ISSN: 2455-3751

Contents lists available at http://www.albertscience.com

ASIO Journal of Microbiology, Food Science & Biotechnological Innovations (ASIO-JMFSBI)

Volume 6, Issue 1, 2021, 09-15

MATHEMATICAL MODELING OF THE GROWTH OF SPECIFIC SPOILAGE MICRO-ORGANISMS IN TILAPIA (*OREOCHROMIS NILOTICUS*) FISH

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ARTICLE INFO

Article History

Received: 7th December, 2021
Accepted: 24th December, 2021

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ABSTRACT

This study examined the growth rate of specific spoilage organisms (SSOs) of tilapia (Oreochromis niloticus) fish and the effect of varying temperature (0, -2 and -4°C) and storage time (24, 48, 72 hours). The growth of SSOs was also modeled using Gompertz function and Linear Regression Models. The results of this study demonstrated that Pseudomonas species, Staphylococcus species, E. coli, Shigella species, Salmonella species, Klebsiella species, Penicillium species, Yeast, Fusarium species, Phytophthora species and Aspergillus species were observed to be SSOs of Oreochromis niloticus fish. They reduce the lifespan of O. niloticus fish at 0, -2 and -4°C temperatures in 24, 48 and 72 hours storage times. On the number of isolated colonies of the microbial growth in tilapia fish -4°C temperature isolates such as Salmonella (2.2×10^8CFU/cm^2), Pseudomonas (2.15×10^8CFU/cm^2) and Staphylococcus (1.8×10^8CFU/cm^2) were observed to be the highest. The experimental data was modeled using the Gompertz modified equation for both bacteria and fungi counts with different temperatures of the isolates in cfu/ml of the samples. The derived parameters of the Gompertz model such as specific growth rate (μ) was observed to increase at temperatures of -4° C with decreasing maximum population density (MPD) and Lag phase duration (LPD) time of the specific spoilage organisms. The test organism's results in tilapia fish at 0, -2 and -4°C temperatures were modeled by the linear regression model (R^2 values were 0.8, 1.0 and 0.5 respectively), showing a strong positive linear relationship. Temperature and time has been established as the two very significant factors that affect the growth of specific spoilage organisms in foods. Hence, modeling the growth of SSO, considering temperature and time enables scientist to predict the possible growth of SSO accurately during processing and storage of tilapia fish.

Keywords: *Oreochromis niloticus fish, Mathematical Models, Specific spoilage organisms, Temperature, Storage time.*

How to cite the article?

Odangowei I. Ogidi, Eruom E. Charles, Chioma C. Okore, Beatrice Wilfred, Lilian M.O.Oguoma, Henshaw E. Carbom, Mathematical modeling of the growth of specific spoilage micro-organisms in tilapia (*oreochromis niloticus*) fish, *ASIO Journal of Microbiology, Food Science & Biotechnological Innovations (ASIO-JMFSBI), 2021, 6(1): 09-15.*

1. INTRODUCTION

Oreochromis niloticus commonly called tilapia is considered as one of the most consumed fish globally with production of 4.200 million tons in 2016 [1]. Tilapia fish is prevalent in Africa and Middle East countries. This provides the prospect of business and domestic protein source, due to their availability and easily cultured practices. Meanwhile wild capture fishes are in high extinction globally [2].

Doi: 10.2016-53692176; https://www.doi-ds.org/doilink/12.2021-57741961/ASIO-JMFSBI/10.2016-53692176/526

O. niloticus is omnivorous, easily growing and breeding in captivity and undemanding under different types of aquatic, climate conditions and more disease resistant than other types of fishes. It can also grow, tolerate and reproduce in saline waters, although, this ability is a bit counterbalanced at high salinity conditions. These unique characteristics make tilapia fish ideal aquaculture species which explains why it has become one of the most significant domesticated fishes all over the world [3].

