ARTICLE
https://doi.org/10.22239/2317-269x.01757

Microscopic quality of lettuce (*Lactuca sativa* L.) from different cultivation forms and minimally processed marketed in municipalities in the northeast and metropolitan regions of the state of São Paulo

Qualidade microscópica de alfaces (*Lactuca sativa* L.) oriundas de diferentes formas de cultivo e minimamente processadas comercializadas em municípios das regiões nordeste e metropolitana do estado de São Paulo

Elaine Cristina de Mattos[I,*]
Jaqueline Pereira Santana[I]
Nathália Oliveira Sgarbosa[II]
Rute Dal Col[I]
Vilma Menezes dos Santos Gaiotto Daros[I]
Sonia de Paula Toledo Prado[II]

[I] Núcleo de Ciências Químicas e Bromatológicas, Centro de Laboratório Regional de Santo André, Instituto Adolfo Lutz, Santo André, SP, Brasil

[II] Núcleo de Ciências Químicas e Bromatológicas, Centro de Laboratório Regional de Ribeirão Preto, Instituto Adolfo Lutz, Ribeirão Preto, SP, Brasil

***** **E-mail:** elaine.mattos@ial.sp.gov.br

Received: 26 Aug 2020
Approved: 29 Apr 2020

ABSTRACT

Introduction: Currently, lettuce (*Lactuca sativa* L.) stands out for being the most consumed vegetable in Brazil. In public health, most etiologic agents of enteric diseases are transmitted through contaminated fruits and vegetables. **Objective:** To verify the quality of lettuces, through microscopic analysis, comparing the results of the different types of cultivation and processing. **Method:** 84 samples of lettuce produced by three cultivation methods (traditional, organic, hydroponic) and minimally processed lettuce, commercialized in municipalities in the northeast and metropolitan regions of the state of São Paulo, were analyzed. The research of macroscopic foreign matter was first carried out; later, to verify the presence of parasitic structures and other microscopic foreign matter, a spontaneous sedimentation technique was used. **Results:** The results revealed that the lettuce samples of the three types of cultivation showed a high occurrence of foreign matter (total of 87.0% positive samples), as well as the presence of parasites (total of 20.0% positive samples). Regarding minimally processed lettuce, the percentage of positivity for the presence of foreign matter and parasites was 58.0%. For this type of product, lower rates were expected, given that they are products previously cleaned and ready for consumption. **Conclusions:** The results of the present study alert to the importance of cleaning procedures for different types of lettuce cultivation prior to consumption. Regarding minimally processed lettuces, the results show that corrective actions must be taken in the sanitation process by producers and / or traders. As for laboratory analysis, it is still necessary to constantly improve analytical methods that enable better detection of parasites in food.

KEYWORDS: Lettuce; Parasites; Quality Control; Microscopy; Food Analysis

RESUMO

Introdução: Atualmente, a alface (*Lactuca sativa* L.) destaca-se por ser a folhosa mais consumida no Brasil. Em saúde pública, grande parte dos agentes etiológicos de enfermidades entéricas é veiculada por meio de hortaliças, legumes e frutas contaminadas. **Objetivo:** Verificar a qualidade de alfaces, através da análise microscópica, comparando os resultados dos diferentes tipos de cultivo e da alface minimamente processada. **Método:** Foram analisadas 84 amostras de alfaces produzidas por três métodos de cultivo (tradicional, orgânico, hidropônico) e alfaces minimamente processadas, comercializadas em municípios das regiões nordeste e metropolitana do estado de São Paulo. Realizou-se primeiramente a pesquisa de matérias estranhas macroscópicas e, para verificar a

presença de estruturas parasitárias e outras matérias estranhas microscópicas, foi utilizada a técnica de sedimentação espontânea. **Resultados:** Os resultados revelaram que as amostras de alfaces dos três tipos de cultivo apresentaram alta ocorrência de matérias estranhas (total de 87,0% de amostras positivas), bem como presença de parasitas (total de 20,0% de amostras positivas). Em relação às alfaces minimamente processadas, a porcentagem de positividade para presença de matérias estranhas e parasitas foi de 58,0%. **Conclusões:** Os resultados do presente estudo alertam para a importância dos procedimentos de higienização dos diferentes tipos de cultivo de alface previamente ao seu consumo. Em relação às alfaces minimamente processadas, os resultados demonstram que devem ser tomadas ações corretivas no processo de sanitização por parte dos produtores e/ou comerciantes. Quanto às análises laboratoriais, ainda se faz necessário o aprimoramento constante de métodos analíticos que possibilitem melhor detecção de parasitas em alimentos.

PALAVRAS-CHAVE: Alface; Parasitas; Controle de Qualidade; Microscopia; Análise de Alimentos

INTRODUCTION

Currently, lettuce (*Lactuca sativa* L.) stands out as the most consumed leafy vegetable in Brazil and the 3rd vegetable in production volume, second only to watermelon and tomato, according to the Brazilian Seed and Seedling Trade Association and data from the Consumer Expenditure Survey (POF), from the Brazilian Institute of Geography and Statistics (IBGE)[1,2].

Lettuce is recognized as a food rich in vitamins, minerals, and fiber that are essential for the functioning of the body. It also has therapeutic properties, being identified as a component of a healthy diet. Another factor that favors the preference is its affordable price[3,4].

