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Quality Evaluation of Honduran Bottled Water for Human Consumption through Experimental Design.

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Abstract - The increase in bottled water consumption in Honduras raises concerns about public safety. This study evaluates the quality of four local brands in Tegucigalpa through an experimental design, revealing potential contaminations, particularly in elevated total coliforms in eight samples. Numerical analyses, including analysis of variance and Fisher's LSD, show significant differences in total dissolved solids (TDS) among the brands (A: 120.50, B: 53.3, C: 23.33, D: 1.33) with units of mh/L, indicating variations in treatment processes. Although some bottled water brands meet certain standard parameters, some samples from different brands analysed in this study exhibit elevated levels of total coliforms without the presence of E. Coli, specifically, highlighting the need to investigate other bacterial genres in these or other brands. The correlation analysis underscores inverse relationships between pH and total coliforms/aerobic bacteria, indicating that higher values of these parameters lead to a decrease in pH, signifying increased acidity. This emphasizes the importance of future research. Validation by experts in experimental design enhances credibility, as both the sample collection and analysis underwent the necessary supervision and rigor to reveal reliable data. The findings emphasize the urgency of maintaining strict quality standards for bottled water in Honduras. This study, enriched with numerical analyses, reinforces the need for continuous safety assessments for the benefit of public health.

***Keywords*: **analysis of variance, correlation coefficient, total dissolved solids, pH, aerobic bacteria, total coliforms, E. Coli

1. Introduction

The problem addressed in this article is as follows: Due to various factors related to different causes, the consumption of bottled water in Honduras has increased. There is documented evidence asserting that this bottled water could have some level of contamination, posing a risk to public health and not complying with the technical regulations for the quality of bottled water and ice for direct and indirect human consumption [1]. Water is vital for human health, and potential contamination of bottled water represents a significant risk. Exposure to contaminants during bottling could include heavy metals, chemicals, and pathogenic microorganisms [2]. The research seeks to determine if existing regulations are adequately met, influencing policies and consumer perception.

In Buenos Aires, Argentina [3], a clinical review was conducted to assess the microbiological risks of drinking water. They used bacterial indicators, including E. coli, to detect fecal contamination, employing the Colilert 18/Quanti-Tray system. This semi-automatic enzyme-based method allows simultaneous detection of coliforms and E. coli within an incubation period of 18-22 hours.

Similarly, in Ecuador [4], 36 water purification plants in Portoviejo were investigated to identify reasons for non-compliance with Hygienic Sanitary Conditions (HSC). The survey of owners and administrators revealed that these plants did not comply with HSC, Levels I and II, according to the analysis with SPSS.

In Honduras itself [5], a student from the Pan-American Agricultural School, Zamorano, investigated the bacteriological quality of bottled water in the central-eastern region. Samples were analyzed in their raw, purified, and bottled states at intervals. Using the Quanty-Tray® method, it was found that 72% of the bottles had total coliforms, and 8% contained E. coli, exceeding the permissible limits for human consumption.

With the above, the general objective of the research was to evaluate bottled water for human consumption from four different brands sold in supermarkets and stores in Tegucigalpa, registered with ARSA, through an experimental design.

The reader will find in Chapter 2 the methodology used, including the approach, scope, variables, and instruments and techniques used; in Chapter 3, the results and analysis of these; Chapter 4, conclusions; Chapter 5, research recommendations; Chapter 6, the applicability of the research; and Chapter 7, proposed future work to deepen the conducted research.

2. Metodology

2.1. Focus and Scope

The research adopted a quantitative approach by collecting data through a single-factor design to assess bottled water from various brands in Tegucigalpa, Honduras [6]. Analysis of variance (ANOVA) and comparative statistical techniques were employed to analyze the data according to the parameters of the Technical Regulation for the Quality of Bottled Water and Ice for Direct and Indirect Human Consumption.