Lifespan or shelf life is used to classify fresh fish quality and its spoilage level which most likely is correlated to the global intensification of aquaculture [4]. The lifespan of fresh fish is commonly affected by many factors. Some direct methods such as chemical and sensory methods are used in evaluating fish quality some decades ago, but are limited by test time and sensitivity. The presence of microorganisms and their activities in fish products are correlated to its spoilage especially with specific spoilage organisms (SSO) which has been established. These SSOs can live and multiply on the fish products and their microbiological metabolic activities can make a strong and very unpleasant smell. With this increasing putrefaction by microbes, modeling of SSOs to predict the lifespan of fish and its products cannot be over emphasized [5]. It has been established that temperature and time are veritable factors which affect the SSO growth in foods. Therefore, modeling the growth of SSO, considering the effect of temperature and time can help in predicting possible growth of SSOs accurately during processing and storage.

Various scientist and researchers have noticed that the microbes linked with seafood directly are correlated with the following factors; storage, fishing ground, harvesting method, transportation and the environment. Moreover, the growth of microorganisms during storage will be depended on various established preservation conditions. Gram and Huss [6] reported that the microbes responsible for spoilage were dominated by psychrotolerant gram-negative bacteria such as *Pseudomonas* species and *Shewanella* species grown on chilled fish.

Modified atmosphere packaging enhanced with carbon dioxide atmosphere slow down respiratory organisms and influences *Photobacterium phosphoreum* and lactic acid bacteria [7]. Bacteria responsible for spoilage in clement water fishes are influenced by psychrotrophic, aerobic or facultative anaerobic gram-negative bacteria commonly known as *Pseudomonas*, *Moraxella, Acinetobacter, S. putrefaciens, Vibrio, Flavobacterium, Photobacterium,* and *Aeromonas* [8]. Gram-positive bacteria such as *Staphylococcus* spp., *Micrococcus*, *Bacillus*, *Clostridium*, *Cornynebacterium, Brochothric thermosphacta,* and *Streptococcus* can be said to be the dominant microflora in tropical marine fish [9].

The study of microbial growth in tilapias by using equations that can predict fish age has been very useful, because they encapsulate information from a data series into a small set of parameters that can be transferred biologically. Microbial growth is a field of microbiology that has been known in the last two decades, and in the last ten years, hundreds of papers has been published with the keyword microbial growth [10 and 11]. This emphasizes the reasons why microorganisms response to different environments by using mathematical models. Mathematical modeling is used to represent and describes real world problems with equation and various mathematical expressions, so that an understanding problems in the real world is obtained more precisely and accurately. Related to natural phenomena, people often need a mathematical model to solve the problem encountered. Therefore, this study was aimed to determine the mathematical modeling of the growth of Specific Spoilage organisms (SSO) in Tilapia (*Oreochromis niloticus*) fish.

2. MATIRIALS & METHODS

2.1. Materials

Sample collection

Nine (9) tilapia (*Oreochromis niloticus*) fish samples were bought from Tombia market and were taken to the microbiological laboratory of The Bayelsa Medical University, Yenagoa for analysis. The fish samples were refrigerated at 0°C, -2°C and -4°C Temperatures for 24, 48 and 72 hours respectively.

Microbiological analysis

Media used: Nutrient agar (NA), Salmonella- Shigalla Agar (SSA), Eosin Methylene blue (EMB), Sabouraud dextrose agar (SDA) and Peptone water.

2.2. Methods

Procedure

The tilapia fish samples was removed from the refrigerator and allowed to get back to room temperature. 1 g of the fish was aseptically cut with a sterile scalpel and weighed into 10ml of buffered peptone water and was left for 30 minutes. Then ten (10) fold serial dilution was carried out and at the tube 10^{-4} (dilution factor). 1 ml was plated out into petri dish and a molten agar cooled at 45°C was poured and mixed with the inoculum (aliquote) to evenly spread. Culture was incubated at 35-37°C in an inverted position in the incubator for 24 hours (for bacteria) and 72-168 hours (for fungi).

Enumeration of bacteria and fungi

The colony forming unit (CFU) was determined after culture incubation using the formula stated below:

$$CFU = \frac{Number\ of\ colony\ counted}{ml\ of\ inoculum} \times Dilution\ factor$$

Mathematical modeling

Modeling, mathematically enable scientist to explain the effects of the main factors affecting the growth parameters of microorganisms. One of the commonly used models in biological sciences is the Gompertz model [12].

