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Microbiological and parasitological quality of *Lactuca sativa* L. sold at farmers' markets in Goiânia, Goiás, Brazil

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Abstract
Fresh lettuce (*Lactuca sativa* L.) is the most consumed leafy vegetable in Brazil. Much purchased from local farmers' markets that lack adequate sanitary conditions. In this context, this study evaluated the quality of lettuce sold in the city of Goiânia, Goiás, Brazil, regarding microbiological and parasitological standards. Results demonstrated microbial contamination with total coliforms, thermotolerant coliforms, and mesophilic aerobic bacteria, with a notable presence of strains from the Enterobacteriaceae family such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter sp*. Additionally, some samples were contaminated with *Entamoeba sp*., *Giardia sp*., and/or nematode larvae, as well as exhibiting a high degree of dirt. Therefore, it is recommended to intensify sanitary inspections and raise awareness among producers and sellers about the importance of good practices from cultivation through commercialization to mitigate potential health risks to consumers.

Keywords: Microbial contamination, *Lactuca sativa* L., farmers' markets, Goiânia, Goiás, Brazil

Introduction

Lactuca sativa L., commonly referred to as lettuce and classified in the Asteraceae family, is the most consumed leafy vegetable in Brazil [1]. Due to its high nutritional value, low caloric content, and being a significant source of vitamins and minerals, lettuce is widely used in various diets. However, to preserve its nutritional qualities, it must be consumed fresh [2].

The consumption of raw foods poses a significant risk to human health, as the presence of fecal-origin infectious agents can act as vectors for various diseases [3]. The detection of fecal microorganisms in foods can indicate the possible presence of pathogens, potential food spoilage, and poor sanitary conditions [4].

Lettuce contamination can occur in numerous ways, primarily by water contaminated with human feces, inadequate hygiene during food handling, or soil contamination due to the use of organic fertilizers containing fecal matter [5]. Other factors that can contribute to vegetable contamination include improper conditions during cultivation, harvesting, storage, transportation, and packaging [6].

Farmers' markets in Brazil play a crucial role in the local economy, especially for small food producers who find in these markets a vital platform for the direct sale of their products. These environments promote circular economy practices and strengthen family farming, enabling farmers to earn a fair income by bypassing intermediaries [7]. Farmers' markets are notable for offering a wide range of fresh products, including fruits, vegetables, greens,

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meats, cheeses, and artisanal products [8].

Foods sold at farmers' markets can exhibit a high degree of contamination due are exposed to the outdoors, making them susceptible to biological changes caused by various organisms. This can occur for insufficient food control measures or improper handling. Additionally, these foods can be contaminated by microorganisms and insects on account of urban pollution [9].

The implementation of parasitological and microbiological monitoring in vegetables and food handlers at farmers' markets would enable the establishment of a preventive and dynamic system to ensure the quality of vegetables consumed by the population [10]. This monitoring would provide essential data on the hygienic and sanitary conditions for products, benefiting Public Health and Sanitary Surveillance [11]. In this context, this study aimed to evaluate the microbiological and parasitological quality of lettuces sold at farmers' markets in the city of Goiânia, Goiás, Brazil.

Methodology

Microbiological analysis

Two lettuce samples of the curly variety were acquired from each of the four selected stalls at five different farmers' markets in Goiânia, Goiás, Brazil, between August 2023 and February 2024, totaling 40 samples. The vegetables were identified and transported under refrigeration (4°C to 8°C) for analysis at the Microbiology Laboratory of Alves Faria University Center (UNIALFA) and at the Microbiology Laboratory of the State University of Goiás (UEG - UnU Itumbiara).

Initially, 25 grams of each sample of *Lactuca sativa* L. were weighed and homogenized with 225 mL of 0.85% sterile saline solution, making this the 10^{-1} dilution. Next, 1 mL from this solution was collected and a new dilution was made in 9 mL of 0.85% sterile saline solution (10^{-2}); this process was repeated for one more dilution (10^{-3}).

The Multiple Tube Fermentation Method (MTFM) was applied to determine the Most Probable Number (MPN) of Total Coliforms (TC) and Thermotolerant Coliforms (TTC) [12]. The analysis was conducted in three distinct stages: presumptive test, test for the determination of total and thermotolerant coliforms, and biochemical test. For the presumptive test, Lactose Broth with inverted Durham tubes was used, with three samples dilutions and five tubes for each dilution. In the first series of tubes, 1 mL of the 10^{-1} dilution was inoculated into 10 mL of Lactose Broth. The second series of five tubes was inoculated with 1 mL of the 10^{-2} dilution, and the third series of five tubes with 1 mL of the 10^{-3} dilution. Tubes were then incubated at 35°C for 48 hours. Tubes of Lactose Broth that showed gas production with bubble formation in the Durham tubes and medium turbidity were considered positive.