The scope of the research was correlational [7], measuring the quality of bottled water in relation to human well-being through physicochemical and microbiological analyses within a single-factor design. This approach addressed the research questions, determining if the bottled water from the evaluated companies is suitable for human consumption and complies with the standards of the Technical Regulation for the Quality of Bottled Water and Ice for Direct and Indirect Human Consumption.

2.2. Population and Sample

The delimited population of the research included four Honduran bottled water brands ranging from 600ml to 750ml, widely consumed in the city of Tegucigalpa, and registered with the Regulatory Sanitary Agency (ARSA) in Honduras. For each brand, six units were selected, each with a different batch number.

The non-probabilistic convenience sampling technique [8] was employed due to economic constraints, limited laboratory availability, restricted communication access, and low market availability.

2.3. Analyzed Variables

The independent variable in the research project is the brand of bottled water sold in Tegucigalpa, denoted as Brands A, B, C, and D. These are kept as variables because ARSA prohibited the disclosure of brand names. The dependent variable is established as the quality of bottled water assessed through the following tests: pH tests, Total Coliforms, Total Dissolved Solids tests, E. Coli tests, and tests for Aerobic Bacteria.

2.4. Instruments and Applied Techniques

For data collection, Microsoft Excel [9] was utilized, along with the statistical comparison mentioned in Specific Objective 2 concerning compliance with the Regulations for Bottled Water and Ice for direct and indirect human consumption. Additionally, the statistical software MINITAB [10] was used to conduct analyses of variances mentioned in Specific Objective 1 and correlation analyses between parameters.

3. Results and Analysis

3.1 Test Measurement and Analysis of Variances

3.1.1 Physicochemical Analysis and Parameter Measurement

Measurements of total dissolved solids were conducted using a TDS meter; total coliforms were measured using the multiple tube technique; Escherichia Coli was measured through the membrane filtration technique; aerobic bacteria were measured using the plate count technique, and pH was measured using a pH meter. Tables 1, presents the results of the tests conducted for each evaluated brand, detailing the outcomes for each with six samples from different batches.

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Table 1 Data Collection

	Dissolved Solids (mg/L)				Total Coliforms			
Brand	A	B	C	D	A	B	C	D
Sample 1	19	112	59.8	2	4.5	<1.8	7.8	<1.8
Sample 2	25	108	48.8	1	<1.8	<1.8	<1.8	<1.8
Sample 3	24	118	50.3	1	<1.8	<1.8	6.8	<1.8
Sample 4	20	139	52	2	<1.8	2	<1.8	<1.8
Sample 5	25	110	50.5	1	<1.8	<1.8	2	2
Sample 6	27	136	59.8	1	2	<1.8	4.5	4.5

	E.Coli				Aerobic Bacterias (UFC)				PH			
Brand	A	B	C	D	A	B	C	D	A	B	C	D
Sample 1	0	0	0	0	6	0	0	38	6.35	8.21	6.63	7.24
Sample 2	0	0	0	0	0	0	0	7	6.4	8.31	6.68	7.26
Sample 3	0	0	0	0	0	0	0	47	6.19	8.43	6.66	6.7
Sample 4	0	0	0	0	0	1.5	0	12	6.25	8.07	6.65	7.3
Sample 5	0	0	0	0	0	0	0	2.5	6.38	8.38	6.64	6.65
Sample 6	0	0	0	0	0.5	0	0	8.5	6.34	8.1	6.64	6.51

3.1.2 Analysis of Variances

The research conducted a one-way analysis of variance (ANOVA) using Fisher's LSD method to assess whether the averages of the four Honduran water brands are equal based on the conducted tests, setting a confidence level of 95%. The hypotheses were defined as follows:

- Null Hypothesis (Ho): The average of all brands is equal.
- Alternative Hypothesis (Ha): At least one brand has a different average.

