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Relationships between Bacterial Zone of Inhibition and In-Situ Characteristics in *Citrus aurantifolia* Fruit Juice: A Linear Regression Perspective

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Abstract: *This study employs linear regression analysis to assess the intricate relationship between the bacterial zone of inhibition and in-situ properties (conductivity, total dissolved solids, salinity, and pH) in Citrus aurantifolia fruit juice. Test organisms, including Salmonella species, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, and Enterobacter aerogenes (dependent variables), were examined alongside in-situ attributes (predictors). Secondary data was utilized, and various linear regression tools were applied, such as Durbin-Watson statistics, R-values, R^2 values, Adjusted R^2, coefficient tables, etc. R-values were found to be higher than 0.40 for every test organism, except Proteus vulgaris, whose value was close to the threshold. R^2 values varied among bacteria types, ranging from 14.2% to 28.8%, indicating the extent to which in-situ parameters explain bacterial occurrence. The Adjusted R^2 values, considering the number of predictors, moderately explained microbial presence (ranging from 0.063 to 0.222). Change Statistics indicated that predictors significantly contributed to the model for all bacteria, highlighting the importance of in-situ variables in determining bacterial presence. Durbin-Watson statistics suggested positive serial correlations between outcomes and predictors, except for Staphylococcus aureus. Analysis of variance results showed varying models, with some statistically significant and others marginally fitting. The intricacy of these relationships was highlighted by the coefficient tables, which showed varying correlation strengths and directions between predictors and zone of inhibition values for various organisms. Therefore, to completely understand the complex relationship between the qualities of Citrus aurantifolia fruit juice and bacterial inhibitory zones, a microbiological study must take into account distinct in-situ features for various bacterial isolates.*

Keywords: *Regression analysis, Food Safety, Food Preservation, In-situ characteristics, Microorganisms, Citrus aurantifolia fruit juice*

1. INTRODUCTION

The process of extending the safety and shelf life of food products by the use of naturally occurring or intentionally created microbiota is called bio-preservation. Unlike traditional food preservation methods that involve the addition of chemicals, bio-preservation utilizes beneficial microbes to prevent the growth of harmful bacteria.

The production of antibiotic compounds by some microbes is one of the primary bio-preservation techniques. For example, because lactic acid bacteria can produce organic acids such as lactic acid, which inhibit the growth of harmful bacteria by forming an acidic environment, they are often used in bio-preservation. Additionally, several microorganisms produce proteinaceous compounds called

bacteriocins, which possess antibacterial properties and can selectively target and halt the progression of specific diseases (Tang et al. 2022).

Another aspect of bio-preservation is manipulating microbial activity to counteract deteriorating species. This could be accomplished by optimizing environmental factors like pH, oxygen levels, and temperature to encourage the growth of helpful bacteria while preventing the growth of rotting microorganisms. Furthermore, the use of certain probiotic bacterial strains for bio-preservation has gathered more attention (Naghmouchi et al. 2020). Probiotics can enhance consumer health in addition to promoting food safety. These microbes may enhance the nutritional profile of the food product and the health of the digestive system overall.

Additionally, plants are a rich source of naturally occurring chemicals that have the potential to be employed as bio-preservatives since they have a wide spectrum of secondary metabolites that include antibacterial properties (Epidi et al. 2016a,b). These compounds not only help plants defend themselves against pests and diseases, but they may also find application in food preservation. Leading examples include rosemary, which is renowned for its antimicrobial components, including essential oils and rosmarinic acid (Nieto et al. 2018). Meat products, oils, and other perishable goods can be preserved with rosemary extracts due to its capacity to efficiently inhibit the growth of bacteria and fungi.

Citrus aurantifolia, also known as lime, is a strong contender for natural food preservation because of its impressive bio-preservative capacity. This citrus species belongs to the Rutaceae family and is well-known for its culinary and medicinal uses (Izah and Odubo, 2023). The bio-preservative properties of *Citrus aurantifolia* fruits, particularly in the peel and juice, are attributed to a range of bioactive compounds found in the fruit.

