

# Microbiological analysis of lettuce (*Lactuca sativa*) from conventional and organic cultivation commercialized at fairs in Brazil

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Research

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## CONFLICTS OF INTEREST

There are no conflicts of interest for any of the authors.

## CITATION

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## ABSTRACT

Brazilian laws established regulatory limits in lettuce only to thermotolerant coliforms (fecal coliforms) and coagulase-positive *Staphylococci* of  $10^2$  MPN  $g^{-1}$  and  $10^3$  CFU  $g^{-1}$  respectively. The objective of this study was to evaluate and compare contamination of lettuce from conventional and organic cultivation, commercialized at fairs in south of Brazil, by the fecal coliforms, coagulase-positive *Staphylococci*, *Salmonella* spp. and *Listeria monocytogenes*. Eighty samples of traditionally and organically produced lettuce each were collected. The contaminated samples percent were 35 and 71.25, for fecal coliforms, 35 and 32,5 for coagulase-positive *Staphylococci*, 0 and 5 for *Salmonella* spp., 2.5 and 2.5 for *Listeria monocytogenes*, in conventional lettuce and organic lettuce, respectively. Results indicated that 35% of the samples of conventionally produced lettuce and 71.25% of the samples of organically produced one presented fecal coliform count above regulatory limits. Coagulase-

positive *Staphylococci* counts above regulatory limits were verified in 28 and 26 samples from the conventional and organic agriculture, respectively. *Salmonella* spp. was present in 5% of organically produced samples and *L. monocytogenes* in 2.5% of lettuce samples from both types of agricultures. Obtained results highlight the importance of good practices from cultivation to commercialization to improve the hygienic-sanitary quality of vegetables.

**Keywords:** Vegetables, microorganisms, hygienic-sanitary quality

## INTRODUCTION

Inclusion of vegetables in the daily diet is widely recommended due to their nutrient content, such as vitamins, minerals and dietary fiber, which are required for proper functioning of the human body [1, 2, 3]. Lettuce (*Lactuca sativa*) is among leafy vegetables popularly consumed in Brazil. This vegetable presents

nutritional benefits for consumers in addition to its low cost and availability [4, 5].

Despite many advantages, lettuce is one of the raw foods with the highest index of contamination, which may be related to failures in one of the stages of the production chain and hygienic-sanitary conditions of the manipulators [6, 7]. There are several factors among the production process that can contribute to the food susceptibility to contamination and make it a risk factor for human health. The vegetable contact with soil and organic residues in fertilizers, which depend on adopted cultivation system, can be a contamination factor [2, 8, 9].

Lettuce can be produced in the conventional, hydroponic and organic culture. In the conventional production system, vegetables are grown in soil with the appropriate application of water, nutrients and fertilizers [10]. Organic farming can be defined as a production system that excludes use of several pesticides, synthetic fertilizers, growth regulators or other contaminants [11].

Regardless of the adopted farming system, all vegetables must be produced following practices that result in products that are safe for human consumption [2]. However, improper manipulation during processing and distribution is a major cause of food-borne diseases [12, 13]. Microorganisms may represent risks to consumer's health. Vegetables consumed *in natura* are commonly associated with the presence of several species of microorganisms, among which are *Salmonella*, *Escherichia coli* and coliforms, of which the last two are indicators of hygienic-sanitary conditions in the production chain and *E. coli* and *coliforms* are indicators of pathogenic contamination of produce [9, 14, 15, 16, 17].

The consumption of raw vegetables is an important means of transmission of infectious and parasitic diseases in the population. Unfavorable sanitary conditions in rural and urban areas favor this contamination, transforming the vegetables into vehicles of transmission of pathogens, as fecal coliforms (in fecal coliforms group exist pathogenic species such as *E. coli*), coagulase-positive *Staphylococci*, *Salmonella* spp. and *Listeria monocytogenes*, causing public health risks.

In view of the above, the objective of this study

was to evaluate and compare the contamination by fecal coliforms, coagulase-positive *Staphylococci*, *Salmonella* spp. and *Listeria monocytogenes* of lettuce from conventional and organic cultivation commercialized at fairs in south of Brazil.

## MATERIAL AND METHODS

Samples of conventionally (n=80) and organically (n=80) produced lettuce (*Lactuca sativa*), variety "American lettuce", were purchased at 20 local fairs in south of Brazil, from January to December of 2016. At each purchase 4 samples of conventionally produced and 4 samples of organically produced lettuce were acquired, in total 20 purchases were made. The organic designation of lettuce is vendor reported and there is an organic fair certification by the city agriculture department.

Microbiological analysis was performed according to the methodology of the *American Public Health Association* (APHA), with modifications [18]. A total of 25 g (weight of sample) were aseptically weighted, identified and serially diluted down to  $10^{-6}$ .