Figures 1 to 8 below, present the analysis conducted for each parameter. Starting with Total Dissolved Solids:

- Total dissolved solids

Análisis de Varianza

Fuente	GL	SC Ajust.	MC Ajust.	Valor F	Valor p
Marca	3	48371	16123.7	292.86	0.000
Error	20	1101	55.1		
Total	23	49472			

Agrupar información utilizando el método LSD de Fisher y una confianza de 95%

Factor	N	Media	Agrupación			
Marca B	6	120.50	A			
Marca C	6	53.53		B		
Marca A	6	23.33			C	
Marca D	6	1.333				D

Fig. 1 Dissolved Solids: ANOVA / Fisher LSD

The null hypothesis (Ho) is rejected, indicating that at least one brand has significantly different average Total Dissolved Solids (SDT). Furthermore, none of the means share a grouping letter in the Fisher's method, confirming their significant differences. This suggests that companies likely employ different treatments for controlling total dissolved solids, and some treatments may be more effective than others.

- Total Coliforms

Análisis de Varianza

Fuente	GL	SC Ajust.	MC Ajust.	Valor F	Valor p
Marca	3	19.27	6.424	2.58	0.082
Error	20	49.80	2.490		
Total	23	69.07			

Agrupar información utilizando el método LSD de Fisher y una confianza de 95%

Factor	N	Media	Agrupación	
Marca C	6	4.08	A	
Marca D	6	2.217	A	B
Marca A	6	2.217	A	B
Marca B	6	1.7500		B

Fig. 2 Total Coliforms: ANOVA / Fisher LSD

The null hypothesis (Ho) is accepted, indicating that the average total coliforms of all brands are statistically equal. This suggests that companies likely employ similar treatments for controlling total coliforms in bottled water. Additionally, the means of the brands share a grouping letter in the Fisher's method, validating the aforementioned.

- E. Coli

  As the data obtained for this parameter were a value of 0, no analysis of variance was conducted.
- Aerobic Bacteria

Análisis de Varianza

Fuente	GL	SC Ajust.	MC Ajust.	Valor F	Valor p
Marca	3	1581	527.07	6.02	0.004
Error	20	1751	87.57		
Total	23	3333			

Agrupar información utilizando el método de Tukey y una confianza de 95%

Factor	N	Media	Agrupación	
Marca D	6	19.17	A	
Marca A	6	1.083		B
Marca B	6	0.250		B
Marca C	6	0.000000		B

Fig. 3 Aerobic Bacteria: ANOVA / Fisher LSD

The null hypothesis (Ho) is rejected, indicating that at least one brand has a different average of aerobic bacterias. According to the Fisher's grouping method, Brand D does not share a letter, suggesting its mean is significantly different from the others.

- PH

Análisis de Varianza

Fuente	GL	SC Ajust.	MC Ajust.	Valor F	Valor p
Marca	3	12.8781	4.29269	108.32	0.000
Error	20	0.7926	0.03963		
Total	23	13.6707			

Agrupar información utilizando el método de Tukey y una confianza de 95%

Factor	N	Media	Agrupación		
Marca B	6	8.2500	A		
Marca D	6	6.943		B	
Marca C	6	6.65000		B	
Marca A	6	6.3183			C

Fig. 4 PH: ANOVA / Fisher LSD

The null hypothesis (Ho) is rejected, indicating that at least one brand has a different average pH. According to the Fisher's method, Brand B and Brand A do not share a grouping letter, making them significantly different; therefore, Brand D and Brand C have statistically equal means.

3.2 Results Analysis

3.2.1 Analysis of Results with Allowable Values

Through comparative statistics, it was identified whether the evaluated parameters comply with the maximum allowable values according to the regulations for Bottled Water and Ice for direct and indirect human consumption. Unmet parameters are marked and identified in table 2.