*Citrus aurantifolia*is a plant that possesses bio-preservative potentials, probably due to its ability to produce organic acids, especially citric acid. Typically, citrus acid raises the acidity of the fruit which inhibits the growth of harmful bacteria and the process of spoiling. Food products have a longer shelf life and are naturally preserved by inhibiting the growth of microorganisms due to their acidic pH (Devlieghere et al. 2004; Reis et al. 2012)

In addition to organic acids, *Citrus aurantifolia*is a substantial source of essential oils. These oils contain bioactive compounds like citral, limonene, and linalool that have antibacterial properties (Lemes et al. 2018). Studies have shown that the essential oils of *Citrus aurantifolia*can inhibit a variety of bacteria and fungi, including those that cause food deterioration and spoilage (Ajayi-Moses et al. 2019). The antimicrobial properties of essential oils make them valuable for bio-preservation applications. The flavonoid and polyphenol content of *Citrus aurantifolia* contributes to its increased bio-preservation capacity (Medeleanu et al. 2023; Izah and Odubo 2023). These materials have been shown to possess antioxidant properties, which may help stop food products from oxidatively decaying. Furthermore, during the fermentation of *Citrus aurantifolia*, lactic acid is produced by lactic acid bacteria, which can be added or exist naturally. Acidification can create unique flavors and textures and increase food product safety by creating an unfavorable environment for bacteria (de Souza et al. 2023).

The bacterial zone of inhibition is a crucial indicator of how effectively *Citrus aurantifolia*fruit juice can prevent bacterial growth and multiplication. The bioactive components of the juice, which include flavonoids, organic acids, and other secondary metabolites, are commonly held accountable for this phenomenon.

In-situ characteristics of *Citrus aurantifolia* fruit juice involves a wide range of parameters, including pH, conductivity, salinity, total dissolved solid, and temperature (Izah and Odubo, 2023). Determining the parameters that contribute to fruit juice's antibacterial action can be greatly aided by knowing the association between these factors and the inhibition exhibited by specific bacteria.

Therefore, this study focuses on the assessment of the association between the bacterial zone of inhibition and in-situ characteristics in *Citrus aurantifolia*fruit juice through a linear regression perspective. The study may have practical implications for food safety and preservation. By creating a quantifiable relationship between the in-situ characteristics of *Citrus aurantifolia* fruit juice and its

inhibitory effects on bacteria, food scientists may be able to predict these effects. This information could be used to develop natural bio-preservatives made from plants and better preservation methods for a variety of food products.

2. METHODOLOGY

Citrus aurantifolia fruit juice has demonstrated antimicrobial properties and potential as a bio-preservative, particularly in nutritive beverages (Izah et al., 2016). In a recent study by Izah and Odubo (2023), the in-situ characteristics, including pH, conductivity, salinity, and total dissolved solids, of *Citrus aurantifolia* fruit juice were measured under different temperature conditions. The recorded values fell within the ranges of 2.52 – 2.74 for pH, 5.06 – 5.85 mS/cm for conductivity, 2.58 – 2.95 ppt for salinity, and 3.58 – 4.29 ppt for total dissolved solids.

The same fruit juices displayed varying degrees of zone of inhibition against several bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Salmonella* species. The minimum inhibitory concentrations and Minimum Bactericidal Concentration values ranged from 12.50% to 50.00% (Izah and Odubo, 2023).

In this study, we utilized the data reported by Izah and Odubo (2023) to investigate the relationship between dependent variables (the test organisms) and predictors (the in-situ characteristics) using a linear regression model. Statistical analysis was performed using SPSS version 20.

