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Generation time, D and Z - values of *pseudomonas fluorescens* under different temperature, water activity and pH conditions

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Abstract— *The aim of this study is to observe and assess the performance of Psychrotrophic Gram-negative bacteria, namely Pseudomonas fluorescens, which pose a significant spoilage problem in food, under different temperature, water activity (aw) and pH conditions. Noting that at aw 0.6 and 0.75 irrespective of temperature or pH and at temperatures 60^0 and 100^0C irrespective of water activity or pH no survival was recorded. The D-value (Decimal reduction time) at 4^0, 25^0, 37^0 and 42^0C differ significantly, with the one at 37^0C was significantly the highest and that at 42^0C was significantly the lowest. The optimum conditions were found to be at pH=6, aw=0.97 and temperature=37^0C showing the significantly lowest generation time (7.69 min). At pH=6, aw=0.97 and temperatures of 4^0 and 25^0C we have significantly different generation times of 46.75 and 10.9 min respectively. Concerning the z-value it was calculated at pH=4 (high acid food), pH=6 (low acid food) and pH=8 (alkaline food), at aw 0.82, 0.93 and 0.97 and from consequent D-values at temperatures 4^0, 25^0, 37^0 and 42^0C and was shown to be 25.31±3.68. Outside of the optimum conditions, Pseudomonas fluorescens died at different rates. Within all pH-values, D-values at 42^0C were significantly the lowest when compared to those at 4^0, 25^0 and 37^0C among the different aw-values. Thus changing only one factor, either storage temperature, pH or aw out of the optimum neighborhood will lead to the reduction of this bacteria, although at different rates.*

Keywords— ***D-Value, Z-Value, low acid food, high acid food, Alkaline food, Generation time, Water activity.***

I. INTRODUCTON

Pseudomonas fluorescens encompasses a group of common, nonpathogenic saprophytes that colonize soil, water and plant surface environments [1]. Pseudomonas has high genetic diversity and poor nutritional needs allowing them to survive in different environments [2]. These characteristics allow them to survive on utensils equipment used in dairy production chain, as milking machines, pipelines, and bulk tanks. These bacteria are among the most common bacteria causing spoilage of food mainly dairy products. It is famous by its ability to cause post pasteurization contamination, and to cause spoilage of food even at low temperature [3]. There are mainly three essential enzymes found in the bacteria involved in spoilage of food mainly, lipases, proteases, and lecithenases. For example, in milk the degradation of casein by proteases will lead to gelation of milk, or when lipases degrade the fat present on the milk into free fatty acids will cause rancidity and bitterness of milk. Pseudomonas grow at pH range of 5.6-7.1 [5], temperature range of 4^0-42^0C, and aw of 0.97 [6].

Pseudomonas are psychrotrophic bacteria, they have the ability to survive in a temperature range of 0^0 - 40^0C [7]. Studies showed that the growth of bacteria is strongly related to the culturing temperature; its maximum growth is found to be at the optimal temperature specific for each bacterium. Concerning *pseudomonas fluorescens*, on its optimal temperature 37^0C, so many important metabolic enzymes are fully active, such as lipases and proteases, and there rate changes with temperature [8]. Psychrotrophs are the most common to cause spoilage at refrigerating

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temperature [9] with Pseudomonas spp being the most common bacteria within them [10].

Goncalves et al, in another study also showed that the pH will decrease the concentration of bacteria when acidity increased, or when it decreases to a value more than the optimal range that a bacteria can tolerate [5]. Water activity is this unbounded water; it is the ratio between vapor pressure of distilled water under identical conditions. Water activity affects bacterial growth and concentration at different levels. However, at water activity below 0.85 no bacteria can grow [11]. Furthermore, moist heat is more effective than dry-heat.

To study the heat resistance of microorganisms we need to study D-values at a reference and different temperatures, and the z-value, the temperature dependence of the thermal inactivation rate. The z-value is an indicator of the microbial inactivation rate based on different temperature dependence. Z-value is the temperature units needed to change microbial inactivation rate by a factor of 10. Z-value is also used to express the degradative reactions dependence on temperature during processing and storage [12].