For the identification of Total Coliforms, aliquots from positive Lactose Broth tubes were collected and inoculated into Brilliant Green Bile Broth (BGB) tubes, which were incubated at 35°C for 48 hours. The quantification of Thermotolerant Coliforms was performed using a Nickel-Chrome loop to collect aliquots from positive Lactose Broth tubes and inoculate them into tubes with *Escherichia coli* (EC) broth and inverted Durham tubes, followed by incubation in a water bath at 45 °C for 48 hours.

For the isolation and identification of *Escherichia coli*, aliquots from positive *Escherichia coli* broth tubes were collected and streaked using a Nickel-Chrome loop onto plates containing Eosin Methylene Blue Agar (EMB) and incubated at 35 °C for 24 hours. Colonies with positive characteristics for *Escherichia coli*, showing a metallic green sheen, were isolated on Tryptic Soy Agar (TSA) and identified through the IMViC tests (Indole, Methyl Red, Voges-Proskauer, Citrate) [13].

To quantify mesophilic aerobic bacteria, the Standard Plate Count (SPC) method was performed using the “Pour Plate” technique. 1 mL of each of the three dilutions of *Lactuca sativa* L. samples was inoculated into 15 mL of Plate Count Agar (PCA). The inoculum was gently homogenized on a flat surface using a figure-eight motion. After the culture medium solidified completely, plates were incubated at 35°C for 24 hours. The procedure was carried out in duplicate for all dilutions [14].

Parasitological analysis

50 g of leaves from each sample of *Lactuca sativa* L. were initially immersed in a neutral detergent solution (5 mL of detergent diluted in 1 liter of 0.9% saline solution) for 20 minutes. Then, samples were blended, and the resulting solution was filtered through sterile hydrophilic eight layers cotton-gauze, collected in an Imhoff cone, and left to settle for 24 hours. Subsequently, the supernatant liquid was discarded, and 10 mL of the lower portion were centrifuged at 2,500 rpm for five minutes at room temperature. The supernatant was again discarded, and a drop of the resulting sediment was added to a slide for optical microscopy, stained with Lugol's solution, and analyzed under 10X and 40X objectives.

Statistical analysis

Data were expressed as central tendency measures (mean) and frequency distributions (absolute and relative). GraphPad Prism 5.01 software was used to perform statistics. And, parametric analysis of variance (one-way or two-way ANOVA) followed by Bonferroni post-hoc with P values <0.05 were adopted.

Results and Discussion

The results of the *Lactuca sativa* L. samples analyzed for Total Coliforms ranged from 2.18×10^{1} MPN/g to 1.52×10^{6} MPN/g among the five farmers' markets in the municipality of Goiânia, Goiás, Brazil, from August 2023 to February 2024. Regarding Thermotolerant Coliforms, the observed values ranged from 1.80×10^{1} MPN/g to 2.25×10^{6} MPN/g; and the microbiological data for Mesophilic Aerobic Bacteria demonstrated a variation from 1.88×10^{1} CFU/g to 7.80×10^{6} CFU/g, $P<0.05$ (Table 1).

Table 1: Most Probable Number (MPN) of Total Coliforms (TC), Thermotolerant Coliforms (TTC), and Mesophilic Aerobic Bacteria on 40 samples of *Lactuca sativa* L. sold at five farmers' markets in the municipality of Goiânia, Goiás, Brazil, from August 2023 to February 2024. *$p<0.05$ for all groups. One and two-way ANOVA followed by Bonferroni post-tests

Sample	MPN/g of TC *	MPN/g of TTC *	Mesophilic Bacteria (CFU/g) *
Farmers' market 1			
1.	2.14×10^{3}	1.75×10^{3}	2.22×10^{4}

2.	3.46 x 10^2	2.70 x 10^2	1.18 x 10^3
3.	2.65 x 10^3	2.05 x 10^3	2.30 x 10^3
4.	5.05 x 10^5	4.79 x 10^5	4.24 x 10^5
Farmers' market 2			
1.	2.18 x 10^1	2.20 x 10^1	2.63 x 10^1
2.	3.54 x 10^3	2.65 x 10^3	1.39 x 10^4
3.	2.24 x 10^1	1.80 x 10^1	1.88 x 10^1
4.	3.22 x 10^3	2.41 x 10^3	4.62 x 10^4
Farmers' market 3			
1.	1.52 x 10^6	3.14 x 10^5	2.69 x 10^6
2.	5.45 x 10^5	5.35 x 10^5	3.81 x 10^5
3.	2.60 x 10^3	1.31 x 10^4	3.33 x 10^5
4.	1.37 x 10^6	2.02 x 10^6	2.63 x 10^6
Farmers' market 4			
1.	5.18 x 10^3	3.41 x 10^4	3.74 x 10^5
2.	4.99 x 10^1	3.74 x 10^1	2.55 x 10^3
3.	2.83 x 10^4	5.65 x 10^5	1.14 x 10^6
4.	3.57 x 10^5	5.02 x 10^5	7.80 x 10^6
Farmers' market 5			
1	5.32 x 10^2	5.35 x 10^2	3.49 x 10^4
2	1.30 x 10^6	2.25 x 10^6	4.33 x 10^5
3	5.09 x 10^2	2.72 x 10^2	4.44 x 10^4
4	3.24 x 10^3	2.89 x 10^3	4.60 x 10^4
CFU: Colony Forming Units			

