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Microbiological evaluation of minimally processed lettuce in food service establishments

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Abstract. Raw foods, such as fruits, vegetables, and legumes, can be subject to microbiological contamination at any stage of the production chain, from cultivation to final handling in the kitchen. Therefore, this study aimed to evaluate the microbiological quality of minimally processed, ready-to-eat lettuce leaves sold in food service establishments in Sinop, MT, Brazil. The parameters analyzed were total coliforms, thermotolerant coliforms, *Escherichia coli*, and *Salmonella* spp. The study included 20 samples collected from 10 restaurants, with two samples per establishment. All samples analyzed showed contamination by total coliforms; 10 were positive for thermotolerant coliforms and *Escherichia coli*, and *Salmonella* spp. was not detected in any of them. Deficiencies in hygienic and sanitary practices were evident.
Keywords: lettuce; food; hygiene; restaurants.

Introduction

Although vegetables are essential components of a healthy diet and their global demand has been growing, outbreaks of foodborne diseases due to the consumption of contaminated vegetables have occurred worldwide, posing a threat to human health (QIUPING et al., 2024).

Vegetable contamination by pathogens is related to irrigation water quality and soil conditions (SCHERER et al., 2016), as well as to inadequate practices throughout the entire production chain: cultivation, distribution, and marketing (MACIEL et al., 2025; AL-MUSAWI et al., 2023). Vendruscolo et al. (2024) confirm this situation by pointing out the lack of hygienic-sanitary conditions and good practices in open-air markets, which, combined with the consumption of raw products, highlights a high risk to consumer health and the need to adopt good agricultural and handling practices.

Among food-contaminating microorganisms, coliform bacteria stand out as critical indicators of the sanitary quality and safety of agricultural environments and associated products (PEREIRA et al., 2020; QIUPING et al., 2024).

Specifically, *Escherichia coli* is used as a marker of fecal contamination because it is present in large quantities in the gastrointestinal tract of humans and warm-blooded animals, being rarely isolated from other niches (SILVA et al., 2006). Studies reveal high coliform contamination in vegetables in different contexts: community gardens (Rosa et al., 2005), traditional and hydroponic crops (Bergamo & Gandra, 2016), minimally processed vegetables (Pereira et al., 2020), restaurants (Guimarães César et al., 2015; Saraiva et al., 2019), and open-air markets (Rodrigues et al., 2021). Al-Musawi et al. (2023) associated high contamination in ready-to-eat salads with handlers' hands, identifying the strain *E. coli* O157:H7, which is considered an emerging pathogen of worldwide importance. Factors such as lack of sanitation, inadequate water, and post-sanitization failures contribute to these findings.

In addition to these indicators, the presence of the pathogen *Salmonella* spp. in food represents a major risk to public health. *Salmonella* is an enteric bacterium belonging to the Enterobacteriaceae family, recognized as one of the main causative agents of serious food poisoning worldwide. Its epidemiological relevance is evidenced by frequent outbreaks recorded in several countries, including Brazil, where a higher incidence of *Salmonella* spp.

infection cases was observed between 2000 and 2015 (Lanza, 2016). Research indicates that contaminations occur due to hygiene failures and cross-contamination during preparation, as animals are the main source of this pathogen and foods of animal origin are the primary transmission route to humans (Silva et al., 2021; Tresseler et al., 2009; Ferrari et al. 2022). This contamination occurs through equipment, utensils, packaging, and handlers. Among the enteritis caused by this bacterium, we can mention salmonellosis, which causes symptoms such as diarrhea, vomiting, and intense nausea, which can appear between 12 and 36 hours after contact with the pathogen (Shinohara et al. 2008).

In a review covering 23 articles published between 2020 and 2025, Raia & Zanin (2023) found the presence of thermotolerant coliforms and *Salmonella* spp. in lettuce at different stages of the production chain in several regions of Brazil. The findings reinforce the need for proper hygiene before consumption and highlight the importance of health education actions by sanitary surveillance agencies targeting producers, traders, and consumers to reduce the microbial and parasitological load of vegetables consumed in the country.