Thus investigating the thermal resistant of bacteria will shed a light on the potential degradation of the food by the studied microbiota. In this study, these factors would guide us to develop a protection policy against the degradation of food during storage by *Pseudomonas fluorescens* in high and low acid food and alkaline food under different temperatures and water activities. In addition, each prevented degradation would lead to the reduction of waste of food and eventually having positive impact on the environment. Noting that losing one gr of beef, for example, means losing the CO_2 needed to produce them, which accounts to 13.3Kg. This is similar to burning 6 liters of petrol [13].

II. MATERIALS AND METHODS

2.1. Bacteria and media

In our study, *pseudomonas fluorescens* was collected from the Lab for Microbiology and Public Health, Faculty of Public Health, Lebanese University in Lebanon, Tripoli. The code of the strain was CMUL 014.

As for the growth-broth, the HIMEDIA Nutrient Broth M002, which complies as per ISO 17025:2005 whose company is situated in Mumbai-India was chosen. It is used for general cultivation of less fastidious microorganisms and can be enriched with blood or other biological fluids.

2.1.1. Developing Medias with different water activities

The different water activities of the media was achieved by dissolving the nutrient broth in distilled water at different rations.

For water activity 0.60: 35g nutrient broth was dissolved in 10 ml distilled water.

For water activity 0.75: 35g nutrient broth was dissolved in 15ml distilled water

For water activity 0.82: 30g nutrient broth was dissolved in 50ml distilled water

For water activity 0.93: 30g nutrient broth was dissolved in 50m distilled water

For water activity 0.97: 0.65g nutrient broth was dissolved in 50ml distilled water

The water activities were validated using the PAWKIT water activity meter and measuring them in triplicates.

2.1.2. Adjustment of pH

The Medias produced were adjusted to different pH values by the addition of (70%) HCl and (1N) NaOH.

For acidic pH (4 and 6), 70% HCl is added until reaching the needed pH.

For basic pH (8), (1N) NaOH is added until reaching the designated pH.

2.2. Equipment used to measure Physico-chemical properties

Brix Value: Brix Value was measured using Portable hand held RFM700 refractometer (Bellingham and Stanley LTD. United Kingdom).

Weight determination: Weight was measured using Portable electronic balance Model 727 was used to measure the weight with an accuracy of ±1 gr (Jata Hogar).

pH: Microcomputer based pH /conductivity /TDS /salinity and temperature pocket meter Model pH/EC80 was used to measure the pH (Jenco VisionP).

Ash content: Ash was determined using the AOAC 942.05 method.

Volume Determination: 10mL glass graduated cylinder, with sub gradations of 0.1mL (Graduated cylinder, tall form, BLAUBRAND®, class A, Boro 3.3, DE-M).

Caloric Value: Bomb calorimeter IKA C200 was used (KA®-Werke GmbH & Co. KG)

Water activity: It was determined using Pawkit water activity meter. Samples were flattened to cover the bottom of the cup and then water activity was measured at room temperature [14].

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2.3. Formulas used for D and Z value and generation time

The microbial destruction rate is defined by D value (equation 1), which is the heating time at a given temperature required to reduce the surviving microbial population by 90% of its initial population [15].

Equation 1 D-Value: Decimal reduction rate.

$$D = \frac{t2 - t1}{Log(N1) - Log(N2)}$$

Where N1 and N2 represents the number of bacteria at a constant temperature measured at t1 and t2 (min) respectively.

The temperature sensitivity indicator is defined as Z (equation 2); a value represents a temperature range, which results in 10-fold change in D values [15].

Equation 2 Z-Value as temperature sensitivity indicator

$$Z = \frac{T2 - T1}{Log(D1) - Log(D2)}$$

Where D1 and D2 (**Eq.** 1) represents the decimal reduction value of bacteria at a measured at T1 and T2 (^oC) respectively.

Generation time (G) is the time needed for a single bacterium to double its number [16] [17] (**Eq.** 3&4).

Equation 3

$$\text{Generation time} = \frac{\text{Time interval}}{\text{Number of generations}}$$

Equation 4

$$\text{Number of generations} = \frac{LogN_1 - LogN_2}{Log(2)}$$

2.4. Procedure

After inoculating the *pseudomonas fluorescens* in the nutrient broth with different water activities, at different pH and incubated at different temperatures, the bacteria was enumerated using the plate count technique every 30 minutes up to 240 minutes (**Fig**. 1).

Please note that there was no growth at temperatures above 42^0C and water activity lower than 0.83. Thus, they will not be included in the statistical analysis.