Fecal coliforms enumeration was done by the Most Probable Number (MPN) technique. The preliminary analysis of coliforms was carried out in Sodium Lauryl Sulphate Broth (SLS) after incubation at 35 °C for 48 hours. The fecal coliforms enumeration was performed in *Escherichia coli* broth (EC) after incubation at 45.5 °C for 24 hours. The results were expressed in MPN g<sup>-1</sup>.

The isolation and quantification of *L. monocytogenes* were performed according to the methodology of the International Organization for Standardization, ISO 11.290-1 - Detection method [19], with modifications. The pre-enrichment stage was performed in *Listeria* Enrichment Broth (LEB) with incubation at 30°C for 24 hours, followed by incubation of an aliquot in Fraser broth (supplemented with SR 0156E Oxoid®) at 35°C for 48 hours. After that, seeding was performed in Oxford Agar (supplemented with SR 0140E Oxoid®) and Palcam Agar (supplemented with SR 0150E Oxoid®) followed by incubation at 35°C for 48 hours. Purified isolates were submitted to phenotypic tests including determination of motility, carbohydrate fermentation capacity (dextrose, xylose, rhamnose and mannitol), and pres-

ence of catalase and  $\beta$ -hemolysin.

The isolation of *Salmonella* spp. was carried out after pre-enrichment in peptonated buffered water at 37 °C for 24 hours, selective enrichment in Rappaport-Vassiliadis Broth at 42 °C for 24 hours and Tetrathionate Broth at 37 °C for 24 hours. Seeding was performed on Deoxycholate-Lysine-Xylose (DLX) agar and Hektoen-Enteric (HE) agar plates, both incubated for 24 hours at 37 °C. Typical colonies were identified biochemically in Triple Sugar Iron Agar (TSI), Lysine Iron Agar (LIA) and Urease Agar at 37 °C for 24 hours. Isolates with characteristic biochemical reactions were submitted to serological identification using the polyvalent anti-somatic and flagellar *salmonella* sera (Probac).

The analysis of positive-coagulase *Staphylococci* was carried out by inoculation of 0.1 ml of each of

serial dilutions, in Baird-Parker Agar using the surface seeding technique, in duplicate. Plates were incubated at  $36 \pm 1$  °C for 24 to 48 hours. Colonies were enumerated and at least five colonies that presented typical morphology and five that presented atypical morphology were selected to perform the test of free coagulase

The likelihood ratio test for the ratio of the prevalence in organic versus conventional for fecal coliforms was used to determine if the prevalence of thermotolerant coliforms is significantly different.

## RESULTS AND DISCUSSION

Results of determination of fecal coliforms, coagulase-positive *Staphylococci*, *Salmonella* spp. and *Listeria monocytogenes* in lettuce samples from conventional and organic cultivation are presented in Table 1.

**Table 1-** Quantification of thermotolerant coliforms, coagulase-positive staphylococci, *Salmonella* spp. and *Listeria monocytogenes* in samples from conventional (n=80) and organic (n=80) cultivation commercialized at fairs in south of Brazil, from January to December 2016. Percentage of contaminated samples relative to the total number of samples analyzed for each production system is shown in the parentheses.

| Samples              | Fecal coliforms *<br>N (%) | Coagulase-positive<br><i>Staphylococci</i> **<br>N (%) | <i>Salmonella</i> spp.<br>N (%) | <i>Listeria monocytogenes</i><br>N (%) |
|----------------------|----------------------------|--|---------------------------------|--|
| Conventional lettuce | 28 (35)                    | 28 (35)  | 0 (0)                           | 2 (2.5)                                |
| Organic lettuce      | 57 (71.25)                 | 26 (32.5)  | 4 (5)                           | 2 (2.5)                                |

N= number of contaminated samples; the number of samples with counts above the regulatory limits of  $10^2$  MPN  $g^{-1}$ \* and  $10^3$  CFU  $g^{-1}$ \*\*

There is a significant difference, in accord to likelihood ratio test for the ratio of the prevalence in organic versus conventional for thermotolerant coliforms, we get a risk ratio of 2.04 (95% CI 1.46, 2.80) with a p-value<0.001. According to microbiological standards established by the National Agency for Sanitary Surveillance (ANVISA) fresh, *in natura*, prepared (peeled, selected or fractionated), sanitized, refrigerated or frozen vegetables for direct consumption, may contain up to  $10^2$  microorganisms  $g^{-1}$  of food of fecal coliforms according to Resolution RDC No. 12, January 2001 [20]. Obtained results show that independently of the production system, fecal coliforms with cell count above  $10^2$  MPN.g.g<sup>-1</sup> are present in

lettuce. Presence of this microorganism was verified in 28 (35%) of the lettuce samples produced in conventional cultivation and in 57 (71.25%) of the lettuce samples produced in organic cultivation (Table 1). Barbosa et al. [3] reported the presence of fecal coliforms in 9 (60%) of 15 samples of conventionally produced lettuce. Coutinho et al. [21] investigated the microbiological quality of lettuce commercialized at open fairs and identified fecal coliform indexes above regulatory limits in all analyzed samples (n=12). In Brazil, different studies have demonstrated a high degree of contamination by fecal coliforms in vegetables during cultivation, which is associated with contamination of water used for irrigation [22, 23, 24, 25].

