**Intrinsic Properties Affecting Microbial Growth**

**1. Introduction**

Microorganisms play a crucial role in humans’ lives, but not always in beneficial impact. Microorganisms linked with foods can be characterized as spoilage, useful or pathogenic (1). Intrinsic factors associated with the food itself can affect microorganisms' growth (2)(3). The Intrinsic factors related to food are inherent, and they include water activity, PH, nutrient content, oxidation-reduction potential, etc. (4). Controlling these intrinsic factors can inhibit the growth of pathogens in food such as ***Escherichia coli K12,* and *Listeria innocua***. These microorganisms cause food-borne illnesses that make it crucial to study them to prevent their growth or include a kill step throw food processing to obtain safe food.

The experiment main objective was to examine the hypothesis of the effect of intrinsic factors such as pH and water activity on ***Escherichia coli K12,* and *Listeria innocua*** growth by using different concentrations of pH and (a w) by making serial dilutions and spread plate technique.

**2. Materials and Methods**

**A. pH effect on microbial growth:**

In the first week, 3 ml of a culture of ***Escherichia coli K12,* and *Listeria innocua*** separately, was provided. 0.1 ml of each culture was inoculated in 10 ml Trypticase Soy Broth (TSB) media presented in five tubes having the following pH levels of 3.0, 5.0, 7.0, 9.0, 11.0 at temperature 23 ℃. Then, the tubes were mixed by vortexing and incubated at 37 ℃ for 48 hours. Observations were recorded for growth (turbidity) and no growth (no turbidity) and were reported as (+) or (-) for each.

**B. Effect of Water Activity (a w) On Microbial Growth:**

3 ml of a culture of ***Escherichia coli K12,* and *Listeria innocua*** separately, was provided. 0.1 ml of each culture was inoculated in 10 ml Trypticase Soy Broth (TSB) media presented in five tubes having sodium chloride (salt) or glucose (sugar).

Salt have the following concentrations in the media 0% (a w 1.00) 1.74% (a w 0.98), 6.57% (a w 0.96), 11.57% (a w 0.94), 14.20% (a w 0.92) at temperature 21 ℃ and for sugar were 0% (a w 1.00), 15.5% (a w 0.98), 35.4% (a w 0.97), 41.0% (a w 0.96), 52.0% (a w 0.94) at temperature 22 ℃. Then, the tubes were mixed by vortexing and incubated at 37 ℃ for 48 hours. Observations were recorded for growth (turbidity) and no growth (no turbidity) and were reported as (+) or (-) for each.

For turbidity checking, the tube sets were observed without shaking it, and results were recorded. For determining the turbidity level, the following scale was used; (No turbidity:(-)0-25% turbidity: (+) 25-50% turbidity: (++) 50-75% turbidity: (+++) 75-100% turbidity: (++++). Then, the inoculated cultures' quantitative analysis was done by performing serial dilutions and spread plating the cultures in duplicate. Tubes of 0.1% peptone were prepared for the specific serial dilutions. Then, 0.1 ml of the inoculum was transferred from the serial dilutions into several Tryptic Soy Agar with Yeast Extract (TSAYE) plates. The Dilutions that were selected to perform spread plating are presented in Tables 1, 2, and 3. From every chosen dilution, spread plating was performed with a clean plastic spreader, and all plates were incubated at 37 ℃ for 48 hours. After the incubation period, the colonies were observed and counted colonies.

**Table 1. Dilutions with different pH levels used for spread plate technique inoculated with *Escherichia coli K12,* and *Listeria innocua***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Organisms | **PH** | | | | |
| **3%** | **5%** | **7%** | **9%** | **11%** |
| E. coli K12 | 0 to -2 | -4 to -6 | -4 to -6 | -4 to -6 | 0 to -2 |
| L. innocua | 0 to -2 | -3 to -5 | -4 to -6 | -4 to -6 | -2 to -4 |

**Table 2. Dilutions with different water activity levels by salt used for spread plate technique inoculated *with Escherichia coli K12,* and *Listeria innocua***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Organisms | **Salt** | | | | |
| **0%** | **15.50%** | **35.40%** | **41.00%** | **52.00%** |
| E. coli K12 | -4 to -6 | -4 to -6 | 0 to -2 | 0 to -2 | 0 to -2 |
| L. innocua | -4 to -6 | -3 to -5 | -3 to -5 | 0 to -2 | 0 to -2 |

**Table 3. Dilutions with different water activity levels by sugar used for spread plate technique inoculated with *Escherichia coli K12,* and *Listeria innocua***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Organisms | **Sugar** | | | | |
| **0%** | **15.50%** | **35.40%** | **41.00%** | **52.00%** |
| E. coli K12 | -4 to -6 | -3 to -5 | 0 to -2 | 0 to -2 | 0 to -2 |
| L. innocua | -4 to -6 | -2 to -4 | 0 to -2 | 0 to -2 | 0 to -2 |