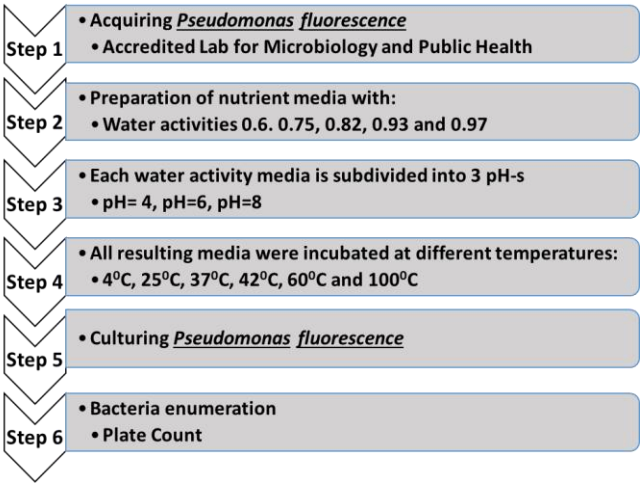

Fig.1 Summary of study flow

2.5. Statistical analysis

All tests and analysis were run in triplicates and averaged. General linear model performed via SPSS (statistical Package for the Social Sciences, version 17.0) was used to study the difference between D and z values at different temperatures, water activity and pH. To study the effect of each factor 2 were fixed and the third one was studied.

Furthermore, for GLM model was applied on generation time we only had growth at temperatures 4^0C, 25^0C and 37^0C, at pH=6 and aw=0.97.

III. RESULTS

3.1 Generation Time

The generation time is the time needed for one single bacterium to double itself [16]. Looking at the growth and death rate data across different temperatures within different water activities and at different pH, it was noticed that the growth of *Pseudomonas fluorescens* occurred only at an optimum pH=6 and aw=0.97 at temperatures 4^0, 25^0 and 37^0C where the last temperature showed the significantly lowest generation time (7.69 min). At temperatures of 4^0 and 25^0C we have significantly different generation times of 46.75 and 10.9 min with the first being significantly the longest and the second being in-between and significantly different from both (**Fig**. 2).

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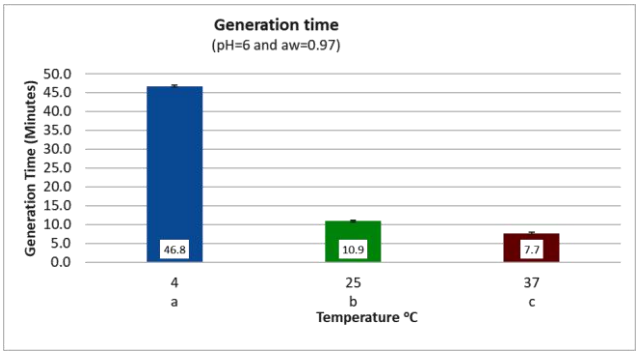

Fig.2: Generation Time at different temperature within pH=6 and aw=0.97

3.2 D-value

3.2.1 D-Values Within different pH at different temperatures and water activity

Within simulated high acid food (pH=4), and 0.82 water activity the temperature 4^0C showed the significantly highest D-value, and the D-value at 42^0C was significantly the lowest with those measured at 25^0C and 37^0C were in between. Within water activity 0.93, there was no significant difference between the different D-values at temperatures 4^0 and 25^0C, with the D-value at 42^0C was significantly the lowest and all were significantly lower than those measured at water activity of 0.82. Except at 37^0C, D-value was significantly the highest within 0.93 water activity at different temperatures and among different water activities. At water activity of 0.97, D-value at 42^0C was significantly the lowest followed by the D-value at 4^0, 25^0 and 37^0C being significantly the highest.

Table 1 D-Values within pH=4 (Simulated high Acid Food) at different water activities (aw) and temperatures

aw	0.82	0.93	0.92
At pH=4	Mean ± SE	Mean ± SE	Mean ± SE
4^0C	$53.60^{a\ 1}$ ±11.28	$13.23^{a\ 2}$ ±21.00	$20.62^{a\ 3}$ ±0.84
25^0C	$31.00^{ab\ 1}$ ±11.28	$14.13^{a\ 2}$ ±21.00	$24.23^{b\ 3}$ ±0.84
37^0C	$28.27^{ab\ 1}$ ±11.28	$264.0^{b\ 2}$ ±21.00	$27.27^{c\ 1}$ ±0.84
42^0C	$9.03^{b\ 1}$ ±11.28	$6.77^{c\ 2}$ ±21.00	$5.13^{d\ 3}$ ±0.84

- Among rows means with different letters are significantly different
- Among Columns: means with different numbers are significantly different

Within the simulated low acid food, at water activity of 0.82 and 0.93 the D-values followed the same pattern to that high acid food. Within the 0.97 and temperatures of 4^0, 25^0, 37^0C there was growth, which is shown in the generation times above. Within 0.97 aw, D-value at 42^0C was significantly the lowest among the different aw values with that at 0.82 being significantly the highest.