In this context, Rossi et al. (2020), when applying a questionnaire to lettuce consumers, concluded that 92.6% do not perform proper hygiene on this food. Of these, 72.5% use only water, 5.4% use vinegar as a disinfecting agent, and only 7.5% perform the hygiene recommended by the National Health Surveillance Agency (2005), which involves washing with treated running water followed by immersion in a 2.5% sodium hypochlorite solution, with 200 ppm active chlorine, for 10 minutes. Thus, the researchers demonstrated a lack of knowledge regarding adequate methodologies for washing and disinfecting fresh market vegetables.

Therefore, the assessment of the microbiological quality of food is essential to identify inadequate hygienic-sanitary conditions, both in water and food, and to enable the adoption of preventive measures. This analysis can be performed by monitoring indicator microorganisms of fecal contamination, especially the coliform group, where *E. coli* is a significant indicator associated with the risk of foodborne diseases (SOUSA, 2006).

Thus, this study aimed to evaluate the microbiological quality of minimally processed, ready-to-eat lettuce (*Lactuca sativa*). Samples were acquired from commercial establishments in Sinop-MT and analyzed for the enumeration of total coliforms, thermotolerant coliforms, the presence of *E. coli*, and the detection of *Salmonella* spp.

Materials and Methods

Samples were randomly collected from self-service food establishments, configured as lunchrooms or restaurants, in the municipality of Sinop-MT. A total of 20 lettuce samples were obtained from 10 establishments, with two samples collected per location, without distinction regarding the variety or color of the vegetable. At the time of collection, samples were handled using the utensils available in the establishments and packed in styrofoam containers provided by the locations. All laboratory analyses were performed in duplicate.

Microbiological Analysis

The method used for counting total coliforms, thermotolerant coliforms, and *Escherichia coli* was the Most Probable Number (MPN), using the methodology of the American Public Health Association (APHA), described in the Compendium of Methods for the Microbiological Examination of Foods (Kornacki; Johnson, 2001).

For each replicate, 25g of the sample was used in 225 mL of diluent (10^{-1} dilution). From the initial dilution (10^{-1}), serial dilutions (10^{-2} and 10^{-3}) were made in tubes with 9 mL of 0.1% peptone water. Subsequently, 1 mL of each dilution was transferred to a series of 3 tubes containing 10 mL of Lauryl Sulfate Tryptose (LST) broth, with inverted Durham tubes, homogenized, and incubated at 35 °C for 24-48 hours for enrichment of lactose-fermenting organisms. In the analysis, gas production in the fermentation tubes (Durham tubes) and turbidity of the medium were observed; these characteristics indicate the presence of coliform microorganisms, leading to the subsequent items of the test.

For the confirmation of total and thermotolerant coliforms, a loopful from each suspected tube was transferred to Brilliant Green Bile 2% (VB) broth and *E. coli* (EC) broth tubes. Observation of growth with gas production in VB tubes after 24-48h of incubation at 35 °C is considered confirmatory for total coliforms. Growth with gas production in EC tubes after 24h of incubation at 45.5 °C is considered confirmatory for thermotolerant coliforms. The MPN/g was determined from positive tubes using the MPN table cited by Silva et al. (2010), appropriate for the inoculated dilutions.

EC tubes positive for thermotolerant coliforms are suspected of containing *E. coli*. For confirmation, a loopful of the culture was streaked onto Eosin Methylene Blue (EMB) Agar plates. The development of typical *E. coli* colonies were isolated for Gram staining and biochemical tests (Indole, Methyl Red, Voges-Proskauer, Citrate - IMViC).

The methodology used for the *Salmonella* analysis was that of the American Public Health Association, described in the Compendium of Methods for the Microbiological Examination of Foods (VANDERZANT and SPLITTSTOESSER, 1992). To assess the presence of *Salmonella*, pre-enrichment was performed in Buffered Peptone Water and selective enrichment in Tetrathionate (TT) Broth and Selenite-Cystine (SC) Broth. After the incubation period, loops from TT and SC broths were streaked onto Hektoen Enteric (HE) Agar and Xylose Lysine Deoxycholate (XLD) Agar plates. Suspected colonies were streaked onto

slants of Lysine Iron Agar (LIA) and Triple Sugar Iron Agar (TSI) for preliminary confirmation.