**3. Results:**

**Table 4. *Escherichia coli K12,* and *Listeria innocua* inoculated for 48 hours at 37 °C in TSB media tubes at different pH and water activity levels with sugar and salt.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **pH** | | **Salt (%)** | | **Sugar (%)** | |
| 3 | **++** | 0 | **++++** | 0 | **++++** |
| 5 | **++** | 1.74 | **+++** | 15.50 | **++** |
| 7 | **+++** | 6.57 | **++** | 35.40 | **+** |
| 9 | **+++** | 11.57 | **+** | 41.00 | **+** |
| 11 | **++** | 14.20 | **-** | 52.00 | **-** |

Microorganisms growth was quantified by the turbidity and based on the results obtained from spread plate technique. The formula used was: **Number of colonies =Average number of colonies/ final dilution.**

**The Effect of pH on microbial growth (Graph 1):** The results obtained showed that Listeria ***innocua*** grew better at pH 9.02 and 6.98, and less at pH 3 while ***Escherichia coli K12*** showed stable growth for pH ranging in between 4.95 and 9.02. The highest growth of ***Escherichia coli K12*** was (9.2 log CFU/ml) at pH 9.02 and it decreased and increased as pH increased or decreased, however, at pH 3 its growth was the least. Average count of ***Escherichia coli K12*** in this range was approximately (8 log CFU/ml). In the degree of the growth rate of Escherichia coli K12, our results were similar to other reported studies, which is Escherichia coli K12 has a higher growth rate than Listeria at pH ranges from (5-9) (6). The results showed that in a high acidic degree (low pH), the less is the microbiological growth for both microorganisms.

**Graph 1. Growth of *Escherichia coli K12, Listeria innocua* observed at different pH levels when plated on TSAYE plate and incubated at 37 ° C for 48 hours.** **Each bar represents the mean for 4 or more replicates. Error bars, standard deviation.**

**Effect of salt on microbial growth (Graph 2): *Escherichia coli K12*** showed highest average growth at salt concentration and water activity at 1.74% (a w 0.98) which is (8.6 log CFU/ml) compared to ***Listeria innocua***, (6.8 log CFU/ml) at 6.57% (a w 0.96)**.** ***Escherichia coli K12*** growth decreased significantly as the water activity decreased. ***Listeria innocua*** showed significant amount of growth (8.3 log CFU/ml) at salt concentration and water activity of 6.57% (a w 0.96) and then decreased significantly from (3.4 log CFU/ml) at water activity of (a w 0.92). Other studies determined the same conclusion that ***Escherichia coli K12*** and ***listeria*** have a different level of tolerance to different water activity levels (7). In brief, rustles concluded that both microorganisms’ growth decreased with the increase of salt concentration.

**Graph 2. Growth of *Escherichia coli K12* and *Listeria innocua* observed at different water activity and salt concentrations levels 0% (a w1.00) 1.74% (a w 0.98), 6.57% (a w 0.96), 11.57% (a w .94), 14.20% (a w 0.92) when plated on TSAYE plate and incubated at 37 ° C for 48 hour. Each bar represents the mean for 4 or more replicates. Error bars, standard deviation.**

**The effect of sugar on microbial growth (Graph 3):** Based on the results both ***Escherichia coli K12* and *Listeria innocua*** showed the highest growth at a water activity of (a w 1.0) and lowest at (a w 0.94), where sugar concentration was the highest (52.0%). As the sugar concentration increased, microbial growth gradually decreased. Escherichia coli K12 showed maximum growth of (8.8 log CFU/ml) at a water activity of (a w 1.0) and zero growth at 52.0% (a w 0.94) when the sugar concentration was the highest. ***Listeria innocua*** at sugar concentration and water activity of 35.4% (a w 0.97), and 41.0% (a w 0.96) was higher than ***Escherichia coli K12*** at the same water activity level and did not decrease significantly when the water activity was the lowest. With the increase of sugar concentration, there was no microbial growth of ***Escherichia coli K12*** at water activity 0.94 than its growth with the increase of salt concentration. Thus, sugar has a more substantial effect on ***Escherichia coli K12*** than salt.

**Graph 3. Growth of *Escherichia coli K12* and *Listeria innocua* observed at different water activity and Glucose concentrations levels 0% (a w 1.00), 15.5% (a w 0.98), 35.4% (a w 0.97), 41.0% (a w 0.96), 52.0% (a w 0.94) when plated on TSAYE plate and incubated at 37 ° C for 48 hour. Each bar represents the mean for 4 or more replicates. Error bars, standard deviation.**

**4. Conclusion**

The experiments' results support the hypothesis of the effect of intrinsic factors, pH, and water activity on ***Escherichia coli K12*** and ***Listeria innocua*** growth rate. By the results obtained, pH and water activity have a significant role in the growth of microorganisms.

The results showed that with low pH, the less is the microbiological growth for both microorganisms. Sugar has more effect than salt in lowering the microbial growth by reducing the water activity on both ***Escherichia coli K12*** and ***Listeria innocua***. Thus, pH at a neutral level and high-water activity enhances microorganisms' growth and increases food spoilage.

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