This fact could justify, in part, the high count of fecal coliforms in lettuce samples evaluated in this study. However, the greater contamination observed for samples from organic cultivation can be associated with the use of organic fertilizers such as fecal manure and also with inadequate hygienic-sanitary conditions during cultivation, handling, storage and transportation.

*Salmonella* spp. was not detected in any lettuce samples from the conventional cultivation, but it was found in 4 (5%) of the organically produced ones (Table 1). According to the Resolution RDC No. 12/2001 of ANVISA, *Salmonella* spp. must be absent in 25 g of fresh, chilled or frozen raw vegetables [20]. Food of animal origin is the main transmitter of *Salmonella* spp., however, several studies have reported the presence of this microorganism in products of plant origin as well [22, 26, 27]. Arbos et al. [2] identified the presence of *Salmonella* spp. in 1 (20%) lettuce sample from organic cultivation. Other studies reported the absence of *Salmonella* spp. in the analyzed lettuce samples, regardless of the place of origin or used production system [9, 28, 29]. According to Oliveira and Junqueira [30], the occurrence of *Salmonella* spp. in organic lettuce samples may be related to use of contaminated water for irrigation or bovine manure as fertilizer, influencing the final microbiological quality of the product.

According to the table 1, there was no difference between the number of lettuce samples from conventional and organic cultivation that had counts of coagulase-positive *Staphylococci* above  $10^3$  CFU g<sup>-1</sup>. Therefore, 28 (35%) and 26 (32.5%) of the analyzed lettuce samples from conventional and organic cultivation, respectively, did not meet the microbiological standards established by the current legislation, which approves a tolerance of  $10^3$  CFU.g<sup>-1</sup> of food [20]. Trindade [31] reported the presence of coagulase-positive *Staphylococci* in 30% of lettuce salad samples analyzed, of which 21% were unsatisfactory or unacceptable for consumption. Monteiro [32] identified the presence of coagulase-positive *Staphylococci* in 22 out of 34 lettuce salad samples studied. Junqueira et al. [33] verified the presence of the pathogen in 32.2% of analyzed lettuce salads (n=31) from restaurants in Rio de Janeiro - RJ with count above  $10^3$  CFU.g<sup>-1</sup>. The investigation of coagulase-positive *Staphylococci* in

food is necessary since some of this microorganism strains can produce thermostable enterotoxins that are responsible for food poisoning with onset within few hours. Manipulators are believed to be the main source of contamination [34]. Therefore, the results obtained in this study are probably related to inadequate food handling, lack of correct hygiene, product storage at inappropriate temperature for long periods of time and, even may be associated with infected manipulators [31, 35].

The occurrence of *L. monocytogenes* was observed in 2 (2.5%) of lettuce samples from both type of production (Table 1). Brandão et al. [36] observed in their study of 97 samples of different types of vegetables the presence of *L. monocytogenes* in only 7 (7.2%) of the samples of vegetables *in natura* and salads. In recent studies, Vongkamjan et al. [37] and Scherer et al. [27] verified the presence of *Listeria* spp. in lettuces samples intended for direct consumption. Bergamo and Gandra [29] verified the presence of *Listeria* spp. in 10 lettuce samples (16.7%), of which 6 were from conventional, 2 from hydroponic and 2 from organic cultivation. This microorganism is part of an important class of human pathogens and, although there is no Brazilian legislation with reference values for it in fresh vegetables, it is widely distributed in the environment and in food. According to the *International Commission on Microbiological Specifications for Foods* [38], the cultivation stage is a primary contamination point by *L. monocytogenes* for leafy vegetables. Thus, the presence of *L. monocytogenes* in lettuce samples suggests their contamination during the production in the field by contaminated soil and/or water, and manipulators hands, or even in the sales establishments.

Several factors may influence the microbial hazards that affect food safety, which can be present at any point of the production chain, such as the growing, harvesting, washing, storage, transport, presentation, commercialization and finally, the consumer table [39, 40]. According to Pacheco et al. [41], the effectiveness of washing and disinfection procedures is essential for the elimination of pathogenic microorganisms present in plants, regardless of the culture system employed. As lettuce is usually consumed raw, in salads or sandwiches, contaminated can cause seri-

ous problems to consumer health and public health in a broader way, it is recommended to sanitize and sanitize with chlorine and subsequent rinse in water free of contaminants [42].

## CONCLUSION

Lettuce samples from both conventional and organic cultivation sold at fairs in south of Brazil, were inadequate according to the established sanitary standard, which was evidenced by the presence of fecal coliforms, *Salmonella* spp., coagulase-positive *Staphylococci* and *L. monocytogenes*. Despite the absence of *Salmonella* spp. in lettuce samples from the conventional cultivation, hygienic-sanitary adjustment are necessary throughout the production chain in order to reduce the potential risks to consumers, contributing to the production of vegetables of good quality.

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