In Brazil, the area planted with leafy vegetables is estimated at 174,061 ac, with 49.9% (86,867 ac) being lettuce, 22.8% (39,685 ac) arugula, 15.3% (26,631 ac) cabbage, and 6.1% (10,774 ac) kale. Of the total lettuce produced, 55% are of the curly variety, 22% of the American variety, 11% of the smooth lettuce and 9% of the mimosa lettuce. The American variety has greater post-harvest durability, and this has contributed to opening the market for use in *fast food chains*[5].

There are at least four lettuce production systems in Brazil: the traditional cultivation, the organic system in the open field, the protected cultivation in the hydroponic system and in the soil. The four systems differ from each other in several aspects of crop management and also in post-harvest handling[6].

The cultivation of lettuce in the field in the traditional system is the most important in terms of area and production, usually concentrated near large urban centers (in beds in the field). There are producers specialized in the cultivation of hardwoods that produce lettuce continuously in the same area during the year, with or without crop rotation, and also small producers that have only a few beds of lettuce together with other vegetable species. The cost of lettuce in traditional cultivation is relatively low when compared to other vegetables, such as tomato, pepper, and hybrid cucumber[6].

The organic method emerged as an alternative to the agro-industrial system and uses modified techniques, such as: organic fertilization, green fertilization, pesticides such as syrups, oils, and natural extracts, among other unique processes in favor of healthy production[7,8].

Hydroponics is a set of techniques for growing plants without the use of soil, so that essential mineral nutrients are supplied to plants through a balanced nutrient solution to meet their nutritional needs. Among the advantages of hydroponics, there are higher crop productivity, precocity, and better product quality and profitability[9].

The production of minimally processed vegetables, also called fresh cut, occurs through a set of post-harvest processing steps, in which the selection, washing, peeling, cutting, and packaging are carried out. This new production category is highly desired by consumers, as the product is fresh and often suitable for immediate consumption. However, these processes need to be carried out in accordance with Good Manufacturing Practices (GMP), as their contamination and deterioration can easily occur at any stage, compromising the quality and safety of fresh foods[10].

Lettuce contamination, both by microorganisms and by foreign matter, can occur from cultivation to commercialization. The use of contaminated water for irrigation of vegetable gardens and sanitization of vegetables, soil contamination by alternative fertilization, failures in hygienic-sanitary conditions of handlers, and inadequate transport and storage favor this condition[11,12,13].

In public health, most of the etiological agents of enteric diseases are transmitted through contaminated vegetables, legumes, and fruits, which are mostly ingested raw, which is a major factor in the epidemiology of intestinal parasites[14].

The verification of the presence of intestinal parasites, especially in vegetables, is of great importance in public health, as it provides subsidies for health surveillance on the quality of these products and indicates conditions in which they were grown[14].

The integrated action between Public Health Laboratories and control bodies such as Sanitary, Epidemiological, and Environmental Surveillance, contributes to outbreak investigations and GMP inspection, for the benefit of promoting consumer safety[15].

It is important to emphasize that, despite being widely spread in the country, minimally processed vegetables do not have vertical legislation (specific for a given product) that define their identity and quality standard, not even those that guide their production, storage, and marketing[16].

In 2009, the Government of the State of São Paulo, through the Resolution of the Secretariat of Agriculture and Supply (SAA) nº 42, of June 19, 2009, recommended the Technical Standard for Minimally Processed and Fresh Cut Vegetables[17], statewide and non-mandatory. This resolution recognizes that the lack of vertical legislation for minimally processed products leaves the producer helpless in terms of quality criteria and other aspects of these products. In addition, its lack can put consumers at risk as these products undergo manipulation and require hygiene standards[16]. In view of the above, the present study aimed to verify the quality of lettuce, through microscopic analysis, comparing the results of different types of cultivation and processing.

METHOD

Eighty-four samples of lettuce (*Lactuca sativa* L.) predominantly of the curly variety were analyzed and, in the absence of these, the American, smooth, and mimosa varieties were analyzed. The lettuces were originated from three methods of cultivation and one of processing: traditional (26 samples), organic (11 samples), hydroponic (23 samples), and minimally processed (24 samples).

The samples were acquired from April 2016 to October 2018 in supermarkets, vegetable gardens, and street markets in the cities of Santo André (São Paulo) and Ribeirão Preto (São Paulo). The criteria for choosing the establishments was made randomly.

A head of lettuce was established as a sample unit, regardless of its weight or size, or a closed package. For samples of the same brand, different batches and expiration dates were chosen.

The samples in their respective wrappers were duly identified and individually packed in polyethylene bags for first use, without manual contact, kept under refrigeration and analyzed in the Food Microscopy Laboratories of the Regional Laboratory Centers - Instituto Adolfo Lutz de Santo André VIII and Ribeirão Preto VI.

First, the search for macroscopic foreign matter was carried out using the macroanalytical method, recommended by the Food and Drug Administration Technical Bulletin nº 5, removing the individual leaves, and examining each one in search of dirt. If positive for the presence of insects, it was verified whether they were alive or dead[18].

Then, to verify the presence of parasitic structures and other microscopic foreign matter, a technique based on the method developed by Matosinhos et al.[19]was used, with a change in the estimated time of spontaneous sedimentation that went to 24h in conical calyx.