Original Gompertz equation:

$$y = a \exp\left[-\exp(b - ct)\right] \quad (1)$$

Where,

a,b and c are Gompertz model parameters

t is time

The original Gompertz model was re-parameterized for the parameters a, b, and c to obtain biologically meaningful parameters, such as the maximum specific

Doi: 10.2016-53692176; https://www.doi-ds.org/doilink/12.2021-57741961/ASIO-JMFSBI/10.2016-53692176/526

growth rate, μm the lag time λ, and the asymptotic value A.

The modified Gompertz equation:

$$y = A \exp\left(-\exp\left[\frac{\mu m e}{A}(\lambda - t) + 1\right]\right) \quad 2$$

Where,
A is asymptotic value
μm is maximum specific growth rate
e is Euler's number (2.7182)
λ is Lag phase
t is time

From this equation, the following derived parameters were obtained: specific growth rate μ = b c/e [log (CFU/cm^2) hours^{-1}], with e = 2.7182; lag phase duration (LPD) = 0.5/μ [hours], maximum population density (MPD) = a + c [log (CFU/cm^2)]. Data fits obtained from Gompertz model were analyzed. When the microbial counts in food remain constant or decrease during storage, it is possible to use the linear regression model [13].

The regression coefficient equation is shown below:

$$r^2 = \frac{a\Sigma y + b\Sigma xy - n\bar{y}^2}{\Sigma y^2 - n\bar{y}^2} \quad 3$$

Where;
a is the intercept of the linear curve
b is the slope of the linear curve
n is the number of variables
x is independent variable
y is the dependent variable

2.2 RESULTS

Microbial growths

Tables 1, 2 and 3 shows the colony morphology of specific spoilage microorganisms of the refrigerated tilapia fish at 0°C, -2°C and -4°C in 24, 48 and 72 hours according to the different media used. Temperature of -4°C were observed to have the highest growth rate for the Isolated specific spoilage microorganisms followed by 0°C temperature as shown in Table 4.

Table 1: Colony morphology of specific spoilage microorganisms of tilapia fish at 0°C in 24, 48 and 72 hours

S/N	Media	Suspected Microorganisms			Colony morphology
		24 hours	48 hours	72 hours	
1	NA	*Pseudomonas sp.*	*Pseudomonas sp.*	*Pseudomonas sp.*	Light blue transparent colonies.
		Staphlococcus sp.	-	-	Yellow-orange small colonies.
2	EMB	No growth	No growth	No growth	No colony formed.
3	SSA	No growth	No growth	No growth	No colony formed.
4	SDA	No growth	*Penicillium sp.*	-	Blue colony with white margin.
			Yeast	-	White cream small colonies.
			Fusarium sp.	*Fusarium sp.*	White fluffy colony.
			Phytophthora sp.	-	White rosette with slightly cottony colonies.
			Aspergillus sp.	*Aspergillus sp.*	White margin colony with dark center

Note: EMB-Eosin Methylene Blue; NA-Nutrient Agar; SSA-Salmonella- Shigella Agar; SDA-Sabouraud Dextrose Agar; S/N- Serial Number

Table 2: Colony Morphology of Specific Spoilage Microorganisms of Tilapia Fish at -2°C in 24, 48 and 72 hours

S/N	Media	Suspected microorganisms			Colony morphology
		24 hours	48 hours	72 hours	
1	NA	*Pseudomonas sp.*	-	-	Light blue transparent colonies.
		-	*Staphlococcus sp.*	*Staphlococcus sp.*	Yellow-orange small colonies.
2	EMB	*Klebsiella sp.*	No growth	-	Light pink colonies (mucoid) with or without dark pink centers.
		Pseudomonas sp.	No growth	*Pseudomonas sp.*	Light blue transparent colonies.
3	SSA	No growth	No growth	No growth	No colony formed.
4	SDA	No growth	*Penicillium sp.*	No growth	Blue colony with white margin.
			Aspergillus sp.		White margin colony with dark center.
			Fusarium sp.		White fluffy colony.

