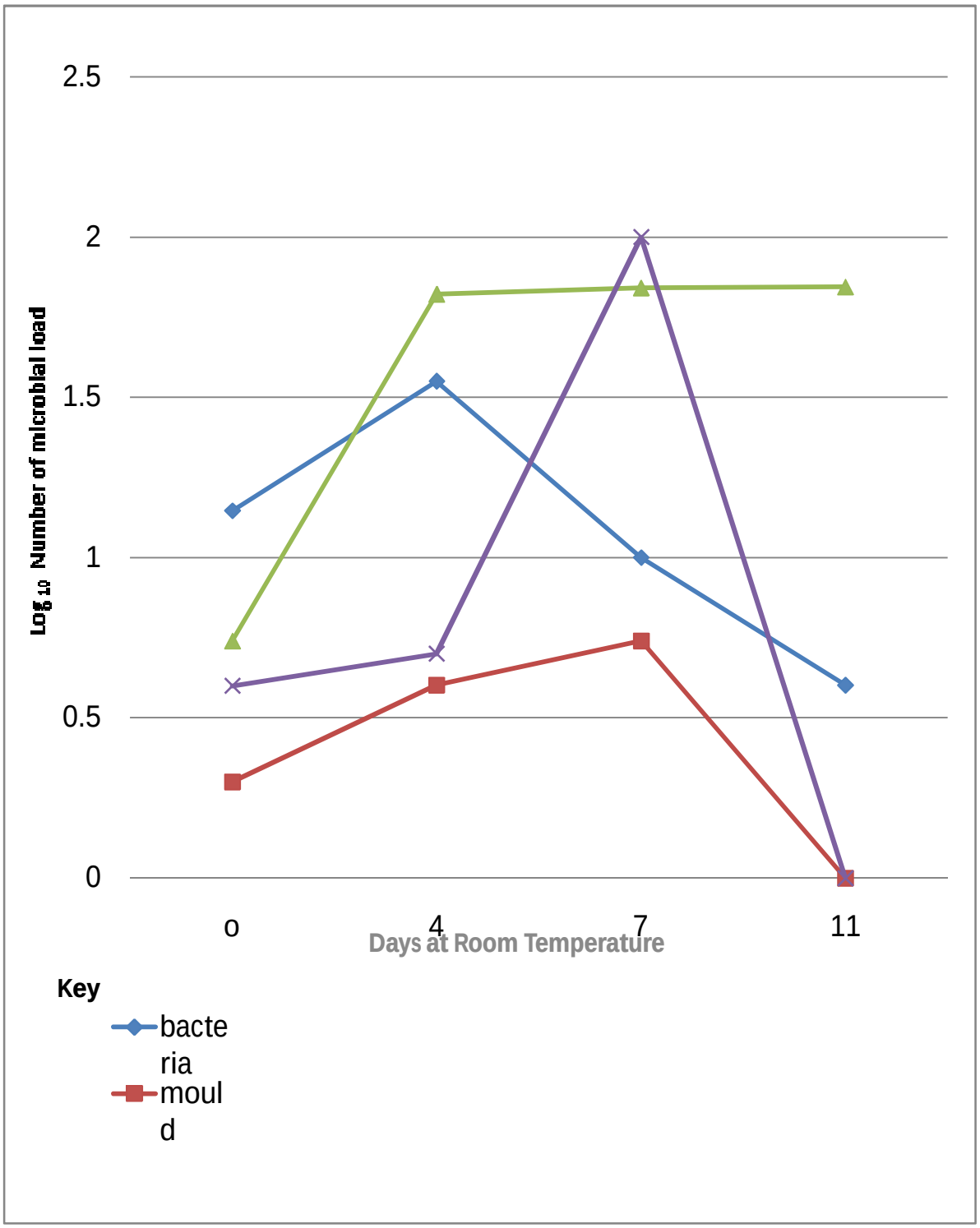

Figure 1: Graphical representation of log10 microbial load of orange from Ekemegbu Market at Room temperature for 11 days.Coliform MPN x10