The samples were weighed (50g each) in a 2 L beaker. Then, 200 mL of glycine 1M was added to the lettuce leaves. The set was manually shaken for 3 min with the aid of a glass rod. Then, the liquid was separated from the sample, transferred to a sedimentation cup, and left to rest for 24h. After the sedimentation time, the supernatant was discarded with the aid of a pipette, leaving a sediment of about 10 mL, transferring it to a 15 mL centrifuge tube. The sedimentation cup was washed with 5 mL of distilled water, added to the same tube, and centrifuged at 2,500 rpm for 5 min. With the aid of a Pasteur pipette, the supernatant was discarded and only the sediment was left. It was homogenized manually and with the pipette a small amount of sediment was transferred to a glass slide, stained with Lugol's solution. Slides were read using an optical microscope at 100X and 400X magnification.

The data obtained were compiled in Excel® for descriptive analysis and the graphs were prepared using the GraphPad Software®, through which a statistical analysis was performed according to Tukey's Test for multiple comparison, in order to compare the observed frequencies and verify if there was a statistically significant difference between the proportions of samples in disagreement. A significance level of 0.05% ($p < 0.05$) was adopted.

RESULTS AND DISCUSSION

The foreign matter found in the macroanalytical analyzes were soil grains, live insects of the order Hemiptera (aphids or aphids), live slugs, and live caterpillars and their droppings, being in percentages of 7.7% in the traditional, 18.0% in organic, 26.0% in hydroponic, and 8.3% in minimally processed. For the latter type of sample, this percentage corresponded to the presence of soil grains.

In the microanalytical analyses, considering all types of cultivation and processing, 87.0% of the samples were positive for at least one type of foreign matter, and the most found was an insect fragment, present in 73.0% of the samples. Parasites classified as hookworms and strongyloids were found in 20.0% of the samples, which is very worrying considering the risks of diseases they can cause.

The Table allows comparing the results of the frequencies of analyzed samples of the three types of cultivation and the minimally processed one, for the presence of foreign matter.

It was found that, for the types of traditional and hydroponic cultivation, there was a high frequency of samples (> 50.0%) with amounts from 1 to 30 for whole insect, insect fragments, free-living larva, and insect egg. For insect larvae, no sample presented a quantity greater than 30, regardless of the type of lettuce. For samples of minimally processed lettuce, it was found that the frequencies for the presence of foreign matter were much lower.

Figure 1 shows the results for the presence of insect fragments, the type of foreign matter most commonly found in the samples, in the analyzed lettuces.

In the samples of traditional cultivation, insect fragments were present in 92.0% of the lettuces, highlighting that one sample had 404 insect fragments in the analyzed aliquot, a point that was outside the curve, therefore it is not shown in Figure 1.

For organic cultivation, this type of foreign matter was found in 82.0%, and one sample showed 378 insect fragments, a point that was also outside the curve and is not included in the graph.

Table. Result of the frequency of analyzed lettuce samples, according to the type of cultivation and processing, for the presence of foreign matter in the microscopic analysis.

Foreign matter		Frequency of samples			
		Traditional	Organic	Hydroponic	Minimally processed
Whole insect	0	15.0%	27.0%	13.0%	92.0%
	1-30	85.0%	64.0%	74.0%	8.0%
	> 30	-	9.0%	13.0%	-
Insect fragments	0	8.0%	18.0%	4.0%	75.0%
	1-30	81.0%	73.0%	74.0%	25.0%
	> 30	11.0%	9.0%	22.0%	-
Mite	0	58.0%	64.0%	22.0%	88.0%
	1-30	42.0%	36.0%	74.0%	12.0%
	> 30	-	-	4.0%	-
Free living larva	0	35.0%	55.0%	13.0%	88.0%
	1-30	50.0%	45.0%	7800%	12.0%
	> 30	15.0%	-	9.0%	-
Insect larva	0	70.0%	82.0%	65.0%	88.0%
	1-30	30.0%	18.0%	35.0%	12.0%
	> 30	-	-	-	-
Insect egg	0	35.0%	55.0%	26.0%	75.0%
	1-30	61.0%	45.0%	70.0%	25.0%
	> 30	4.0%	-	4.0%	-
Parasites	0	65.0%	82.0%	7800%	96.0%
	1-30	31.0%	18.0%	22.0%	4.0%
	> 30	4.0%	-	-	-

Source: Elaborated by the authors, 2020.

Hydroponic cultivation was the one that presented the greatest variety of foreign matter. Insect fragments were present in 96.0% and two samples had more than 50 fragments (97 and 81 fragments), also outside the curve shown in Figure 1.

Regarding minimally processed lettuce, 25.0% were positive for insect fragments, a very high rate, considering that these samples underwent a cleaning process and are supposedly ready for consumption.

Figure 2 shows the results of the samples analyzed for the presence of parasites.

Of the total analyzed, regardless of the type of cultivation and processing, the positivity index for the presence of parasites was 58.0%.