Table 2 D-Values within pH=6 (Simulated low Acid Food) at different water activities (aw) and temperatures

aw	0.82	0.93	0.92
At pH=6	Mean ± SE	Mean ± SE	Mean ± SE
4^0C	$86.8^{a\ 1}$ ±11.3	$19.5^{a\ 2}$ ±21.0	n.a.
25^0C	$55.1^{b\ 1}$ ±11.3	$21.6^{a\ 2}$ ±21.0	n.a.
37^0C	$17.2^{c\ 1}$ ±11.3	$44.8^{b\ 2}$ ±21.0	n.a.
42^0C	$4.8^{c\ 1}$ ±11.3	$4.1^{c\ 2}$ ±21.0	$2.5^{a\ 3}$ ±0.8

- n.a.= Not applicable, in these conditions there is growth.
- Among rows means with different letters are significantly different
- Among Columns: means with different numbers are significantly different

Within the simulated alkaline food, at 0.82 aw D-value at 42^0C was significantly the lowest, at 37^0C was significantly the highest with those at 4^0 and 25^0C being in between. At 0.93 aw, D-vale at 42^0C was significantly the lowest, and that at 4^0C was significantly the highest, threst being in between and significantly different from each other.

Table 3 D-Values within pH=8 at different water activities (aw) and temperatures

aw	0.82	0.93	0.92
At pH=8	Mean ± SE	Mean ± SE	Mean ± SE
4^0C	$36.70^{a\ 1}$ ±22.29	$35.23^{a\ 2}$ ±0.37	$20.62^{a\ 3}$ ±1.04
25^0C	$18.03^{a\ 1}$ ±22.29	$11.43^{b\ 2}$ ±0.37	$24.23^{b\ 3}$ ±0.85
37^0C	$406.0^{b\ 1}$ ±22.29	$16.07^{c\ 2}$ ±0.37	$27.27^{c\ 1}$ ±0.85
42^0C	$4.67^{c\ 1}$ ±22.29	$5.30^{c\ 2}$ ±0.37	$5.13^{d\ 3}$ ±0.85

- Among rows means with different letters are significantly different
- Among Columns: means with different numbers are significantly different

3.2.2 D-value within different temperatures at different pH and water activity

At simulated fridge storage (4^0C), within aw=0.82, pH outside the optimum (pH= 6) showed no significant differences both have D-Values which are significantly

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lower. Within aw=0.93, D-values at pH=4, 6 and 8 differ significantly from each other with that at 4 being the lowest and at 8 was significantly the highest (table 4). Concerning the significance within different pH between different aw, D-values tended to be lower the higher the aw value.

Table 4 D-Values within temperature (T=4^{0}C) at different water activities (aw) and temperatures

aw	0.82	0.93	0.97
At T=4^{0}C	Mean ± SE	Mean ± SE	Mean ± SE
pH=4	31.27$^{a\ 1}$ ±2.61	13.23$^{a\ 2}$ ±0.62	20.62$^{a\ 3}$ ±0.97
pH=6	86.87$^{b\ 1}$ ±2.61	19.57$^{b\ 2}$ ±0.62	n.a.
pH=8	36.70$^{a\ 1}$ ±2.61	35.17$^{c\ 1}$ ±0.62	13.10$^{d\ 2}$±20.97

- n.a= Not applicable, in these conditions there is growth.
- Among rows means with different letters are significantly different
- Among Columns: means with different numbers are significantly different

At simulated room temperature storage (25^{0}C), within the different aw, D-values recorded at pH=8 were significantly the lowest and at pH=6 were significantly the highest (Table 5). Noting that at aw=0.97 and pH=6 there is no death rate but growth rate (Table 5).