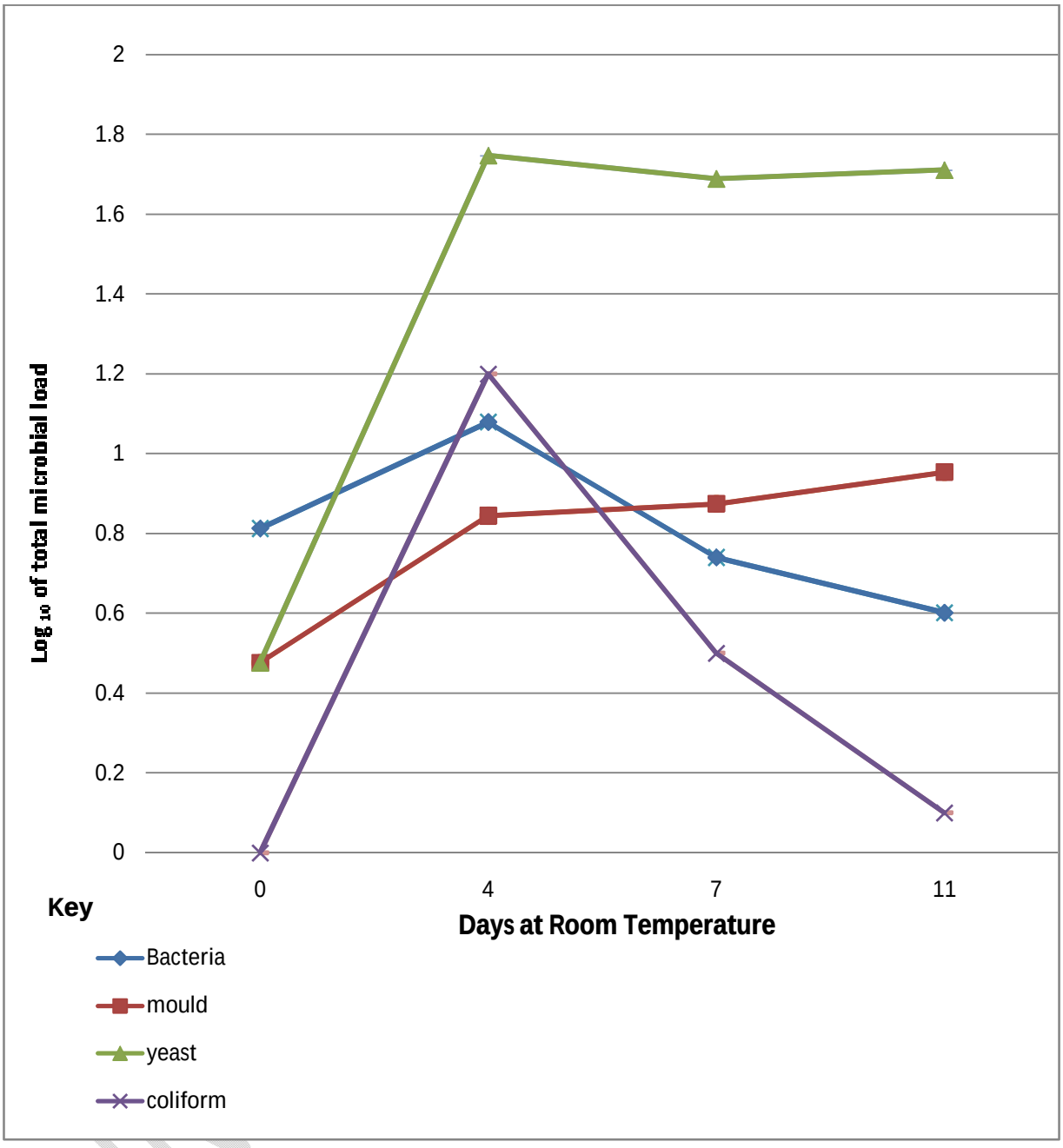

Figure 2: Graphical representation of log10 microbial load 0f Orange from Akwakuma Market at Room temperature for 11 day. Coliform MPN x10