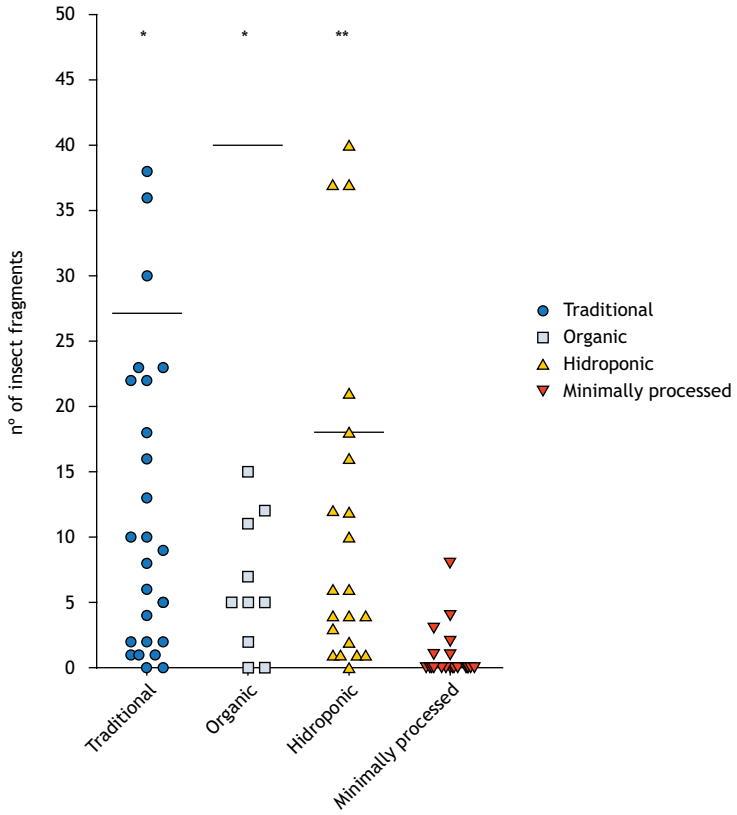
For the traditional cultivation method, there was 35.0% positivity, and in one sample 72 parasitic forms were found, a point that was outside the curve. In organic lettuce, parasites were found in only two samples, corresponding to 18.0%. As for the hydroponic cultivation, positivity for parasites was verified in 22.0% (five samples). Finally, in the minimally processed samples evaluated, parasites were found in only one sample, which is equivalent to 4.0%.

Even though in the descriptive analysis it is possible to perceive differences between the four types of lettuce, according to Tukey's Test for multiple comparison, there was no statistically significant difference between the different types of cultivation and processing for the presence of insect fragments, nor for the presence of parasites.

In addition to the aforementioned parameters, the following foreign matter was also found: insect pupa, synthetic fibers, fungi (filamentous, spores, and yeasts), sand (earth grains), animal hair, pollen grains, starch, algae, and free-living organisms. Figure 3 shows some foreign matter present in the lettuce samples analyzed.

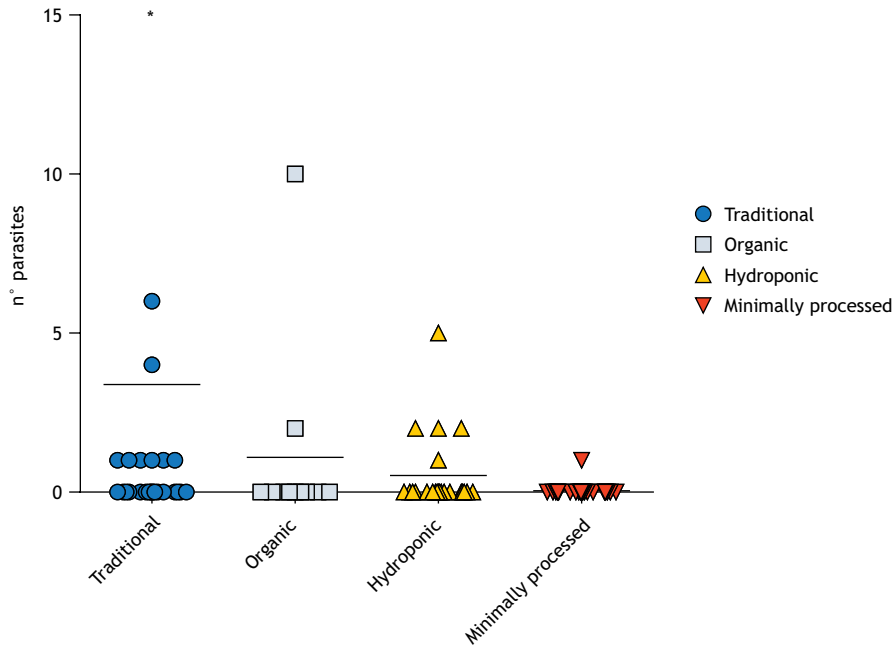
Regarding the research of foreign matter in lettuces, there are few data in the literature. In the work carried out by Guimarães et al.[20], all samples analyzed showed at least one type of contamination, a result similar to that of the present study, however the percentage of samples with the presence of light dirt did not exceed 50.0%, a value much lower than what we found. On the other hand, up to 50.0% of samples collected in open markets showed nematode larvae.