Note: EMB-Eosin Methylene Blue; NA-Nutrient Agar; SSA-Salmonella- Shigella Agar; SDA-Sabouraud Dextrose Agar; S/N- Serial Number

Doi: 10.2016-53692176; https://www.doi-ds.org/doilink/12.2021-57741961/ASIO-JMFSBI/10.2016-53692176/526

Table 3: Colony Morphology of Specific Spoilage Microorganisms of Tilapia Fish at -4°C in 24, 48 and 72 hours

S/N	Media	Suspected microorganisms			Colony morphology
		24 hours	48 hours	72 hours	
1	NA	*Staphlococcus sp.*	No growth	No growth	Yellow-orange small colonies.
		Pseudomonas sp.			Light blue transparent colonies.
		Penicillium sp			Blue colony with white margin.
2	EMB	*E. coli*	No growth	No growth	Pink colonies with colourless centers.
		Pseudomonas sp.			Light blue transparent colonies.
3	SSA	*E. coli*	No growth	No growth	Pink colonies with colourless centers.
		Shigella sp.			Colourless colonies.
		Salmonella sp.			Colourless colonies with dark centers.
4	SDA	*Fusarium sp.*	-	-	White fluffy colonies.
		Yeast *(candida)*	-	-	Cream milk budding colonies.
		Phytophthora sp.	-	-	White rosette with slightly cottony colonies.
		-	*Penicillium crustosum*	*Penicillium crustosum*	Brownish white mycelia at the margin.

Note: EMB-Eosin Methylene Blue; NA-Nutrient Agar; SSA-Salmonella- Shigella Agar; SDA-Sabouraud Dextrose Agar; S/N- Serial Number

Table 4: Number of colonies of microbial analysis of Tilapia fish in CFU/cm² at 0, -2 and -4°C

S/N	Specific Spoilage Microorganism	Number of colonies at different refrigerated temperatures in CFU/cm²		
		0°C	-2°C	-4°C
1	*Pseudomonas sp.*	1.8×10^{7}	5.2×10^{7}	2.15×10^{8}
2	*Klebsiella sp.*	1.3×10^{7}	2.6×10^{7}	1.5×10^{8}
3	*Staphylococcus sp.*	1.3×10^{8}	1.7×10^{7}	1.8×10^{8}
4	*E. coli*	1.0×10^{8}	1.2×10^{7}	2.0×10^{8}
5	*Salmonella sp.*	1.2×10^{8}	1.4×10^{7}	2.2×10^{8}
6	*Shigella sp.*	1.3×10^{8}	1.5×10^{7}	1.9×10^{8}
7	*Fungi*	1.0×10^{6}	1.5×10^{7}	2.31×10^{7}

Note: S/N- Serial Number

Mathematical modelling

Tables 5 - 7 shows the mathematical modeling of the growth of specific spoilage of microorganisms of tilapia fish at 0, -2, and -4°C temperature. The derived parameters of Gompertz models such as specific growth rate (μ) as observed to increase at temperature of -4° with decreasing Lag phase duration and maximum population density. Figures 4.1-4.3 shows the linear regression results of 0, -2 and -4°C of tilapia fish samples with R^2 values of 0.8, 1.0 and 0.5 respectively.

Table 5: Mathematical modelling of growth of microorganisms of Tilapia fish at 0ºC

S/N	Specific spoilage microorganisms	Gompertz Parameters			Derived Parameters		
		a	b	c	μ	LPD	MPD
1	*Pseudomonas sp.*	4.87	1.17	0.88	2.75	0.1818	41.75
2	*Klebsiella sp.*	5.2	1.16	0.83	2.52	0.1984	42.87
3	*Staphylococcus sp.*	2.9	1.28	1.48	5.65	0.0885	35.52
4	*E. coli*	3.16	1.26	1.36	5.04	0.0992	36.16
5	*Salmonella sp.*	2.98	1.27	1.44	5.44	0.0919	35.80
6	*Shigella sp.*	2.9	1.28	1.48	5.65	0.0885	35.52
7	*Fungi*	7.76	1.11	0.55	1.35	0.3703	49.86