3. RESULTS AND DISCUSSION

Tables 1, 2, 3, and 4 present the Pearson correlation, model summary, analysis of variance, and coefficient statistics of the linear regression analysis of bacterial isolates. Thus, *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Staphylococcus aureus* (dependent variables), and in situ characteristics, thus, total dissolved solid (TDS), pH, salinity, and conductivity (predictor variables) of the *Citrus aurantifolia* fruit.

Table1. *Pearson correlation of the bacterial isolates and the in-situ characteristics of the Citrus aurantifolia fruit juice. N=48.*

		Bacterial isolates	pH	Conductivity	Salinity	TDS
Pearson Correlation	*Escherichia coli*	1.000				
	pH	-.279	1.000			
	Conductivity	.281	-.218	1.000		
	Salinity	.283	-.189	.655	1.000	
	TDS	.060	-.154	.664	.411	1.000
Sig. (1-tailed)	*Escherichia coli*	.				
	pH	.028				
	Conductivity	.026	.068	.		
	Salinity	.026	.099	.000		
	TDS	.343	.148	.000	.002	.
Pearson Correlation	*Salmonella* species	1.000				
	pH	-.440	1.000			
	Conductivity	.070	-.218	1.000		
	Salinity	.035	-.189	.655	1.000	
	TDS	.092	-.154	.664	.411	1.000
Sig. (1-tailed)	*Salmonella* species	.				
	pH	.001				
	Conductivity	.319	.068	.		
	Salinity	.406	.099	.000		
	TDS	.266	.148	.000	.002	.
Pearson Correlation	*Pseudomonas aeruginosa*	1.000				
	pH	.327	1.000			
	Conductivity	-.375	-.218	1.000		
	Salinity	-.398	-.189	.655	1.000	
	TDS	-.301	-.154	.664	.411	1.000
Sig. (1-tailed)	*Pseudomonas aeruginosa*	.				
	pH	.012	.			
	Conductivity	.004	.068			
	Salinity	.003	.099	.000	.	
	TDS	.019	.148	.000	.002	.

Table1. *(Contd): Pearson correlation of the bacterial isolates and the in-situ characteristics of the Citrus aurantifolia fruit juice. N=48*

		Bacterial isolates	pH	Conductivity	Salinity	TDS
Pearson Correlation	*Enterobacteraerogenes*	1.000				
	pH	-.516	1.000			
	Conductivity	.154	-.218	1.000		
	Salinity	.035	-.189	.655	1.000	
	TDS	.168	-.154	.664	.411	1.000
Sig. (1-tailed)	*Enterobacteraerogenes*	.				
	pH	.000				
	Conductivity	.149	.068	.		
	Salinity	.408	.099	.000		
	TDS	.126	.148	.000	.002	.
Pearson Correlation	*Proteus vulgaris*	1.000				
	pH	-.361	1.000			
	Conductivity	.097	-.218	1.000		
	Salinity	.008	-.189	.655	1.000	
	TDS	.111	-.154	.664	.411	1.000
Sig. (1-tailed)	*Proteus vulgaris*	.				
	pH	.006				
	Conductivity	.257	.068	.		
	Salinity	.477	.099	.000		
	TDS	.226	.148	.000	.002	.
Pearson Correlation	*Staphylococcus aureus*	1.000				
	pH	.015	1.000			
	Conductivity	-.401	-.218	1.000		
	Salinity	-.468	-.189	.655	1.000	
	TDS	-.107	-.154	.664	.411	1.000
Sig. (1-tailed)	*Staphylococcus aureus*	.				
	Ph	.459				
	Conductivity	.002	.068	.		
	Salinity	.000	.099	.000		
	TDS	.235	.148	.000	.002	.