Santana et al.[21] carried out a survey on the physical, microbiological, and parasitological quality of lettuce, in which they



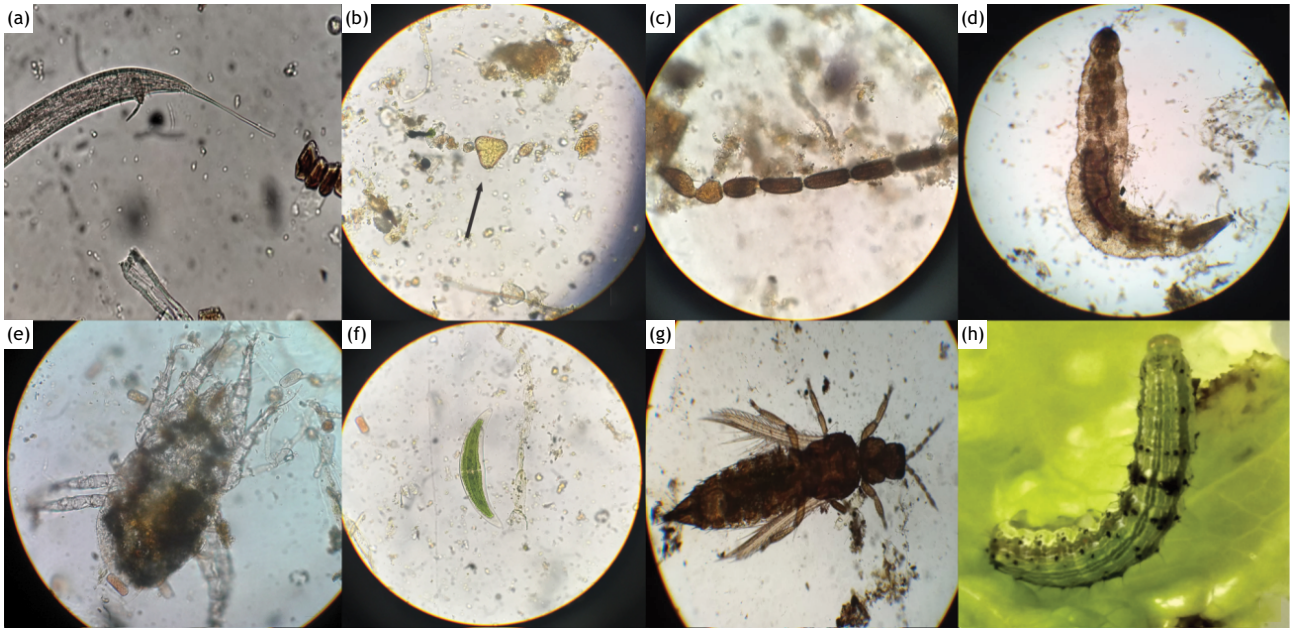
Source: Elaborated by the authors, 2020.
* Points outside the curve.

Figure 1. Insect fragments found in lettuce samples from the three cultivation modes (traditional, organic, and hydroponic) and minimally processed, in absolute numbers.



Source: Elaborated by the authors, 2020.
* Points outside the curve.

Figure 2. Parasites found in lettuce samples from the three cultivation modes (traditional, organic, and hydroponic) and minimally processed, in absolute numbers.



Source: Elaborated by the authors, 2020.
A: *Strongyloides* spp larva with detail for the presence of spicule; B: pollen grain; C: insect fragment; D: insect larva; E: mite; F: microalgae of the genus *Closterium* sp; G: whole insect of the order Thysanoptera; H: caterpillar.

Figure 3. Examples of foreign matter found in lettuce samples analyzed under optical microscope, 100x to 400x magnification.

worked with the same cultivation systems of the present study and obtained percentages very similar to those found in this work for the presence of insect fragments (83.3%) and for strongyloid parasites (33.3%), with more expressive values in the organic lettuces.

In the research carried out by Dantas et al.[22], the presence of insects was verified by macroscopic analysis in up to 67.0% of the analyzed lettuce samples. The same authors revealed the presence of parasites in up to 77.0% of the lettuces.

The similar levels of contamination of the three lettuce cultivation systems seem to be fundamentally associated with the sanitary conditions of the environment in which they are cultivated, even different in each production system, according to the cultivation practices used.

The presence of light soiling in lettuce samples is expected considering that the products come from the field and do not undergo any cleaning procedure. However, it serves as an indicator of the presence of possible pathogens, including parasites, in addition to the possibility of characterizing a transmission profile of intestinal parasites.

As for the minimally processed samples, the presence of dirt indicates, in addition to failures of good practices, a potential health risk, due to the possibility of also containing pathogens and because they are ready-to-eat foods.

Free-living insects, mites, and larvae that are identified as important carriers of pathogenic agents were also found in the work developed by Silva et al.[23], although the quantitative data were not disclosed, which reinforces the need for adequate hygiene to leave the lettuces in conditions of consumption[23,24].

Intestinal parasitosis still constitute a serious public health problem in Brazil. They have a higher prevalence in populations of lower socioeconomic status and precarious conditions of basic sanitation, which result in high rates of contamination. Considering the importance of consuming vegetables for health and that they are foods eaten *in natura*, the occurrence of parasites in this type of product exposes a large part of the population to parasitic diseases.

The importance of this theme is noticeable by the number of published works on the incidence and frequency of parasites in vegetables.

Internationally, studies carried out in Ghana, Egypt, and Colombia revealed 61.0%, 45.5%, and 100.0%, respectively, of positivity for parasites in lettuce samples, values much higher than those of the present study[13,25,26].

In Brazil, research carried out in all regions of the country showed different results for the presence of parasites in lettuce samples.