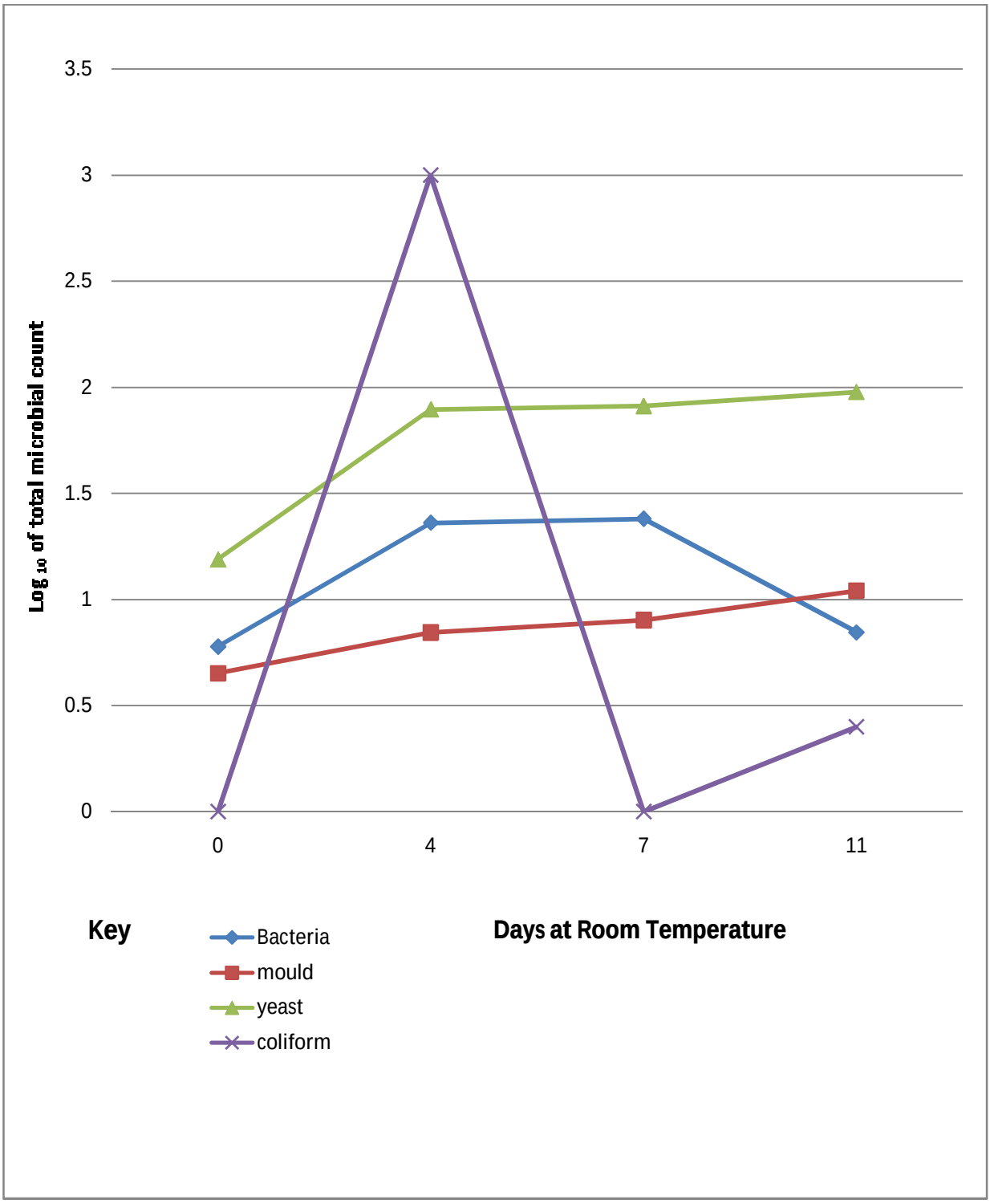

Figure 3: Graphical representation of microbial count of orange from Ekeonunwa Market at room temperature for 11 days.Coliform MPN x10