Table 2 Parameter compliance

	Brand A						Brand B						Brand C						Brand D					
Parameters	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6	L1	L2	L3	L4	L5	L6
TDS																								
Total Coliforms	■												■		■			■						■
E. Coli																								
Aerobic Bacterias																								
PH	■	■	■	■	■	■																		

- Total dissolved solids: The permissible value is <500 mg/L, and all brands comply with the parameter for total dissolved solids established by the aforementioned regulations. In case of exceeding these levels, water with high levels of dissolved solids may taste unpleasant to consumers, and individuals consuming water with a high level of these solids may experience gastrointestinal irritation [11].
- Total Coliforms: The permissible value is <2 MPN/100ml, and it is observed that Sample 1 of Brand A, Samples 1, 3, and 6 of Brand C, and Sample 6 of Brand D exceed this value. Therefore, this can be a cause for concern, as exceeding the maximum permissible value indicates a possible route of contamination between bacterial sources [12].
- E. Coli: The permissible value is 0 CFU/100mL, and all samples comply with this value. The presence of E. Coli in water is a public health concern due to its ability to transmit diseases, particularly diarrheal strains. Detection

suggests possible recent contamination by sewage or animal waste, increasing the risk of pathogens and emphasizing the need for continuous water quality monitoring to protect public health [13].

- Aerobic Bacteria: The permissible value is <500 CFU/mL, and it is observed that all samples for each brand evaluated comply with the established parameter for aerobic bacteria. Finding aerobic bacteria exceeding the maximum permissible levels for consumption may indicate poor handling during the bottle packaging process [14].
- pH: The permissible pH range is between 6.5 and 8.5, and the pH of all 24 samples complies with the parameter established by the regulations. Therefore, they are within permissible values. Water with a pH below 6.5 is more acidic, and above 8.5 is more alkaline [15]. It is important to note that pH changes can alter concentrations of other substances in the water that may affect toxicity levels; this can be caused by a significant temperature change or human activities.

3.2.2 Correlation Between Parameters

A correlation analysis was conducted to identify whether the quantity of aerobic bacteria/total coliforms could influence the pH of the evaluated samples, either towards more acidic or basic pH levels. Additionally, due to the discrepancy found in the values of Brand D concerning low total dissolved solids and high aerobic bacteria, a correlation analysis was performed to determine if the quantity of aerobic bacteria could affect the total dissolved solids data. The results obtained are presented in Figures 5, 6 and 7.

- Total Coliforms vs. pH:

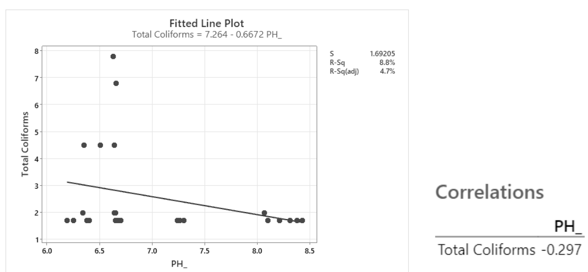


Fig. 5 Scatter graph and correlation coefficient T. coliforms vs. PH

The correlation coefficient is negative, indicating a weak negative relationship between pH and total coliforms. This could mean that higher total coliform values in the samples correspond to lower pH values. Lower pH values indicate more acidity.

- Aerobic Bacteria vs. pH

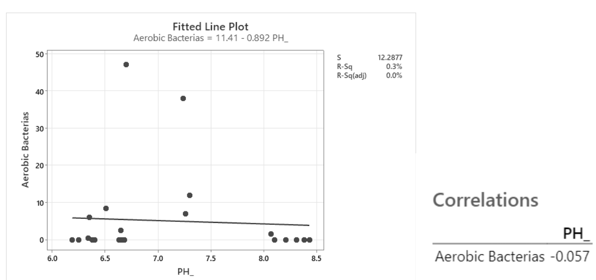
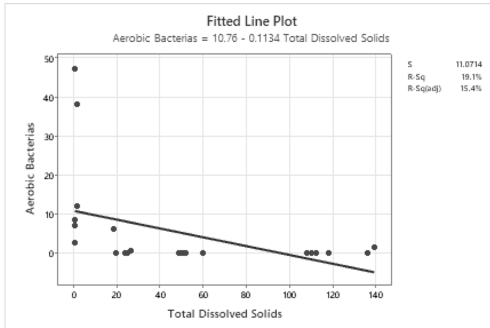


Fig. 6 Scatter graph and correlation coefficient Aerobic Bacteria vs. PH

The correlation coefficient is negative, signifying a weak negative relationship between pH and aerobic bacteria. This suggests that higher values of aerobic bacteria in the samples correspond to lower pH values. Lower pH values indicate more acidity.