Simões et al.[27], working with samples from Campinas (São Paulo), Takayanagui et al.[28], analyzing samples from Ribeirão Preto (São Paulo), and Aquino[27], in a research with lettuce sold in the Sertão of Paraíba, found percentages of positivity for parasites of 6.4%, 7.0%, and 4.2%, respectively, values close to those found for the minimally processed samples of the present study.

On the other hand, some studies revealed 100.0% of lettuce samples with the presence of parasites, such as the publication by Cantos et al.[30], who worked with vegetables sold in the city of Florianópolis (Santa Catarina), Nakashima et al.[31], who analyzed samples of lettuce after treatment with different fertilizers, and also Oliveira Filho et al.[32], who carried out an evaluation of the parasitological prevalence of lettuce sold in street markets in a municipality in the interior of Minas Gerais.

Indices of contamination with parasites above 40.0% in lettuce samples were verified, including their identification, such as *Entamoeba* spp, *Isospora* spp, *Strongyloides stercoralis*, *Toxocara* spp, *Eimeria* spp, *Giardia* spp, among others, in studies developed in several regions of Brazil[33,34,35,36].

One factor that favors this high rate of contamination of lettuces is their own anatomy. The wide flexible leaves, juxtaposed and compact structure present a greater possibility of contamination, because, during its cultivation, there is a greater contact with the polluted soil and water, consequently, a greater adhesion of the parasitic evolutionary forms, conferring a greater resistance to the hygiene methods[37].

Percentage data similar to those found in lettuces grown in the traditional, organic, and hydroponic modes of this work for the presence of parasites were also reported. In studies carried out in the Southeast region of Brazil, Colli et al.[38] revealed 18.0% of samples positive for *Giardia duodenalis*. Lellis et al.[39] found a percentage that ranged from 18.2% to 36.3% for units collected in supermarkets and 8.3% to 22.2% for rates from street markets. Lopes and Santos[40] also demonstrated that 20.0% of the lettuces contained enteroparasites. Lastly, Pacífico et al.[41] reported that 12.0% of the products analyzed had parasites.

In the Northeast region, there are also reports of the occurrence of parasites in lettuce samples at values similar to those of this study. Esteves and Figueirôa[42] found 23.8% positivity, while Mesquita et al.[43] obtained a percentage rate of 34.1%.

The same occurs in the Midwest Region, where Ferro et al.[44] and Peres Junior et al.[12] showed that 11.0% and 20.0%, respectively, of the analyzed lettuce samples had parasites. In the North of Brazil, Medeiros et al.[45] observed that the level of contamination of the vegetable was similar in the different seasonal periods, being 36.7% during the dry season and 38.7% in the rainy season. Finally, in the South Region, in a study carried out by Montanher et al.[46] it was shown that only 10.0% of the samples contained parasites.

It is pointed to the positivity of the samples, the vulnerability for contamination of fecal origin in the soil of the garden, contaminating the vegetables. Agricultural practices, ecological conditions, transport, and handling, storage, and marketing conditions are factors that also contribute to the occurrence of contaminating agents for this vegetable[23].

Factors such as high humidity, temperature between 20°C and 30°C and good oxygenation are essential for the development of helminth eggs and favor the processes of embryogenesis, larvae formation, and hatching[45]. Perhaps for reasons related to the climate, the results of the different studies, in different regions, are so discrepant: from absence to 100% of samples analyzed with the presence of parasites.

In general, the method used for the investigation of dirt and parasites in most of the aforementioned works was spontaneous sedimentation with water for 24h[12,13,22,23,29,30,32,40,43,45]. There are some variations related to the extractor liquid, some authors use detergent[3,20,31,33,35,36,39], 33% zinc sulfate[20,21,27,31,42], *tween*[41,44], or saline[25,26,35,46] solution, in order to facilitate the detachment of dirt and parasitic forms from the leaves.

In the present work, glycine 1 M was used to wash lettuce leaves and recover foreign matter, including parasites, as recommended in the methodology proposed by Matosinhos et al.[19]. Cook et al.[48] demonstrated that glycine 1 M presented a percentage greater than 100% in the recovery of oocysts from *Cryptosporidium parvum* from lettuce and raspberries samples, being the best of all extractor liquids studied.

The fact is that all methodologies used supposedly proved to be efficient to recover this foreign matter, including parasites, and measure their occurrence.

It is important to mention the difficulty in laboratory diagnosis of the genus and species of different structures such as eggs and larvae of the various parasites, due to the morphological similarity that they present when visualized by light microscopy. Numerous species of superfamilies are parasites of other animals and free-living beings and pose no danger to human health. However, it must be considered that some free-living nematodes may play an important role in the transmission of pathogens such as *Cryptosporidium parvum*[49].

In general, regardless of the source of contamination of vegetables, washing before consumption is the most important part to eliminate possible parasites and dirt that may be present in the food, as recommended by the Ministry of Health[31,50].

CONCLUSIONS

The laboratory analysis of vegetables is of great importance for providing data on the hygienic-sanitary conditions related to the different stages of the production chain and as a control of the transmission of enteroparasitoses, pointing to the risks of infection that the population is exposed to, in addition to contributing to the taking of adequate prophylactic measures. In the case of minimally processed vegetables, data on their quality are even more relevant as the products are ready to eat and may pose an even greater risk to consumers.

