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Determinación de *E. coli /coliformes* en muestras de lechugas iceberg obtenidas del Mercado 27 de febrero, agosto 2023

Determination of E. coli/coliforms in iceberg lettuce samples obtained from the "February 27" market, August 2023

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Palabras claves:
lechuga iceberg, E. coli, coliformes, mercados, Compact Dry.

Resumen

Introducción: La contaminación generada en los alimentos se ha convertido en un problema de salud pública al producir grandes patologías en el hombre, en donde se estudia las distintas colonias microbiológicas como *E. coli y coliformes*. Estos grupos de bacterias se pueden identificar en distintos tipos de alimentos como las lechugas iceberg, por sus parámetros de producción como las malas prácticas agrícolas. **Objetivo:** Determinar la presencia de *E. coli /coliformes* en lechugas iceberg expendidas en el mercado 27 de febrero de la ciudad de Cuenca. **Métodos:** Estudio de carácter observacional descriptivo, de corte transversal en el período agosto 2023 en el cual se recolectaron 30 muestras para la identificación de *E. coli/coliformes* en lechugas iceberg. **Resultados:** La determinación de *E. coli/coliformes* en las 30 muestras de lechuga analizadas del mercado 27 de febrero de la ciudad de Cuenca nos indica un crecimiento del 30% para *E. coli* que determinan algún grado de contaminación y el 100% para coliformes que pasa los límites establecidos por la Normativa Sanitaria de Perú. **Conclusiones:** Se determinó la frecuencia de *E. coli/coliformes* en las muestras recolectadas de lechuga iceberg expendidas por los comerciantes del mercado 27 de febrero de la ciudad de Cuenca procedente de diversos factores de contaminación a lo largo de su producción, uno de ellos la calidad del agua de riego que está en contacto directo con este producto alimenticio. **Área de estudio general:** Bioquímica y Farmacia. **Área de estudio específica:** Microbiología de Alimentos. **Tipo de estudio:** Artículo original / Original article.

Keywords:
iceberg lettuce, E. coli, coliforms, markets, Compact Dry.

Abstract

Introduction: Contamination in food has become a public health problem, causing significant pathologies in humans. Different microbiological colonies, such as E. coli and coliforms, are studied. These groups of bacteria can be identified in different types of foods, including iceberg lettuce, due to their production parameters and poor agricultural practices. Objective: To determine the presence of E. coli / coliforms in iceberg lettuce sold in the “27 de Febrero” market in Cuenca. Methods: A descriptive, observational, cross-

sectional study in August 2023 was conducted with 30 samples collected to identify E. coli / coliforms in iceberg lettuce. Results: The determination of E. coli/coliforms in the 30 samples of lettuce analyzed from the “27 de Febrero” market in Cuenca indicates a growth of 30% for E. coli, which determines some degree of contamination, and 100% for coliforms that passes the limits established by the Peruvian Sanitary Regulations. Conclusions: The frequency of E. coli/coliforms was determined in samples collected from iceberg lettuce sold by traders at the “27 de Febrero” market in Cuenca from various contamination factors throughout its production, one of them being the quality of the irrigation water in direct contact with this product.

Introduction

Foodborne diseases (FBDs), considered of global interest due to their impact on humans, are defined as the fact that one or more people become ill after ingesting water or any type of contaminated food, supported by the analysis of indicators that this was the responsible party (1, 2). According to the Centers for Disease Control and Prevention (CDC) "they estimate that every year 48 million people contract a foodborne illness, 128,000 are hospitalized and 3,000 die" (3).

The symptoms of foodborne illness include gastrointestinal illness, causing nausea, vomiting, stomach cramps and diarrhea; however, this varies depending on the bacteria transmitted by the food (4, 5). These pathologies arise due to an extensive bacterial load of the microorganism, which will be able to produce toxins in the food and at the same time will sustain its growth and multiplication so that it can finally enter the human body and attack its defenses (6, 7).