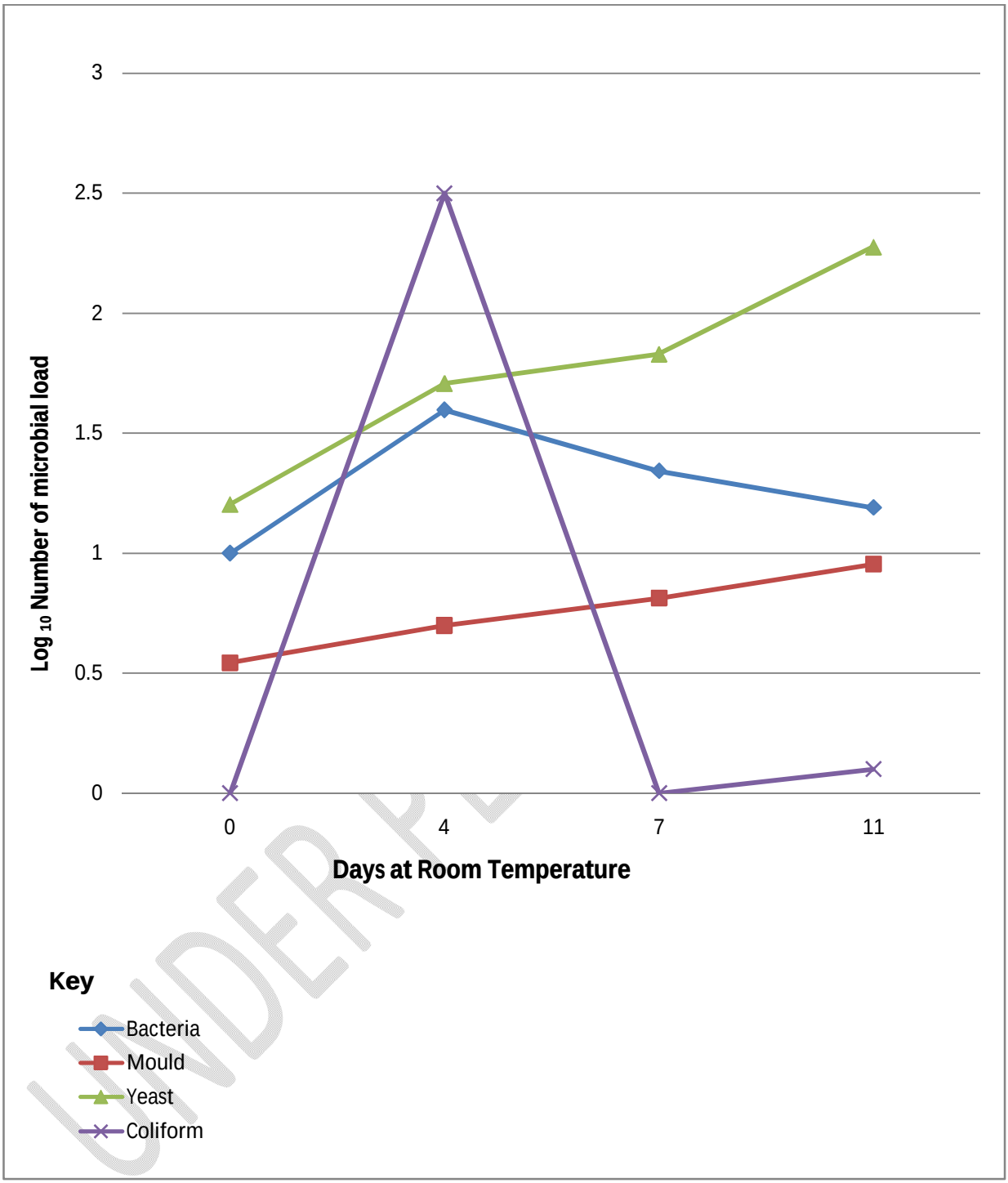

Figure 4: Graphical representation of log_{10} microbial load of orange from Relief Market at room temperature for 11 days. Coliform MPN x10