The Pearson correlation showed that *Escherichia coli* positively correlates statistically ($p = 0.05$) with conductivity, and salinity, and negatively with pH at $p<0.05$. *Salmonella* species, *Proteus vulgaris,* and *Enterobacter aerogenes* showed no statistical relationship at $p = 0.05$ with the in situ characteristics except for pH, which negatively correlates significantly with the test isolate at $p = 0.01$. The *Pseudomonas aeruginosa* correlates negatively with all the in situ characteristics at $p = 0.05$ except pH it positively correlates with. *Staphylococcus aureus* showed no statistical association with pH and total dissolved solids but correlated negatively with salinity and conductivity (Table 1).

The correlation helps in understanding how these bacterial zones of inhibition respond to specific in situ characteristics, which can be valuable information for various fields such as microbiology, ecology, or food science. The p-value and the direction (positive and negative) indicate the strength and direction of the relationships between different bacterial zones of inhibition and in-situ characteristics. For example, *Escherichia coli* tends to increase in concentration with higher conductivity and salinity but decreases as pH becomes more alkaline. On the other hand, *Pseudomonas aeruginosa* generally decreases with changes in the in-situ characteristics except for pH, where it increases. *Staphylococcus aureus,* however, does not show a significant relationship with pH and total dissolved solids but tends to decrease with higher salinity and conductivity.

The R-value shows the correlation between the dependable variables (bacterial isolates) and predictors (in situ parameters) for each type of test organism. When the R-values are greater than 0.40, they can be taken for analysis. However, in this study, the R-values are 0.413 (*Escherichia coli*), 0.446 (*Salmonella* species), 0.494 (*Pseudomonas aeruginosa*), 0.537 (*Enterobacter aerogenes*), 0.377 (*Proteus vulgaris),* and 0.532 (*Staphylococcus aureus*). However, the R-value for *Proteus vulgaris*is less than 0.400, but it was considered for the study since it was close to the limit (Table 2).

The proportion of variance in the dependent variables for test organisms that is explained by the predictors (in situ characteristics) is measured by the value of R squared (R^2). The R^2 values for each bacterium (*Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Staphylococcusaureus*) indicate the proportion of variability in their occurrence that can be explained by the in situ characteristics. The R^2 was calculated to be 17.0% (*Escherichia coli*),

19.9% (*Salmonella*species), 24.4% (*Pseudomonas aeruginosa*), 28.8% (*Enterobacter aerogenes*), 14.2% (*Proteus vulgaris)*, and 21.6% (*Staphylococcus aureus*). Thus, the values show the level at which the data fit the linear regression model, although the best isolates that fit the model are in the following order: *Enterobacter aerogenes*>*Staphylococcus aureus*>*Pseudomonas aeruginosa*> *Salmonella* species>*Escherichia coli*>*Proteus vulgaris.* Based on the R^2 values, it shows that the predictors (in situ parameters) do not show much variability in the dependable variable (test bacterial*)* despite the statistical dissimilarity. Thus suggesting a moderate explanatory power of the model.

The Adjusted R^2 values provide a more conservative measure, considering the number of predictors in the model. The Adjusted R^2 ranges from 0.063 to 0.222, suggesting that, after adjusting for the number of predictors, the model still explains a moderate amount of variability in microbial occurrence.

The Change Statistics for R^2Change and F Change provide insights into the contribution of each predictor (TDS, pH, Salinity, Conductivity) to the model. For all bacteria, the predictors collectively contribute significantly to the model. This suggests that these in situ characteristics play a role in determining the presence of bacteria in the fruit juice.

To determine whether the variables were still related to one another once the regression model was finished, the Durbin-Watson statistics were also run, and the values were 1.101 (*Escherichia coli*), 1.469 (*Salmonella* species), 0.828 (*Pseudomonas aeruginosa*), 1.240 (*Enterobacter aerogenes*), 1.080 (*Proteus vulgaris)*, and 1.606 (*Staphylococcus aureus*). These values are an indication of a positive serial correlation between the probable outcome and the predictors, except for *Staphylococcus aureus,* which is acceptable because of no serious autocorrelation concern. The analysis of variance revealed that the statistical variation: F-value = 2.207, p-value = 0.084 for *Escherichia coli*, F-value = 2.670, P-value = 0.045for *Salmonella* species, F-value = 3.469, P-value = 0.015 for *Pseudomonas aeruginosa,* F-value = 4.357, p-value = 0.005 for *Enterobacter aerogenes*,F-value = 1.785, p-value = 0.149 for *Proteus vulgaris,* and F-value = 4.238, p-value = 0.006 for *Staphylococcus aureus*.