Microbiological contamination of lettuce may begin during sowing, harvesting, processing, storage or distribution; during production lettuce is usually fertilized with animal waste, generating biological contamination (7). Another factor is irrigation, which involves sources of contamination in agriculture; the consumption of this vegetable favors the exposure of several people to foodborne pathogens such as coliform bacteria or E. coli, called "indicator organisms." This group indicates a direct route of water-food contamination, guaranteeing entry into the organism in case the food does not meet the sanitary quality of production (8, 9).

In Ecuador, there is no regulation that indicates the parameters for quality control in lettuce, so this study was based on the Sanitary Standards of Peru. The purpose of this research is to determine the degree of microbiological contamination of iceberg lettuce sold in the 27 de Febrero market in the city of Cuenca, to determine and identify the presence of E. coli/coliforms to generate relevant data on the degree of contamination of this food consumed daily by the people of Cuenca.

Methodology

This study is of a descriptive, observational nature, cross-sectional in the period from August 2023.

Study universe, sample treatment and sample (applicable, only for epidemiological studies)

The study was carried out in the 27 de Febrero market in the city of Cuenca, located on the streets of Ave. 10 de Agosto and Adolfo Torres; where 12 stalls are dedicated to the sale of vegetables (lettuces specifically) (10). To carry out this study, consent was obtained by submitting a request to the market manager, thus obtaining the corresponding permits.

Sampling: Based on INEN 1529-2:2013 regulations, lettuces were obtained directly and under aseptic conditions; initially, a quantity of 100 g of each sample was placed in sterile, sealed, hermetically sealed bags, to be collected in a container at 5 °C (11). The samples were transferred to the Food Microbiology laboratories of the Biochemistry and Pharmacy Department of the Catholic University of Cuenca within a maximum time of one hour to perform the respective analysis.

Sample preparation: the dilutions in this study were carried out according to INEN 1529-2:2013 regulations.

-First dilution 1/10: Using sterile forceps, 10 g of the lettuce leaf sample was placed with 90 mL of peptone water in a stomacher, then it was homogenized in the blender for 30 seconds, without exceeding two minutes to avoid overheating of the blades (1/10);

-Second dilution 1/100: Using a sterile pipette, place 1 mL of the first dilution (stock dilution) in a tube containing 9 mL of peptone water (1/100);

-Third dilution 1/1000: Using a sterile pipette, place 1 mL of the second dilution in a tube containing 9 mL of peptone water (1/1000) (11).

-*Identification of Escherichia coli/coliforms*

For microbiological culture, specific Compact Dry EC plates were used for the detection of E. coli/coliforms, where 1 mL of each dilution was poured with the help of a sterile pipette until covering the surface of the plate. These were then incubated at a temperature of 35-37 °C for 24 to 48 hours. The presence of blue/purple-blue colonies for the presence of Escherichia coli and a red/pink/purple color for coliforms spp. was then observed and recognized. The CFU was counted to identify the approximate quantity of said microorganisms. If no colonies were observed after the established time (24-48 h), the test was terminated, and the absence of both E. coli and coliforms was reported (12,13).

Processing, analysis, summary and presentation of information

The lettuce samples were taken at the 27 de Febrero market in the city of Cuenca, and were then transferred for study to the Food Microbiology laboratories of the Biochemistry and Pharmacy program at the Catholic University of Cuenca. The results obtained were stored in the Excel application to determine by descriptive statistics and frequency analysis in the form of a frequency polygon graph and double entry tables to reflect the presence or absence of E. coli/coliforms, the number of CFU in each sample analyzed, the number of samples that do or do not comply with the guidelines of the Peruvian Technical Standard RM No. 615-2003 for food and beverages for human consumption, Section 14.1: Fresh fruits and vegetables (without any treatment) (14), sold at the 27 de Febrero market in the city of Cuenca-Ecuador.

Results

A total of 30 samples of iceberg lettuce were collected, from which the following results were obtained:

Table 1. Sample distribution according to type of E. coli growth from the 27 de febrero market in the city of Cuenca – Ecuador

Dilution	Positive growth	Negative growth	Total
1:10	9 (30%)	21 (70%)	30 (100%)
1:100	4 (13.3%)	26 (86.7%)	30 (100%)
1:1000	2 (6.7%)	28 (93.3%)	30 (100%)

Table 1 shows the growth of E. coli in the different dilutions; 1:10, 30% of the samples showed development of the microorganism; 1:100, 13.3% of the samples showed growth and finally 1:1000, 6.7% of the samples showed growth.