Note: LPD- Lag phase duration, MPD- maximum population density, μ- specific growth rate, a: log (CFU cm^{-2}), c: log (CFU cm^{-2}), b: hours^{-1}, μ: log (CFU cm^{-2}) hours^{-1}, MPD: (log (CFU cm^{-2}), LPD: (hours), S/N- Serial Number

Table 6: Mathematical modelling of growth of microorganisms of Tilapia fish at -2ºC

S/N	Specific spoilage microorganisms	Gompertz Parameters			Derived Parameters		
		a	b	c	μ	LPD	MPD
1	*Pseudomonas sp.*	3.82	1.21	1.12	3.85	0.129	38.14
2	*Klebsiella sp.*	4.51	1.18	0.95	3.05	0.164	40.46
3	*Staphylococcus sp.*	4.93	1.17	0.87	2.71	0.185	41.93
4	*E. coli*	5.28	1.15	0.81	2.43	0.206	43.24
5	*Salmonella sp.*	5.13	1.16	0.84	2.56	0.195	42.69
6	*Shigella sp.*	5.06	1.16	0.85	2.61	0.192	48.88
7	*Fungi*	5.06	1.16	0.85	2.60	0.192	42.43

Note: LPD- Lag phase duration, MPD- maximum population density, μ- specific growth rate, a: log (CFU cm^{-2}), c: log (CFU cm^{-2}), b: hours^{-1}, μ: log (CFU cm^{-2}) hours^{-1}, MPD: (log (CFU cm^{-2}), LPD: (hours), S/N- Serial Number

Doi: 10.2016-53692176; https://www.doi-ds.org/doilink/12.2021-57741961/ASIO-JMFSBI/10.2016-53692176/526

Table 7: Mathematical modeling of growth of microorganisms of Tilapia fish at -4ºC

S/N	Specific spoilage microorganisms	Gompertz Parameters			Derived Parameters		
		a	b	c	μ	LPD	MPD
1	*Pseudomonas sp.*	2.39	1.34	1.79	7.35	0.068	34.82
2	*Klebsiella sp.*	2.75	1.29	1.56	6.06	0.083	35.26
3	*Staphylococcus sp.*	2.58	1.32	1.66	6.66	0.075	35.02
4	*E. coli*	2.47	1.33	1.74	7.07	0.071	34.94
5	*Salmonella sp.*	2.37	1.34	1.81	7.44	0.067	34.86
6	*Shigella sp.*	2.52	1.32	1.70	6.82	0.073	34.86
7	*Fungi*	4.63	1.18	0.93	2.97	0.168	40.92

Note: LPD- Lag phase duration, MPD- maximum population density, μ- specific growth rate, a: log (CFU cm^{-2}), c: log (CFU cm^{-2}), b: hours^{-1}, μ: log (CFU cm^{-2}) hours^{-1}, MPD: (log (CFU cm^{-2}), LPD: (hours), S/N- Serial Number