- Aerobic Bacteria vs. Total Dissolved Solids (TDS)



Correlations

	Aerobic Bacterias
Total Dissolved Solids	-0.437

Fig. 7 Scatter graph and correlation coefficient Aerobic Bacteria vs TDS

The correlation coefficient is negative, indicating a negative relationship between total dissolved solids (TDS) and aerobic bacteria. This could mean that higher values of aerobic bacteria in the samples correspond to lower TDS values.

3.3 Expert triangulation

The research was validated by three experts in industrial engineering specializing in experimental design. Correlation and Fisher analyses were conducted in response to their recommendations, providing a deeper understanding of relationships and highlighting differences between brands. The rationale behind the sample size was specified, acknowledging its importance for future research. These corrections strengthened the methodology, ensuring the quality of results and providing a solid foundation for future studies in industrial engineering, especially in experimental design.

4. Conclusion

4.1. Specific Objective 1 Conclusions

Through analysis of variances and Fisher's LSD method, it was determined that the means of TDS with unit mg/L for each brand are significantly different: Brand A with a mean of 120.50, Brand B 53.3, Brand C 23.33, and Brand D 1.33. The means of total coliforms and E. coli are statistically equal. The mean of aerobic bacteria for Brand D is 19.17 and is significantly different from the other brands. The pH of Brand B and Brand A has significantly different means; therefore, Brand D and Brand C have statistically equal means with a value of 6.65. These results may indicate that brands may have different treatments and parameter controls, with some being more efficient than others.

4.2 Specific Objective 2 Conclusions

The parameters of pH, TDS, and aerobic bacteria are within the maximum allowable values according to the Regulation of Bottled Water and Ice for direct and indirect human consumption in Honduras. In the total coliform tests with a unit of NMP/100ml, there is a discrepancy in the levels, as two samples of Brand A have levels of 4.5 and 2; 1 sample of Brand B with a level of 2; 3 samples of Brand C have levels of 7.8, 6.8, 2, and 4.5; and 2 samples of Brand C with levels of 2 and 4.5. These levels exceed the maximum allowable of <2 NMP/100ml despite having samples free of E. coli, warranting further investigation into other bacterial genera such as Enterobacter.

A correlation analysis revealed negative coefficients, indicating a weak inverse relationship between total coliforms/aerobic bacteria and the pH of the samples, as well as between aerobic bacteria and total dissolved solids (TDS). These findings highlight the importance of further research to understand the relationship between these parameters and their impact on the quality of bottled water.

4.3 Specific Objective 3 Conclusion

The research was validated through triangulation by experts in industrial engineering, specializing in the design of experiments, strengthening its credibility and rigor. This provides a solid foundation for future more detailed and precise investigations in the field of industrial engineering, especially in the design of experiments.

5. Research Recommendations

- Before deciding how many brands of water to evaluate, it is crucial to consider the most marketed ones and obtain precise data through contact with ARSA. It is also essential to investigate methods used in previous studies as a reference for the present research project.
- To ensure ongoing relevance, collaboration with public health experts and government regulators is suggested, integrating new perspectives, and updating the methodology with emerging water quality technologies and practices.
- To strengthen the research, it is recommended to expand the scope of the study by including more brands of bottled water available in Honduras. This would offer a more comprehensive perspective on water quality in the country, identifying broader patterns and enriching the validity and representativeness of the results.

6. Applicability

What has been done in this research was implemented in the bottled water industry, and the results obtained can be considered for a future evaluation of bottled water quality using the parameters established by the Regulation of Bottled Water and Ice for direct and indirect human consumption.

The experimental design used for this research can be replicated or used as a guide for Honduran companies in the water industry, whether potable, for consumption, or even from beaches and rivers to measure their quality. It is important to consider the established parameters for each type as permissible values may vary. It can also be implemented in bottling companies for different liquids.

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