Table 5 D-Values within temperature (T=25^{0}C) at different water activities (aw) and temperatures

aw	0.82	0.93	0.97
At T=4^{0}C	Mean ± SE	Mean ± SE	Mean ± SE
pH=4	31.00$^{a\ 1}$ ±2.07	14.13$^{a\ 2}$ ±0.44	24.23$^{a\ 3}$ ±0.28
pH=6	69.77$^{b\ 1}$ ±2.07	21.60$^{b\ 2}$ ±0.44	n.a.
pH=8	18.03$^{c\ 1}$ ±2.07	11.43$^{c\ 1}$ ±0.44	18.73$^{b\ 1}$ ±0.28

- n.a.= Not applicable, in these conditions there is growth.
- Among rows means with different letters are significantly different
- Among Columns: means with different numbers are significantly different

At 37^{0}C as shown by Table 6, two three digit D-values were noticed at pH=4 and aw=0.93 and pH=8 and aw=0.82. Within the same pH (4, 6 and 8) the D-values differ significantly at different water activities.

Table 6 D-Values at different water activities (aw) and temperatures

aw	0.82	0.93	0.97
At T=4^{0}C	Mean ± SE	Mean ± SE	Mean ± SE
pH=4	28.27$^{a\ 1}$ ±1.90	264.00$^{a\ 2}$ ±1.97	27.27$^{a\ 3}$±0.88
pH=6	17.23$^{b\ 1}$ ±1.90	44.83$^{b\ 2}$ ±1.97	n.a.
pH=8	406.00$^{a\ 1}$ ±1.90	16.07$^{c\ 2}$ ±1.97	25.57$^{d\ 3}$ ±0.88

- n.a.= Not applicable, in these conditions there is growth.
- Among Columns: means with different letters are significantly different
- Among rows: means with different numbers are significantly different

At incubation temperature of 42^{0}C (Table 7), within the water activities 0.82 and 0.93, D-values at pH=4 were significantly the highest, while the D-values measured at aw=0.97 were significantly highest at pH=8 and significantly the lowest at pH=6.

Table 7 D-Values within temperature (T=42^{0}C) at different water activities (aw) and temperatures

aw	0.82	0.93	0.97
At T=4^{0}C	Mean ± SE	Mean ± SE	Mean ± SE
pH=4	9.03$^{a\ 1}$±0.03	6.77$^{a\ 2}$±0.04	5.13$^{a\ 3}$±0.03
pH=6	4.87$^{b\ 1}$±0.03	4.13$^{b\ 2}$±0.04	2.57$^{b\ 3}$±0.03
pH=8	4.67$^{c\ 1}$±0.03	5.30$^{c\ 2}$±0.04	7.80$^{c\ 3}$±0.03

- Among rows means with different letters are significantly different
- Among Columns: means with different numbers are significantly different

3.3 Z-Value

The temperature sensitivity indicator is defined as Z (equation 2); a value represents a temperature range, which result in 10-fold change in D values [15]. There was no significant difference between z values recorded within and among different pH (4, 6 and 8), within and among different water activities (0.82, 0.93 and 0.97) out of the growth neighbpourhood (pH=6 and 4^{0}C, 25^{0}C, and 37^{0}C) and it was found to have a mean value of 25.31±3.68.

3.4 Construction of D and generation time values rubric

Combining the values of this study we end up in a rubric that shows we have growth only at pH=6 and aw=0.97. Furthermore, it shows clearly that at aw=0.75 and lower, and at temperatures 60^{o}C and higher *pseudomonas fluorescens* would not survive (Fig. 3). Furthermore, at

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42^0C at water activities of 0.82, 0.93 and 0.97 we have a one digit D-Value. At 4^0C, 25^0C and 37^0C incubation temperatures we have two digit D-values (Fig. 3). Only at aw=0.93 and pH=4 and at aw=0.82 and pH=8 we recorded a three digit D- values (Fig. 3).

Water Activity	pH	Recorded *Pseudomonas fluorescens* Response					
	4	21	24	27	5	TS	TS
0.97	6	GT: 47	GT: 11	GT: 8	3	TS	TS
	8	13	19	26	8	TS	TS
	4	13	13	264	7	TS	TS
0.93	6	20	20	45	4	TS	TS
	8	35	35	16	5	TS	TS
	4	31	31	28	9	TS	TS
0.82	6	87	70	17	5	TS	TS
	8	37	18	406	5	TS	TS
	4	TS	TS	TS	TS	TS	TS
0.75	6	TS	TS	TS	TS	TS	TS
	8	TS	TS	TS	TS	TS	TS
	4	TS	TS	TS	TS	TS	TS
0.65	6	TS	TS	TS	TS	TS	TS
	8	TS	TS	TS	TS	TS	TS
Temperature ^{0}C		4	25	37	42	60	100

Fig.3 D-values (minutes) and Generation time (GT - minutes) of Pseudomonas fl. at different water activities and acidity levels (pH) at different incubation temperatures

- TS: D-Value too short to be detected in this study
- All decimals are rounded up.