The present study demonstrated that the lettuce samples of the three types of cultivation showed a high frequency of foreign matter, as well as the presence of parasites, which highlights the importance of hygiene procedures for this type of food before consumption. In relation to minimally processed lettuce, the results, even at lower frequencies, call for corrective actions to be taken in the sanitization process by producers and/or traders.

The performance of the Health Surveillance in the places of production and distribution of vegetables is essential for the maintenance of food quality and safety, as well as the elaboration of specific legislation at the national level, considering that the consumption of vegetables is increasing by the population that seeks a balanced and healthy diet. There is also a notable expansion of food services, in addition to the increase in consumption of minimally processed foods.

REFERENCES

1. Portal do Agronegócios. Alface é a folha mais consumida no Brasil. MS Notícias. 16 abr 2015[acesso 5 jun 2020]. Disponível em: http://www.msnoticias.com.br/editorias/agronegocios/um/57176/
2. Instituto Brasileiro de Geografia e Estatística - IBGE. Pesquisa de orçamentos familiares POF: tabelas: análise do consumo alimentar pessoal no Brasil. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2020[acesso 5 jun 2020]. Disponível em: https://www.ibge.gov.br/estatisticas/sociais/educacao/9050-pesquisa-de-orcamentos-familiares.html?edicao=9051&t=resultados
3. Silva AAV, Costa AFM, Freitas RMS, Santos MBSV, Lourenço ALN, Malta AS et al. Qualidade parasitológica e condições higiênico-sanitárias de sururu (*Mytella charruana*) e alface (*Lactuca sativa*) comercializados em um mercado público de Maceió-AL. Rev Ciênc Farm Basica Apl. 2015;36(4):525-9.
4. Resende FV, Saminêz TCO, Vidal MC, Souza RB, Clemente FMV. Cultivo de alface em sistema orgânico de produção. Circular Técnica Embrapa 56. nov 2017.
5. Vilela NJ, Luengo RFA. Produção de hortaliças folhosas no Brasil. Campo e Negócios Online. 20 jul 2017.
6. Henz GP, Suinaga F. Tipos de alface cultivados no Brasil. Comunicado Técnico Embrapa 75. 2009[acesso 5 jun 2020]. Disponível em: https://www.cnph.embrapa.br/paginas/serie_documentos/.../cot_75.pdf
7. Silva AMNCP, Ferreira RLF, Araújo Neto SE, Tavella LB, Solino AJS. Qualidade de alface crespa cultivada em sistema orgânico, convencional e hidropônico. Hortic Bras. 2011;29(2):242-5. https://doi.org/10.1590/S0102-05362011000200019
8. Sediyama MAN, Santos IC, Lima PC. Cultivo de hortaliças no sistema orgânico. Rev Ceres. 2014;61(Supl.):829-37. https://doi.org/10.1590/0034-737X201461000008
9. Bezerra Neto E, Barreto LP. As técnicas de hidroponia. An Acad Pernamb Cienc Agron. 2011;8:107-37.
10. Maistro LC. Alface minimamente processada: uma revisão. Rev Nutr. 2001;14(3):219-24. https://doi.org/10.1590/S1415-52732001000300008
11. Arbos KA, Freitas RJS, Stertz SC, Carvalho LA. Segurança alimentar de hortaliças orgânicas: aspectos sanitários e nutricionais. Ciênc Tecnol Aliment. 2010;30(Supl.1):215-20. https://doi.org/10.1590/S0101-20612010000500033
12. Peres Junior J, Gontijo EEL, Silva MG. Perfil parasitológico e microbiológico de alfaces comercializadas em restaurantes *self-service* de Gurupi-TO. Rev Cient ITPAC. 2012;5(1):1-8.
13. Polo GA, Benavides CJ, Astaiza JM, Vallejo DA, Betancourt P. Determinación de enteroparásitos en *Lactuca sativa* en fincas dedicadas a su producción en Pasto, Colombia. Rev Biomed. 2016;36(4):525-34. https://doi.org/10.7705/biomedica.v36i4.2914
14. Panza SGA, Fagan RFV. Levantamento das condições de higienização de vegetais em restaurantes do município de Maringá/PR. Hig Aliment. 2008;22(164):19-24.
15. Ristori CA, Sakuma H, Paula AMR, Rowlands REG, Lopes GIL, Pisani B et al. Elucidação de surtos de doenças transmitidas por alimentos, no estado de São Paulo, no período de julho de 2002 a dezembro de 2004. Bol Inst Adolfo Lutz. 2006;16(1):14-5.
16. Kluge RA, Silveira Gomez AC, Inestroza-Lizardo C, Berno ND. Processamento mínimo de hortaliças: princípios e práticas. Piracicaba: Universidade de São Paulo; 2016.
17. Secretaria de Agricultura e Abastecimento do Estado de São Paulo - SAA. Resolução Nº 42, de 19 de junho de 2009. Recomenda norma técnica para produtos hortifrutícolas minimamente processados e frescos cortados. Diário Oficial do Estado. 20 jun 2009.
18. US Food and Drug Administration - FDA. Macroanalytical procedures (MPM). FDA Technical Bulletin 5. 1984[acesso 8 jun 2020]. Disponível em: http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/MacroanalyticalProceduresManualMPM/default.htm
19. Matosinhos FC, Valenzuela VC, Silveira JA, Rabelo EM. Standardization of a method for the detection of helminth eggs and larvae in lettuce. Parasitol Res. 2016;115(5):1827-34. https://doi.org/10.1007/s00436-016-4922-8
20. Guimarães AM, Alves EGL, Figueiredo HCP, Costa GM, Rodrigues LS. Freqüência de enteroparasitas em amostras de alface (*Lactuca sativa*) comercializadas em Lavras, Minas Gerais. Rev Soc Bras Med Trop. 2003;36(5):621-3. https://doi.org/10.1590/S0037-86822003000500014
21. Santana LRR, Carvalho RDS, Leite CC, Alcântara LM, Oliveira TWS, Rodrigues BM. Qualidade física, microbiológica e parasitológica de alfaces (*Lactuca sativa*) de diferentes sistemas de cultivo. Ciênc Tecnol Aliment. 2006;26(2):264-9. https://doi.org/10.1590/S0101-20612006000200006
22. Dantas MM, Lima EQ, Queiroga Filho E. Avaliação parasitária em hortaliça comercializada no semiárido da Paraíba, Brasil. Braz J Develop. 2020;6(1):2667-84. https://doi.org/10.34117/bjdv6n1-193
23. Silva J, Moura VG, Silva MJM, Chaves CC, Silva AV, Sousa PB et al. Ocorrência de enteroparasitas em alface crespa (*Lactuca sativa*) de cultivo convencional comercializadas em supermercados e hortas comunitárias de Teresina, Piauí. Rev Eletronica Acervo Saude. 2019;11(17):1-7. https://doi.org/10.25248/reas.e1728.2019