Resolution RDC No. 724/2022 [15], which provides for the microbiological standards of fresh vegetables in Brazil, establishes the tolerance limit of the Most Probable Number (MPN) of Total Coliforms (TC) and Thermotolerant Coliforms (TTC) at a maximum of $1x10^2$ MPN/g; and $1.0×10^5$ CFU/g for Mesophilic Aerobic Bacteria. According to these microbiological sanitary standards, in general, lettuce from the five different farmers' markets studied is unfit for consumption, with the presence of even *Escherichia coli*, indicating possible fecal contamination. The exceptions are stalls 1 and 3 at farmers' market 2; and stall 2 at farmers' market 4, in which microbiological and parasitological standards for lettuce are within the accepted limit for human consumption.

Similar studies were conducted with samples of *Lactuca sativa* L. sold at farmers' markets in various regions of Brazil. Teixeira *et al.* (2013) [16] observed bacterial and parasitological contamination levels above the allowed limits for all lettuce samples analyzed in Juazeiro do Norte, Ceará. França *et al.* (2014) [17] found that lettuce sold at farmers' markets in Uberlândia, Minas Gerais showed high contamination of mesophilic aerobic microorganisms, with counts exceeding 10^6 CFU/g, indicating the possible presence of pathogenic microorganisms, resulting from inadequate or poor hygiene during production and handling. Similarly, Coutinho *et al.* (2015) [18] analyzed curly lettuce from farmers' markets in Sobral, Ceará, and found that 100% of the samples had thermotolerant coliform levels above limits, identifying ten species of bacteria from the Enterobacteriaceae family. In contrast, Bobco *et al.* (2011) [19], in studies evaluating the hygienic conditions of lettuce sold in Erechim, Rio Grande do Sul, observed that all samples met acceptable standards for human consumption regarding thermotolerant coliforms.

As shown in Table 2, from the forty samples examined, 43 strains were isolated, all belonging to the Enterobacteriaceae family. *Escherichia coli* was identified in 18 different *Lactuca sativa* L. samples (41.86%); *Klebsiella pneumoniae* in 7 lettuces (16.28%); *Enterobacter sp*. represented 13.95% of the findings (n = 6); *Serratia sp*., 11.62% (n = 5); *Pseudomonas sp*. was present in three lettuces (6.98%); *Shigella sp*. and *Salmonella sp*. were found in 2 samples each (4.65%), $P<0.05$.

Table 2: Bacterial strains from the Enterobacteriaceae family identified on 40 samples of *Lactuca sativa* L. sold at five farmers' markets in the municipality of Goiânia, Goiás, Brazil, from August 2023 to February 2024. *$p<0.05$ for all groups. One-way ANOVA and Bonferroni post-tests

Bacterial agents*	**n = 43**	
	n	**%**
Escherichia coli	18	41.86
Klebsiella pneumoniae	7	16.28
Enterobacter sp.	6	13.95
Serratia sp.	5	11.62
Pseudomonas sp.	3	6.98
Shigella sp.	2	4.65
Salmonella sp.	2	4.65

The Enterobacteriaceae family comprises a diverse group of Gram-negative, rod-shaped bacteria that are widely distributed in the environment, including soil, water, and plants, as well as in the intestinal tracts of animals and humans [20]. These bacteria are facultatively anaerobic and hold significant clinical and ecological importance [21]. Among the most well-known genera are *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, and *Enterobacter*. Many species within the Enterobacteriaceae family are opportunistic pathogens, responsible for urinary, gastrointestinal, and systemic infections, especially in immunocompromised individuals [22]. Additionally, antimicrobial resistance is a major concern within this group, with multi-resistant strains emerging and complicating infection treatments [23].