IV. DISCUSSION

The generation time revealed the conditions at which we should worry for our product. If we have food with pH=6, water activity= 0.97 and storage temperature between 4^0C and 37^0C we should be careful. The higher the temperature lower the generation time thus at 37^0C we would reach the critical level of *pseudomonas fluorescens* around 1.4 times faster than that stored at 25^0C and around 6 times faster than that stored at 4^0C (Fig. 2) (Fig. 3). Furthermore, it was noticed that all growth outside these neighborhood was zero and actually was replaced by death rate Classification of commercial apple juices based on multivariate analysis of their chemical

profiles [18]. At pH=4 food is considered high acid food. As shown in D-value results and the overall summary table (Fig. 3), at this pH there is no growth but a recorded death rate which is lowest in value at temperature = 42^0C and non-existent at Temperatures higher than that. In this category of food we can look at pomegranate (pH=4.04), grape verijuice (pH=2.84), Sour apple juice (pH=3.64) [19]. These foods might be considered unsusceptible for this bacterium.

Considering other food category, namely the low acid food with pH range between 4.6 and 7 they are more susceptible. Since at pH=6 and aw=0.97 we noticed growth. For this type of foods to be safer from this bacterium the pH should be lowered to make it high acid food or increased to fall in the alkaline food category, and/or lower the water activity. Example of this food category are the dairy products, which are known to be the most susceptible to this bacterium, which is in accordance with the results of this study. Since dairy products are sensitive to changes in pH and/or aw since both change the properties of the product, extra care should be taken to prevent cross contamination. Especially that it can grow under fridge conditions at aw=0.97.

As for the alkaline foods, pH>7, it followed the same trend of the high acid food where the lowest D-value was recorded at 42^0C and temperatures of 60^0C and 100^0C the D-values were too short to be recorded by this study. Food that fall under this category are Alkaline-fermented foods food products that are widely consumed in Southeast Asia and African countries and has a fermentation culture based on the dominant microorganisms, Bacillus spp., which hydrolyze proteins into amino acids and ammonia [20].

After collecting the data of the *Pseudomonas fl.* a rubric table was constructed as a preliminary guide to see the susceptibility of the food to this bacterium (Fig. 3). We need to know, however, the aw, pH and the storage temperature. All foods with aw<0.83 are safe. Furthermore, outside of the growth zone and at incubation temperature=42^0C D-values were one digit, thus less than 10 minutes. If we take 10 as the representative value, it means within 2 hours we would reach the total death time of 12D. In addition to that, at 37^0C and water activities of 0.82 and 0.93 the D-values were 405 and 264 minutes. These values should be validated with further studies. However, if we take them as basis the total death time of 12 D would be 3.3 to 2.2 days respectively. The rest of the D-values are less than 45 min. considering the maximum as representative number 9 hours would be needed to reach the total death time of 12D.

V. CONCLUSION

Low acid food of water activity near 0.97 and stored in fridge room temperature and slightly warm room temperature are the most susceptible to the deterioration due to *Pseudomonas fluorescens.* Foods with aw less than 0.83, like jams, are not susceptible irrespective of pH or storage temperature. The D-value rubric table might serve as a quick guide for categorization of the degree of susceptibility of food in question based on pH, aw and temperature of incubation.