For *Escherichia coli*, the F-value is relatively small, indicating a moderate overall fit of the model. The associated p-value (0.084) is greater than 0.05, suggesting that the overall model might not be statistically significant at the conventional significance level. The F-value for *Salmonella* species is larger than in the previous case, indicating an improved fit of the model. The associated p-value (0.045) is less than 0.05, suggesting that the overall model is statistically significant. The F-value for *Pseudomonas aeruginosa* is further increased, indicating a better fit of the model. The associated p-value (0.015) is less than 0.05, indicating that the overall model is statistically significant. The F-value for *Enterobacter aerogenes* is higher, suggesting a strong fit of the model. The associated p-value (0.005) is less than 0.05, indicating a statistically significant overall model. The F-value for *Proteus vulgaris* is relatively small, indicating a modest fit of the model. The associated p-value (0.149) is greater than 0.05, suggesting that the overall model might not be statistically significant. The F-value for *Staphylococcus aureus* is high, indicating a strong fit for the model. The associated p-value (0.006) is less than 0.05, indicating a statistically significant overall model.

Table2. *Analysis of Variance model summary of the microbes and in situ characteristics of the Citrus aurantifolia fruit juice*

Model Summary										
Microbes	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics					Durbin-Watson
					R Square Change	F Change	df1	df2	Sig. F Change	
Escherichia coli	.413[a]	.170	.093	4.14238	.170	2.207	4	43	.084	1.101
*Salmonella*species	.446[a]	.199	.124	3.94276	.199	2.670	4	43	.045	1.469
Pseudomonas aeruginosa	.494[a]	.244	.174	3.84231	.244	3.469	4	43	.015	.828
Enterobacteraerogenes	.537[a]	.288	.222	3.38239	.288	4.357	4	43	.005	1.240
Proteus vulgaris	.377[a]	.142	.063	5.00097	.142	1.785	4	43	.149	1.080
Staphylococcus aureus	.532[a]	.283	.216	2.86256	.283	4.238	4	43	.006	1.606

a. Predictors: (Constant), TDS, pH, Salinity, Conductivity

b. Dependent Variable: bacterial isolates (Escherichia coli, Salmonellaspecies, Pseudomonas aeruginosa, Enterobacteraerogenes, Proteus vulgarisand Staphylococcus aureus)

Overall, the F-value is usually used to show an enhancement in the prediction of the variable by fitting the model after considering the inaccuracy present in the model. An F-value higher than 1 yields an acceptable model. For the study, the values were higher than 1 for each of the test organisms (Table 3). This suggests that the values are acceptable as a predictive tool, and hence the data employed in the statistical analysis were acceptable, especially the ones that showed statistical deviation based on the analysis of variance.

Table3. *ANOVA Table for the linear regression analysis of the in situ characteristics of the Citrus aurantifolia fruit juice*