The detection of *Escherichia coli* in water and food is indicative of fecal contamination. Thus, it can be concluded that at some stage of the production chain, transport, storage, or handling of *Lactuca sativa* L. samples, exposure to fecal material of human and/or animal origin occurred. The genera *Enterobacter* and *Klebsiella* are widely used as indicators of sanitary conditions and the quality control of water and food due to their prevalence and resilience in various environments [24]. Studies have demonstrated that the presence of these bacteria is frequently associated with inadequate hygiene and sanitation practices, indicating fecal contamination [25]. For instance, *Enterobacter sp*. has been identified in surface waters and supply systems as a marker of fecal pollution and deficiencies in water treatment [26]. Similarly, *Klebsiella sp*. are recognized as important indicators of contamination in food products, especially fresh vegetables and dairy products, reflecting failures in production and handling processes [27]. The detection of *Enterobacter* and *Klebsiella* in aquatic and food environments underscores the need for strict sanitary controls and hygiene practices to ensure public safety.

In Brazil, since the 1970s, there has been specific legislation that establishes and regulates that vegetables must be free from dirt, parasites, and larvae [28]. In this study, however, it was observed that some vegetables researched are not in compliance with this standard, meaning they are in unsanitary conditions for consumption. The parasitological analysis showed that 30% of the samples were contaminated with *Entamoeba sp.*, *Giardia sp.*, and/or nematode larvae, as well as a high degree of dirt, indicating failures in the hygiene of *Lactuca sativa* L. during harvesting, storage, and/or at the point of sale. Parasites were identified at stall 4 from farmers' market 1, stalls 1 and 4 from farmers' market 3, stalls 3 and 4 from farmers' market 4, and stall 2 from farmers' market 5. Furthermore, 20% of the samples evidenced mixed contamination, with the identification of two or more different parasites, especially at farmers' market 3 and farmers' market 4 (Table 3).

Table 3: Parasites identified on 40 samples of *Lactuca sativa* L. sold at five farmers' markets in the municipality of Goiânia, Goiás, Brazil, from August 2023 to February 2024. **p*<0.05 for all groups. One-way ANOVA and Bonferroni post-tests

Sample	***Entamoeba sp.***		***Giardia sp.***		**Nematode larvae**	
	n = 6*		**n = 4***		**n = 2***	
	n	**%**	**n**	**%**	**n**	**%**
Farmers' market 1						
1.	-	-	-	-	-	-
2.	-	-	-	-	-	-
3.	-	-	-	-	-	-
4.	1	100	1	100		
Farmers' market 2						
1.	-	-	-	-	-	-
2.	-	-	-	-	-	-
3.	-	-	-	-	-	-
4.	-	-	-	-	-	-
Farmers' market 3						
1.	1	33.33	-	-	-	-
2.	-	-	-	-	-	-
3.	-	-	-	-	-	-
4.	2	66.66	1	100	1	100
Farmers' market 4						
1.	-	-	-	-	-	-
2.	-	-	-	-	-	-
3.	1	100	1	50	-	-
4.	-	-	-	-	-	-
Farmers' market 5						
1.						
2.	1	100	-	-	-	-
3.	-	-	-	-	-	-
4.	-	-	-	-	-	-

In the study by Coutinho *et al.* (2015) [18], it was observed that 91% of samples were contaminated by parasites, and in 41% of the lettuce samples, this contamination was mixed, with up to three different parasites. Carvalho *et al.* (2019) [29], evaluating samples of *Lactuca sativa* sold at farmers' markets and supermarkets in Patos, Paraíba, described that 72% of these samples contained *Entamoeba coli*, *Giardia lamblia*, *Endolimax nana*, *Iodamoeba butschlii*, and nematode larvae. Ramos *et al.* (2014) [30], evaluating lettuce samples sold in the municipality of Umuarama, Paraná, identified a low contamination rate, only 7.5%. In a recent study, Ulsenheimer *et al.* (2024) [31] found that 80% of *Lactuca sativa* samples analyzed from Ijuí, Rio Grande do Sul, contained the protozoan *Tritrichomonas foetus*. Thus, in general terms, investigations established that the hygienic and sanitary standards of *Lactuca sativa* sold in various regions of Brazil, including those analyzed in this study, are below expectations due to a lack of efficient microbiological control. Therefore, it is necessary to implement and apply sanitary education programs so that people who handle and cultivate vegetables can provide a safe product that does not pose health risks to consumers.

Conclusion

It is concluded that some curly lettuce samples (*Lactuca sativa* L.) sold at farmers' markets in Goiânia, Goiás, Brazil, presented microbial contamination for total coliforms, thermotolerant coliforms, and mesophilic aerobic bacteria, with a notable presence of strains from the Enterobacteriaceae family. Additionally, samples contaminated with *Entamoeba sp*., *Giardia sp*., and/or nematode larvae, as well as a high degree of dirt, were observed. Therefore, it is recommended to intensify sanitary inspections and raise awareness among producers and sellers about the importance of good practices from cultivation through commercialization to mitigate potential health risks to consumers.

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Declaration of interest statement
Authors declare no conflict of interest.

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