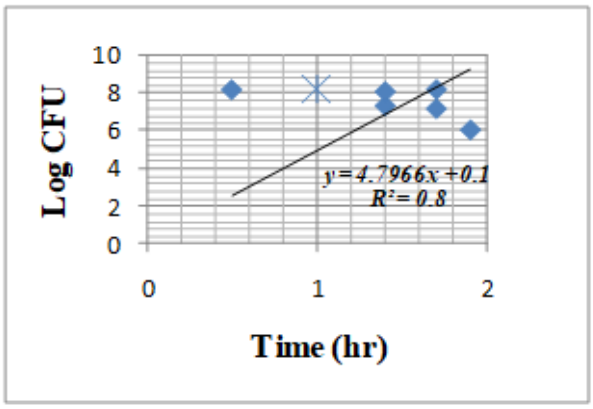

Fig. 1: Linear Regression Model for 0°C temperature of tilapia fish samples

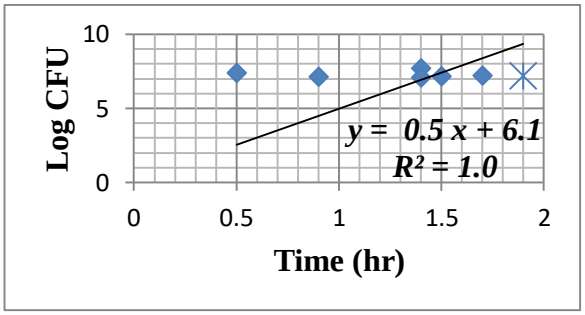

Fig. 2: Linear Regression Model for -2°C temperature of tilapia fish samples

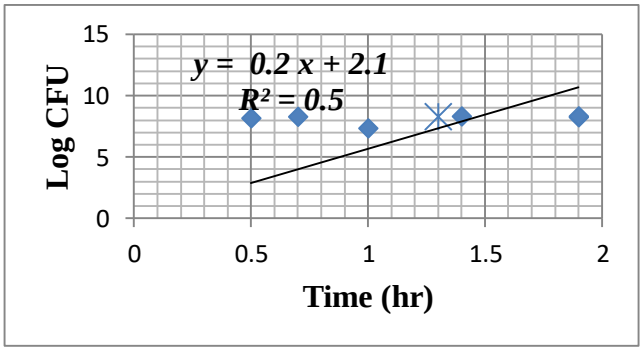

Fig. 3: Linear Regression Model for -4°C temperature of tilapia fish samples

3. DISCUSSION

Isolation of Specific spoilage microorganisms in tilapia fish

The shelf life of fresh fish is commonly affected by many factors. Some direct methods such as chemical and sensory methods are used in evaluating fish quality some decades ago, but are limited by test time and sensitivity. The presence of microorganisms and their activities in fish products are correlated to its spoilage especially with specific spoilage organisms (SSO)

In this research different types of specific spoilage organisms that are pathogenic in nature had been isolated from tilapia fish. Pathogens have been established to contribute very significant diseases globally which includes pneumonia, caused by *Streptococcus* and *Pseudomonas*, and foodborne illnesses caused by *Shigella* and *Salmonella.*

The SSO in tilapia fish at 0°C were bacteria (*Pseudomonas sp. and staphylococcus sp.)* and fungi (*Penicillium sp.,* Yeast, *Fusarium sp., Phytophthora sp. and Aspergillus sp.)* isolates as shown in Table 1. At temperature -2°C the Tilapia SSO was observed to be Bacteria (*Pseudomonas sp., staphylococcus sp. and Klebsiella sp.)* and fungi (*Penicillium sp., Fusarium sp. and Aspergillus sp.)* strains as shown in Table 2. While Table 3 shows the tilapia fish SSO at -4°C, which was bacteria (*Pseudomonas species, staphylococcus species, E. coli, Shigella species and Salmonella species)* and fungi (*Penicillium species,* Yeast (candida), *Fusarium sp.*and *Phytophthora sp.)*. The specific spoilage organisms found in tilapia fish are in conformity with the studies of [14 and [15].

On the number of isolated colonies of the microbial growth in tilapia fish -4°C temperature isolates such as *Salmonella* (2.2×10^8CFU/cm^2), *Pseudomonas* (2.15×10^8CFU/cm^2) and *Staphylococcus* (1.8×10^8CFU/cm^2) were observed to be highest (Table 4). The high presence of *Salmonella species* can be correlated to contaminated waters used by the retailers of the tilapia fish. *Salmonella* has been isolated often from the fish market environments and gastrointestinal tract of all farmed and wild animals [16 and 17]. High presence of *Salmonella species* in different raw food samples in local markets had also been reported by another study which supports this study [18]. These microorganisms natural flora of their skin and mucous membranes of animals and humans causes contamination in fish [17]. The result of this study is in accord with [19 and 14].