Table 2 Sample distribution according to coliform growth type in the 27 de febrero market in the city of Cuenca – Ecuador

Dilution	Positive growth	Negative growth	Total
1:10	30 (100%)	0 (0%)	30 (100%)
1:100	30 (100%)	0 (0%)	30 (100%)
1:1000	30 (100%)	0 (0%)	30 (100%)

Table 2 shows 100% coliform growth in all dilutions. MNP was reported in most cases, except for some plates from the 1:1000 dilution.

Table 3. Distribution of samples with coliforms according to maximum quantification of the Peruvian Health Standard NTS N° 071-N-MINSA/DIGESA-V.01 XlV.1

Dilution	<10^2 CFU/g	> 10 ^ 2 CFU/g	Total
1:10	0 (0%)	30 (100%)	30 (100%)
1:100	0 (0%)	30 (100%)	30 (100%)
1:1000	0 (0%)	30(100%)	30 (100%)

Table 3 shows that in the 1:10, 1:100 and 1:1000 dilutions, a growth of total coliforms developed outside the limits established by the Peruvian Health Standard NTS No. 071-N-MINSA/DIGESA-V.01 XlV.1.

Table 4. Distribution of samples with E. coli according to maximum quantification of the Peruvian Health Standard NTS No. 071-N-MINSA/DIGESA-V.01 XlV.1

Dilution	0%	<10^2 CFU/g	Total
1:10	21 (70%)	9 (30%)	30 (100%)
1:100	26 (86.7%)	4 (13.3%)	30 (100%)
1:1000	28(93.3%)	2(6.6%)	30 (100%)

Table 4 shows that in the 1:10 dilutions, 30% is within the minimum limit; in the 1:100 dilution proportion, 13.3% does not exceed the minimum limit and in the 1:1000 dilution, 6.6% is within the minimum range for E. coli.

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Figure 1. Growth result of the different dilutions made from the samples collected from the 27 de febrero market in the city of Cuenca

Figure 1 shows the growth of E. coli (blue color) and coliforms (pink/purple color) in the dilutions of the iceberg lettuce samples from the 27 de febrero market in the city of Cuenca.

Discussion

In the present study, the presence of E. coli/coliforms in iceberg lettuce, also called Lactuca sativa var. Capitata, sold in the 27 de febrero market in the city of Cuenca, Ecuador, was examined; resulting in the growth of E. coli within the ranges in the different dilutions performed, with 30% being representative, compared to the Peruvian Health Regulations that establish the minimum level 10 ^ 2 CFU/g and the maximum level 10 ^ 3 CFU/g in fruits and vegetables respectively (15, 16).

The presence of these microorganisms, despite being at a minimal level, reflects a risk of contamination of these pathogenic bacteria in iceberg lettuce sold in the market; it indicates that there are poor agricultural practices, handling and that hygiene conditions are vulnerable during harvesting, processing, packaging, transportation and storage (17, 18).

According to a study carried out in Ica, Peru, where 48 lettuce samples were analyzed, which showed a percentage of 56.3% of E. cola; compared to 30% that reflected a degree of contamination; confirming that there is greater contamination in Ica, Peru than in Cuenca, Ecuador (19).

This is due to the fact that the cultivation methods used in Ica are inadequate, since manure is used as fertilizer in the sowing field, river water or waste water for irrigation of vegetables, in addition to an adequate dosage of herbicides and pesticides. However, the contamination of the lettuce used for this study arises at a certain level due to poor practices on the part of farmers, however, emphasis is placed on an adequate means of transport, along with poor hygiene of the dispenser and seller (16).

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Another study carried out in the city of Loja, which analyzed 80 lettuce samples, established that 33.75% presented some degree of contamination (19); finally, a study carried out in Brazil with the objective of determining the microbiological quality of 162 vegetables including lettuce; determined that the degree of contamination for Escherichia coli represented 53.1%, thus generating a call to the population to improve hygienic measures when dealing with lettuce (20).