4.0. DISCUSSION

The roots of the function (when TC = 0) gives t = 1.13 days and 9.21 days. This implies that the bacterial contamination of oranges lasts for about 10.34 days.The samples became infected about 1.13 days before the observation. Within this 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 infection. This was followed by a gradual slowdownwhich led to almost a total disappearance of the bacteria 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 had the highest number of viable bacteria count with 174 bacterial count. This was followed by Ekemegbu market with 127 bacterial count, and then Ekeonuwa market with 53 bacterialcount. Strikingly *Baciliussp* was present in all the markets' orange fruit samplesand had the highest number when compared with other bacterial species such as *Staphylococcus aureus* and *Erwinia*sp. It was also 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 orangeswhich increased yeast count (*Candida tropicalis*) over bacteria. This is in line with the reports of Mandoza*et al.*, [10],Suaad and Eman, [11],Correa de souza*et al.*, [12] and Tournas et *al.*, [13], 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 yeasts thrive well in acidic medium. The isolation of Aspillusniger corroboratesthe report by Rashad *et al.*,[14].

The presence of these microorganisms in orange fruit pose serious health challenges to consumers especially those orange fruits stored for a long period of time. Such health challenges include food poisoning, pneumonia, and wound infections. Although, these conditions are not common.

Conclusion

The results of this study also suggest that an 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, ripe orange fruits should not be stored for long.In fact,this should be consumed immediately.For the poorly ripped ones, the storage time should be shortened.This would help avoid the consumption of unhealthy orange fruits which might look healthy outwardly, but harbour microorganisms inside. Thus, one gets infected with microorganisms instead of being nourished with vitamin C for which humans consume oranges.Fruits not preserved by techniques such as waxing, refrigerating, and others, 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 much ripped. This is to ensure that the purpose of consumption is maximally achieved.

REFERENCES

[1] Gustavsson J, Cederberg C, Sonesson U. Global Food Losses and Food Waste: Extent, Causes and Prevention; Food and Agriculture Organization of the United Nations: Rome, Italy, 2011.

[2] Pitt JI. Hocking AD. Fungi and Food Spoilage; Springer: Boston, MA, USA, 2009.

[3] AgriosGN. Plant Pathol., Academic Press, New York, pp. 922, 2005.

[4] Barth H, Hankinson M, Zhuang TR,Breidt H. “Microbiological spoilage of fruits and vegetables”,Compendium of the microbiological spoilage of foods and beverages, food microbiology and food safety.2009; 135-183.

[5] Morton J. Fruits of Warm Climate. Miami, Florida. 1987.

[6] Nicolosi E, Deng ZN, Gentile A, Lamalta S, Continella G, Tribulato E. “Citrus Phylogeny and genetic origin of important species as investigated by molecular markers’ TAG. Theoretical and applied genetic. 2000, 100(8): 1155-1166.

[7] Bailey H, Bailey E.Hortus Third. Cornell University Macmillan.N.Y. 1976.

[8] Kimball, DA. Citrus processing: A complete Guide (2nd ed).New York: Springer. 1999.

[9] Webber I,Herbt J: rev Waller Reuther and harry W. Lawton, willandifodgson. The citrus industry, art cultural varieties of citrus. Riverside (A: University of California Division of agricultural sciences). 1943.

[10] Mandoza SL, Montemagon LA, Boscan J, BarriereoJA. Microflora in Pasteurized Fruit Juice in Venezuela. Arch LatinoamNutr. 1982; 32:617-629.

[11] Suaad SA,Eman AHA. Microbial growth and chemical analysis of mineral content in bottled fruits juice and drinks in Riyadh, Saudi Arabia Res. J., Microbial. 2008; 3:319-325.

[12] Correa de Souza MC, BanassiMT, Meneghei FA. Stability of unpasteurized and refrigerated Orange Juice Braz. Arch Bio technol. 2004; 47:391-397.

[13] Tournas VH. Moulds and Yeasts in Fresh and Minimally Processed Vegetables and Sprouts. International Journal of Food Microbiology. 2005; 99: 71-77.

[14] Rashad A, Ahmed RA, Saleh AM. “Isolation and identification of some fruit spoilagefungi:screening of plant cell wall degrading enzymes”, African J. of Micro. 2011; 5(4): 443-448.