			ANOVA[a]			
Microbes		Sum of Squares	Df	Mean Square	F	Sig.
Escherichia coli	Regression	151.461	4	37.865	2.207	.084[b]
	Residual	737.851	43	17.159		
	Total	889.312	47			
Salmonella species	Regression	166.028	4	41.507	2.670	.045[b]
	Residual	668.451	43	15.545		
	Total	834.479	47			
Pseudomonas aeruginosa	Regression	204.842	4	51.211	3.469	.015[b]
	Residual	634.824	43	14.763		
	Total	839.667	47			
Enterobacteraerogenes	Regression	199.369	4	49.842	4.357	.005[b]
	Residual	491.943	43	11.441		
	Total	691.313	47			
Proteus vulgaris	Regression	178.563	4	44.641	1.785	.149[b]
	Residual	1075.417	43	25.010		
	Total	1253.979	47			
Staphylococcus aureus	Regression	138.898	4	34.724	4.238	.006[b]
	Residual	352.352	43	8.194		
	Total	491.250	47			

a. Predictors: (Constant), TDS, pH, Salinity, Conductivity

b. Dependent Variable: bacterial isolates (Escherichia coli, Salmonella species, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus vulgarisand Staphylococcus aureus)

The coefficient table is usually used to indicate the strength of the correlation that exists between the dependable variable and predictors, the implication of the variable in the model, and the magnitude with which it impacts the dependable variable (Henseler and Fassott, 2010).

Based on the coefficient tables, the regression model's constant term was found to be 22.365, 72.820, 36.553, 71.598, 71.012, and 66.952 for *Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, and *Staphylococcus aureus*, respectively (Table 4). The dependable variable for the regression model was determined to be test organisms.

For *Escherichia coli*, the unstandardized coefficient for PH is -9.583, indicating that a one-unit decrease in pH is associated with a 9.583 unit increase in *Escherichia coli* count. However, the result is not statistically significant (p = 0.125). The unstandardized coefficient FOR conductivity is 5.034, suggesting that a one-unit increase in conductivity is associated with a 5.034 unit increase in *Escherichia coli* count. Again, the result is not statistically significant (p = 0.211). The unstandardized coefficient for salinity is 5.184, but the result is not statistically significant (p = 0.435). The unstandardized coefficient for Total Dissolved Solids is -4.745, and the result is not statistically significant (p = 0.234).

Furthermore, similar interpretations can be made for *Salmonella* species, where pH has a significant negative association (coefficient: -18.619, p = 0.003), and other factors (Conductivity, Salinity, and Total Dissolved Solids) are not statistically significant.

For *Pseudomonas aeruginosa*, A one-unit increase in pH is associated with a 10.178 unit increase in *Pseudomonas aeruginosa* count, but the result is not statistically significant (p = 0.080). The unstandardized coefficient for conductivity is -1.530, and the result is not statistically significant (p = 0.679). The unstandardized coefficient for salinity is -8.748, and the result is not statistically

significant (p = 0.159). The unstandardized coefficient for total dissolved solid is -2.065, and the result is not statistically significant (p = 0.574).

For, *Enterobacter aerogenes*, A significant negative association is observed (coefficient: -19.427, p < 0.001) for pH. The unstandardized coefficient for conductivity is positive (1.123), but the result is not statistically significant (p = 0.730). Both salinity and total dissolved solid are not statistically significant in *Enterobacter aerogenes* count.

For *Proteus vulgaris*, A significant negative association is observed (coefficient: -18.444, p = 0.016) for PH. The unstandardized coefficient for conductivity is positive (1.026), but the result is not statistically significant (p = 0.831). Both salinity and total dissolved solid are not statistically significant in *Proteus vulgaris* count.

For *Staphylococcus aureus*, the unstandardized coefficient of pH is -2.881, and the result is not statistically significant (p = 0.500). The unstandardized coefficient for conductivity is -4.770, but the result is marginally significant (p = 0.089). A significant negative association is observed (coefficient: -9.447, p = 0.044) for salinity. The unstandardized coefficient for total dissolved solid is positive (4.213), but the result is not statistically significant (p = 0.128).

Typically, a positive unstandardized coefficient for a predictor variable means that there is a positive relationship between that predictor and the dependent variable (Menard 2011; Sharma and Rani 2013). In other words, as the value of the predictor variable increases by one unit, the expected change in the dependent variable is in the same direction (an increase). Conversely, a negative unstandardized coefficient for a predictor variable indicates a negative relationship between that predictor and the dependent variable (Wood 2006; Shanock et al. 2010). In this case, as the value of the predictor variable increases by one unit, the expected change in the dependent variable is in the opposite direction (a decrease).