REFERENCES

[1] Scales, Brittan S., Dickson, Robert P., LiPuma, John J., and Huffnagle, Gary B. (2014). Microbiology, genomics, and clinical significance of the Pseudomonas fluorescens species complex, an unappreciated colonizer of humans. Clinical microbiology reviews, 27(4), 927-948. doi:10.1128/CMR.00044-14

[2] Garrido-Sanz, Daniel, Arrebola, Eva, Martínez-Granero, Francisco, García-Méndez, Sonia, Muriel, Candela, Blanco-Romero, Esther, Martín, Marta, Rivilla, Rafael, and Redondo-Nieto, Miguel. (2017). Classification of Isolates from the Pseudomonas fluorescens Complex into Phylogenomic Groups Based in Group-Specific Markers. 8(413). doi:10.3389/fmicb.2017.00413

[3] Rajmohan, S., Dodd, C. E., and Waites, W. M. (2002). Enzymes from isolates of Pseudomonas fluorescens involved in food spoilage. J Appl Microbiol, 93(2), 205-13. doi:10.1046/j.1365-2672.2002.01674.x

[4] Gonçalves, Letícia Dias dos Anjos, Piccoli, Roberta Hilsdorf, Peres, Alexandre de Paula, and Saúde, André Vital. (2017). Predictive modeling of Pseudomonas fluorescens growth under different temperature and pH values. Brazilian Journal of Microbiology, 48(2), 352-358. doi:https://doi.org/10.1016/j.bjm.2016.12.006

[5] Lin, Hao, Shavezipur, Mohammad, Yousef, Ahmed, and Maleky, Farnaz. (2016). Prediction of growth of Pseudomonas fluorescens in milk during storage under fluctuating temperature. Journal of Dairy Science, 99(3), 1822-1830. doi:https://doi.org/10.3168/jds.2015-10179

[6] Dagorn, A., Chapalain, A., Mijouin, L., Hillion, M., Duclairoir-Poc, C., Chevalier, S., Taupin, L., Orange, N., and Feuilloley, M. G. (2013). Effect of GABA, a bacterial metabolite, on Pseudomonas fluorescens surface properties and cytotoxicity. Int J Mol Sci, 14(6), 12186-204. doi:10.3390/ijms140612186

[7] Guillou, C and Guespin-Michel, J F. (1996). Evidence for two domains of growth temperature for the psychrotrophic bacterium Pseudomonas fluorescens MF0. 62(9), 3319-3324.

[8] Kasana, R. C. (2010). Proteases from psychrotrophs: an overview. Crit Rev Microbiol, 36(2), 134-45. doi:10.3109/10408410903485525

[9] Food Spoilage by Pseudomonas spp.—An Overview, in Foodborne Pathogens and Antibiotic Resistance. p. 41-71.

[10] Fraser, Angela and Barron, Felix, Acidified Foods: Food Safety Considerations for Food Processors. 2013. p. 231-239.

[11] Peleg, Micha. (2019). Modeling Degradation Kinetics in Dry Foods Storage under Varying Temperature and Moisture Content—Theoretical Evaluation. Food Engineering Reviews. doi:10.1007/s12393-018-9185-y

[12] Andreas, Grabolle, Tanja, Loitz. (2007). Pendos CO2-Zähler (1 ed.). Berlin: Pendo.

[13] Nielsen, S. Suzanne. (2017). Food Analysis: springer.

[14] KJ Valentas, E Rotstein, RP Singh (1997). Handbook of food engineering practice: CRC press.

[15] Painter, Page. (1975). Generation Times of Bacteria. Journal of general microbiology, 89, 217-20. doi:10.1099/00221287-89-2-217

[16] Jun, Suckjoon, Si, Fangwei, Pugatch, Rami, and Scott, Matthew. (2018). Fundamental principles in bacterial physiology-history, recent progress, and the future with focus on cell size control: a review. Reports on progress in physics. Physical Society (Great Britain), 81(5), 056601-056601. doi:10.1088/1361-6633/aaa628

[17] Włodarska, Katarzyna, Pawlak-Lemańska, Katarzyna, Górecki, Tomasz, and Sikorska, Ewa. (2017). Classification of commercial apple juices based on multivariate analysis of their chemical profiles. International Journal of Food Properties, 20(8), 1773-1785. doi:10.1080/10942912.2016.1219367

[18] Dimassi, Ossama, Aboulhosn, Ghenwa, Akiki, Raymond, and Rached, Mohammad. (2019). Sour Apple Concentrate: processing essentials and organoleptic properties. International Journal of Environment, Agriculture and Biotechnology, 4, 1738-1744. doi:10.22161/ijeab.46.18

[19] Choi, Kwon-Young, Wernick, David, Tat, Christine, and Liao, James. (2014). Consolidated conversion of protein waste into biofuels and ammonia using Bacillus subtilis. Metabolic Engineering, 23. doi:10.1016/j.ymben.2014.02.007