These results generated in Brazil are manifested by various factors such as poor hygiene on the part of the farmer or probably due to the use of highly contaminated raw materials or simply due to a lack of good hygiene practices both in transport and sale. However, when comparing the contamination generated in lettuce sold in the city of Loja, mention is made of the residual water used by farmers, in addition to a lack of hygiene on the part of the sellers of these vegetables (19, 20).

On the other hand, with respect to coliforms in iceberg lettuce, it was determined that the 30 samples collected in the 27 de febrero market in the city of Cuenca, showed growth of 100%, the same value that exceeded the 10^2 CFU/g established by the Peruvian Health Regulations for vegetables. It should be emphasized that this group is considered as indicator microorganisms, which implies that the food was exposed to general contamination (14, 16).

In the province of Quillacollo, Cochabamba, Bolivia, a study was carried out in which the microbiological quality of 21 lettuce samples was analyzed, yielding results between 10 ^ 4 CFU/g and 10 ^ 7 CFU/g, which compared to this research are much higher levels of contamination. However, both studies agree that more than 75% of the lettuce analyzed present some degree of microbiological contamination, in relation to total coliforms (19).

Analyzing that the factors involved in the contamination generated in the province of Quillacollo are due to a shortage of drinking water that forces the farmer to use waste water, in addition to the poor handling of both the farmer and the seller, along with the contamination generated by the waste of domestic animals.(20).

In Piura, a study was conducted, analyzing 100 lettuce samples to investigate the presence of total and fecal coliforms; the study shows that 69% of the samples exceed the limits of total coliforms, similar to the results obtained in this study (19). According to Toledo et. al in their study called "Determination of Escherichia coli/coliforms in lettuce sold in the 10 de agosto market in the city of Cuenca" E. coli did not show growth in any dilution compared to this study where there was growth; while 96.7% showed growth of coliforms in the 1:100 dilution specifically, data that resemble this study and reaffirm the presence of these indicator microorganisms in the markets of the city (21).

This study is of great academic and social importance because it offers updated information on the microbiological quality of lettuce from the 27 de Febrero market in the city of Cuenca, thereby minimizing the risk of foodborne illnesses by focusing on reinforcing proper food handling, such as proper hand washing by food handlers and the food itself.

Conclusions

- The results collected in the period August 2023 determined the presence of E. coli and coliforms in iceberg lettuce in the 27 de febrero market in the city of Cuenca, which are indicative of contamination through untreated water, irrigation with wastewater or contamination with feces, in addition to poor handling practices within the market, these aspects contribute to the transmission and appearance of ETA, which is why controls and hygiene measures must be reinforced by both sellers and consumers.
- Using Compact Dry EC plates, the presence of both E. coli and coliforms was identified in the different dilutions of the 30 samples collected. In addition, quantification was carried out according to INEN 1529-2, giving us as the most relevant result the 1:10 dilution for E. coli with 30% and 100% in all dilutions for coliforms, the latter being reported in their majority as too numerous to count in comparison with the Peruvian Health Standard NTS N° 071-N-MINSA/DIGESA-V.01 XlV.1.
- Microbiological studies carried out on iceberg lettuce aim to evaluate the overall quality of this food for direct consumption, which is why the Department of Hygiene and Market Control of the Illustrious Municipality of Cuenca recommends strictly monitoring agricultural practices, as well as those of producers and retailers, to prevent and control outbreaks of illness due to the consumption of contaminated fresh food.

Conflict of interest

The authors declare that there are no conflicts of interest.

Authors' contribution statement

Mgs. Silvia Monserrath Torres Segarra: responsible for reviewing the methodological process, results, discussion, introduction, writing of the document and conclusions.

Emily Tamara Aguilar Ortiz: Responsible for the execution of the practical part, writing of the document, results, discussion, conclusions, summary, keywords, abstract and keywords

Jenny Alexandra Saquicaray Morocho: Responsible for the execution of the practical part, writing, discussion, conclusions, summary, keywords, abstract and keywords

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