Results and Discussion

All samples were positive for total coliforms, with values exceeding 1,100 MPN/g. Although legislation does not establish specific standards for total and thermotolerant coliforms, the count of these microorganisms reflects the sanitary conditions and safety of agricultural products (QIUPING et al., 2024). These results are in agreement with those obtained by Perondi et al. (2013), whose study demonstrated that approximately 88% of lettuce samples collected from food services were positive for total coliforms, with values exceeding 1,100 MPN/g, highlighting deficiencies in hygienic-sanitary practices, such as proper sanitization with sodium hypochlorite.

The studies by Almeida (2006) and Cruz & Santos (2018) corroborate these findings by demonstrating that 100% of lettuce samples collected in restaurants showed high counts of total coliforms. These results reflect failures in hygiene practices during product handling and preparation. The adoption of good hygiene practices at all stages, from production to distribution and marketing, as well as preventive measures such as installing protective screens in gardens, could minimize contamination by agents such as animal feces that eventually access cultivation sites.

For thermotolerant coliforms, 50% of the samples showed values exceeding 1,100 MPN/g. The presence of these microorganisms is directly related to hygiene failures, which can occur from planting to the commercialization of the vegetable. The results found are in agreement with those of Rosa et al. (2005), Confessor et al. (2021), and Maciel et al. (2025), who obtained positive results for thermotolerant coliforms.

Regarding the presence of *E. coli* and *Salmonella*, the results were compared to the microbiological standards established by Normative Instruction No. 161 of 2022, of the National Health Surveillance Agency (ANVISA, 2022), which defines standards for *E. coli* and *Salmonella* for the category "Prepared (whole, peeled, or fractionated), sanitized, blanched, refrigerated, or frozen, which do not require effective heat treatment before consumption".

The results indicated that 50% of samples from five specific establishments were positive for *E. coli*. Current legislation establishes a maximum limit of 10 CFU/g for indicative samples for sanitized raw-consumed vegetables. Although counting was not performed, the presence of *E. coli* is concerning, as it is a relevant indicator of microbiological contamination of fecal origin, directly associated with the occurrence of foodborne diseases.

These findings are in line with those of Guimarães César et al. (2015), who, in their research on the microbiological evaluation of lettuce salads in restaurants, found that 61.1% of samples had high counts of thermotolerant coliforms, and the presence of *E. coli* was confirmed in 5.6% of samples.

Regarding *Salmonella* spp., it was not detected in any of the samples. This result complies with current legislation, which requires absence in 25 grams of sample. Similar results were found by Fagiani et al. (2018) when evaluating minimally processed lettuce. In contrast, Andrade et al. (2022) reported the presence of *Salmonella* spp. in 100% of the lettuce samples analyzed, while Confessor et al. (2021) identified 12% of lettuce samples positive for the pathogen. Recognized worldwide as one of the main causative agents of serious food poisoning, research for *Salmonella* is indispensable as an indicator of microbiological safety in vegetables.

Therefore, reducing contamination and risks to public health depends on simple and effective measures, such as the proper use of sanitizers, the implementation of quality programs in production, the training of handlers, and public awareness. Investing in prevention is essential to guarantee food safety and avoid overloading the Unified Health System (SUS) with waterborne and foodborne diseases.

Conclusion

All analyzed samples showed contamination by total coliforms, and ten of them were positive for thermotolerant coliforms and *E. coli*, evidencing deficiencies in hygienic-sanitary practices. Regarding *Salmonella* spp., it was not detected in any of the samples.

Given the role of *E. coli* as an indicator of fecal contamination associated with foodborne diseases, there is a need to assess the quality of food offered in food services and the potential risks to public health.

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