The total fungi load of the study for 0, -2 and -4°C are shown in Table 4. Temperature -4°C was also observed to be highest in total fungal count (2.31×10^7CFU/cm^2), though the count is low when compared with the various bacterial counts. This might be because of their slow growth and relatively poor ability to compete with bacteria successfully, for they are most likely to be found in the foods in which the environment is less favorable for bacterial growth, e.g. low pH, low -aw- , high salt or sugar content , low storage temperature, the presence of antibiotics, or exposure to irradiation. These findings were synonyms to the reports of [20].

Doi: 10.2016-53692176; https://www.doi-ds.org/doilink/12.2021-57741961/ASIO-JMFSBI/10.2016-53692176/526

Mathematical modeling

Food qualities are frequently evaluated by microbial growth. Mathematical models are used in predicting the change in food quality in respect to temperature and time which can be used in estimating the shelf life of foods. It can be used also to reduce the required amount of time and expensive challenge testing. It may also help in designing more effective methods of challenge testing, and finally can be used for distribution chain optimization. Another very significant characterization of models is the acquisition of enhanced knowledge of the components that defines food quality.

Gompertz´s original and derived parameters showing the growth of specific spoilage organisms (SSOs) isolated from the surfaces of tilapia fish samples at 0, -2 and -4°C with storage time of 24, 48 and 72 hours is shown in Tables 5-7. The experimental data was modeled using the Gompertz modified equation for both bacteria and fungi counts with different temperatures of the isolates in cfu/ml of the samples. The derived parameters of the Gompertz model such as specific growth rate (μ) was observed to increase at temperatures of -4° C with decreasing maximum population density (MPD) and Lag phase duration (LPD) time of the specific spoilage organisms. These findings were in agreement with the works of [14, 19 and 21].

The test organism's results in tilapia fish at 0, -2 and -4°C temperature were modeled by the linear regression model (R^2 values were 0.8, 1.0 and 0.5 respectively), showing a strong positive linear relationship (Fig 1, 2 and 3). This linear regression model results were in accordance with the works of [22]. Temperature and time has been established as the two very significant factors that affect the growth of specific spoilage organisms in foods. Hence, modeling the growth of SSO, considering temperature and time enables scientist to predict the possible growth of SSO accurately during processing and storage.

DECLARATIONS

Funding: None

Conflict of interest: The authors declare that they have no competing interests.

Ethical approval: Not applicable

CONCLUSION

This research results demonstrated and shows that *Pseudomonas species, staphylococcus species, Escherichia coli, Shigella species, Salmonella species, Klebsiella species, Penicillium species,* Yeast, *Fusarium species, Phytophthora species* and *Aspergillus species* are Specific Spoilage Organisms (SSOs) of Tilapia (*Oreochromis niloticus*) fish. They reduce the lifespan of *Oreochromis niloticus* at 0, -2 and -4°C temperatures in 24, 48 and 72 hours storage times. On the number of isolated colonies of the microbial growth in tilapia fish -4°C temperature isolates such as *Salmonella* (2.2×10^8CFU/cm^2), *Pseudomonas* (2.15×10^8CFU/cm^2) and *Staphylococcus* (1.8×10^8CFU/cm^2) were observed to be highest. The intense presence of *Salmonella sp*ecies in the study can be correlated to contaminated waters used by the retailers of the tilapia fish. The experimental data was modeled using the Gompertz modified equation for both bacteria and fungi counts with different temperatures of the isolates in cfu/ml of the samples. The derived parameters of the Gompertz model such as specific growth rate (μ) was observed to increase at temperatures of -4° C with decreasing maximum population density (MPD) and Lag phase duration (LPD) time of the specific spoilage organisms. Temperature and time has been established as the two very significant factors that affect the growth of specific spoilage organisms in foods. Hence, modeling the growth of SSO, considering temperature and time enables scientist to predict the possible growth of SSO accurately during processing and storage. The test organism's results in tilapia fish at 0, -2 and -4°C temperature were modeled by the linear regression model (R^2 values were 0.8, 1.0 and 0.5 respectively), showing a strong positive linear relationship.

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