Tolerance is the proportion of variance in a predictor variable that is not explained by the other predictor variables in the model (Mohammadi et al. 2003; Lavery et al. 2019). A lower tolerance value indicates higher collinearity. For all predictor variables in each model (*Escherichia coli*, *Salmonella* species, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Staphylococcus aureus*), pH tolerance is 0.948, suggesting that 94.8% of the variance in pH is not explained by the other predictors. Conductivity tolerance is 0.380, indicating that 38.0% of the variance in conductivity is not explained by the other predictors. Salinity tolerance is 0.567, meaning that 56.7% of the variance in salinity is not explained by the other predictors. Total Dissolved Solids tolerance is 0.558, indicating that 55.8% of the variance in total dissolved solids is not explained by the other predictors.

Variance Inflation Factor (VIF) is a measure that quantifies how much the variance of the estimated coefficients is increased due to multicollinearity (Tamura et al. 2019; Zainodin et al. 2015). A VIF value greater than 1 suggests some level of multicollinearity, with higher values indicating more severe collinearity (Ovharhe et al. 2022). Thus, VIF (1.054) for PH suggests that the variance of the pH coefficient is not substantially inflated by multicollinearity. The VIF (2.634) for conductivity, indicates moderate inflation of the variance of the conductivity coefficient. The VIF (1.763) for salinity suggests moderate inflation of the variance of the salinity coefficient. The VIF (1.792) for total dissolved solids indicates moderate inflation of the variance of the total dissolved solids coefficient. The values for pH have high Tolerance (close to 1) and low VIF, indicating low multicollinearity. pH does not share much variance with the other predictors. The values for conductivity, salinity, and total dissolved solids show moderate multicollinearity, as reflected by lower Tolerance values and higher VIF values. This suggests that these variables might share some variance with other predictors.

PH appears to be a significant predictor for the counts of *Salmonella* species, *Enterobacteraerogenes*, and *Proteus vulgaris*. Conductivity, salinity, and total dissolved solids show varying degrees of association with bacterial counts, but the significance varies across different bacteria types. Furthermore, not all predictors are statistically significant for each bacterium, emphasizing the importance of considering specific in situ characteristics for different bacterial isolates.

Table4. *Linear regression coefficient table of the in situ characteristics of the Citrus aurantifolia fruit juice*

Coefficients													
Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.	95.0% Confidence Interval for B		Correlations			Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
Escherichia coli	(Constant)	22.365	25.271		.885	.381	-28.599	73.328					
	pH	-9.583	6.122	-.223	-1.565	.125	-21.929	2.763	-.279	-.232	-.217	.948	1.054
	Cond	5.034	3.965	.286	1.270	.211	-2.962	13.029	.281	.190	.176	.380	2.634
	Sal	5.184	6.585	.145	.787	.435	-8.096	18.464	.283	.119	.109	.567	1.763
	TDS	-4.745	3.932	-.224	-1.207	.234	-12.674	3.185	.060	-.181	-.168	.558	1.792
Salmonella species	(Constant)	72.820	24.053		3.028	.004	24.313	121.327					
	pH	-18.619	5.827	-.448	-3.195	.003	-30.371	-6.868	-.440	-.438	-.436	.948	1.054
	Cond	-.734	3.774	-.043	-.195	.847	-8.345	6.877	.070	-.030	-.027	.380	2.634
	Sal	-1.770	6.268	-.051	-.282	.779	-14.410	10.870	.035	-.043	-.039	.567	1.763
	TDS	1.493	3.742	.073	.399	.692	-6.054	9.040	.092	.061	.054	.558	1.792
Pseudomonas aeruginosa	(Constant)	36.553	23.440		1.559	.126	-10.718	83.825					
	pH	10.178	5.679	.244	1.792	.080	-1.274	21.630	.327	.264	.238	.948	1.054
	Cond	-1.530	3.678	-.090	-.416	.679	-8.947	5.887	-.375	-.063	-.055	.380	2.634
	Sal	-8.748	6.108	-.252	-1.432	.159	-21.066	3.570	-.398	-.213	-.190	.567	1.763
	TDS	-2.065	3.647	-.101	-.566	.574	-9.420	5.290	-.301	-.086	-.075	.558	1.792
Enterobacteraerogenes	(Constant)	71.598	20.634		3.470	.001	29.985	113.211					
	pH	-19.427	4.999	-.513	-3.886	.000	-29.508	-9.346	-.516	-.510	-.500	.948	1.054
	Cond	1.123	3.237	.072	.347	.730	-5.406	7.652	.154	.053	.045	.380	2.634
	Sal	-4.799	5.377	-.152	-.892	.377	-15.642	6.045	.035	-.135	-.115	.567	1.763
	TDS	1.935	3.210	.104	.603	.550	-4.539	8.410	.168	.092	.078	.558	1.792
Proteus vulgaris	(Constant)	71.012	30.508		2.328	.025	9.486	132.539					
	pH	-18.444	7.391	-.362	-2.495	.016	-33.350	-3.539	-.361	-.356	-.352	.948	1.054
	Cond	1.026	4.787	.049	.214	.831	-8.627	10.680	.097	.033	.030	.380	2.6 34
	Sal	-5.178	7.950	-.122	-.651	.518	-21.210	10.855	.008	-.099	-.092	.567	1.763
	TDS	1.832	4.747	.073	.386	.701	-7.740	11.405	.111	.059	.055	.558	1.792
Staphylococcus aureus	(Constant)	66.952	17.463		3.834	.000	31.735	102.170					
	pH	-2.881	4.231	-.090	-.681	.500	-11.413	5.651	.015	-.103	-.088	.948	1.054
	Cond	-4.770	2.740	-.365	-1.741	.089	-10.296	.755	-.401	-.257	-.225	.380	2.634
	Sal	-9.447	4.551	-.356	-2.076	.044	-18.624	-.269	-.468	-.302	-.268	.567	1.763
	TDS	4.213	2.717	.268	1.551	.128	-1.267	9.692	-.107	.230	.200	.558	1.792

a. Dependent Variable: bacterial isolates (Escherichia coli, Salmonellaspecies, Pseudomonas aeruginosa, Enterobacteraerogenes, Proteus vulgarisand Staphylococcus aureus)

4. CONCLUSION

The study assessed the relationships between the in-situ characteristics of *Citrus aurantifolia* fruit juice and the bacterial zone of inhibition using a linear regression technique. The study yielded some fascinating findings on the association between bacterial zone of inhibition and in-situ variables such as conductivity, salinity, pH, and total dissolved solid. Many test species had R-values (shows the strength of the association) greater than the 0.40 limit. *Proteus vulgaris* was included in the analysis since its R-value was so near to the cutoff. In addition, the R^2 values showed that some isolates fit the model fairly well, while others only displayed weaker associations. The findings of the study also demonstrated the significance of certain predictors, such as pH, conductivity, salinity, and total dissolved solids, in influencing the bacterial zone of inhibition. While pH was found to be a significant predictor for several different species of bacteria, other predictors varied in significance, indicating the need to consider specific in-situ parameters for different bacterial isolates. The analysis also revealed multi-collinearity in several predictors, emphasizing how important it is to understand how these factors interact to predict bacterial zones of inhibitions.

Since the study provides valuable insights into the complex relationship between the number of bacteria in *Citrus aurantifolia* fruit juice and its in-situ characteristics, it is crucial to have a thorough understanding of these associations and how they impact the assurance of food safety and quality.

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