24. Neves DP. Parasitologia humana. 12a ed. São Paulo: Atheneu; 2011.
25. Duedu KO, Yarnie EA, Tetteh-Quarcoo PB, Attah SK, Donkor ES, Ayeh-Kumi PF. A comparative survey of the prevalence of human parasites found in fresh vegetables sold in supermarkets and open-aired markets in Accra, Ghana. BMC Res Notes. 2014;7:1-6. https://doi.org/10.1186/1756-0500-7-836
26. Eraky MA, Rashed SM, Nasr MES, El-HamsharyS, El-Ghannam AS. Parasitic contamination of commonly consumed fresh leafy vegetables in Benha, Egypt. J Parasitol Res. 2014;2014:1-7. https://doi.org/10.1155/2014/613960
27. Simões M, Pisani B, Marques EGL, Prandi MAG, Martini MH, Chiarini PFT et al. Hygienic-sanitary conditions of vegetables and irrigation water from kitchen gardens in the municipality of Campinas, SP. Braz J Microbiol. 2001;32(4):331-3. https://doi.org/10.1590/S1517-83822001000400015
28. Takayanagui OM, Capuano DM, Oliveira CAD, Bergamini AMM, Okino MHT, Silva AAMCC et al. Análise da cadeia de produção de verduras em Ribeirão Preto, SP. Rev Soc Bras Med Trop. 2006;39(2):224-6. https://doi.org/10.1590/S0037-86822006000200018
29. Aquino VVF. Qualidade higiênico: sanitária de alfaces (*Lactuca sativa*) comercializadas em feiras livres do sertão da Paraíba [dissertação]. Campina Grande: Universidade Federal de Campina Grande; 2019.
30. Cantos GA, Soares B, Maliska C, Gick D. Estruturas parasitárias encontradas em hortaliças comercializadas em Florianópolis, Santa Catarina. Rev NewsLab. 2004;66:154-63
31. Nakashima HH, Faglioni CM, Aguera RG, Santos RU, Ecker AEA, Ecker ABS. Análise parasitológica pós colheita de *Lactuca sativa* em diferentes fertilizantes orgânicos. Uningá Rev J. 2019;34(3):1-13.
32. Oliveira Filho JES, Caixeta BT, Silva AL, Monteiro EM, Ribeiro AC, Dias RLA. Avaliação da prevalência parasitológica de alfaces (*Lactuca sativa*) comercializadas por feiras livres em um município do interior de Minas Gerais. Rev Psicol Saude Debate. 2019:5(1):10-8. https://doi.org/10.22289/2446-922X.V5N1A2
33. Coelho LMP, Oliveira SM, Milman MHSA, Karasawa KA, Santos RP. Detecção de formas transmissíveis de enteroparasitas na água e nas hortaliças consumidas em comunidades escolares de Sorocaba, São Paulo, Brasil. Rev Soc Bras Med Trop. 2001;34(5):479-82. https://doi.org/10.1590/S0037-86822001000500012
34. Oliveira DCS, Brito JK, Maia MC. Avaliação parasitológica em amostras de alfaces (*Lactuca sativa*) comercializadas em supermercados de Ipatinga, Minas Gerais. Rev Dig Nutr. 2012;6(11):933-44.
35. Soares B, Cantos GA. Qualidade parasitológica e condições higiênico-sanitárias de hortaliças comercializadas na cidade de Florianópolis, Santa Catarina, Brasil. Rev Bras Epidemiol. 2005;8(4):377-84. https://doi.org/10.1590/S1415-790X2005000400006
36. Soares B, Cantos GA. Detecção de estruturas parasitárias em hortaliças comercializadas na cidade de Florianópolis, SC, Brasil. Braz J Pharm Sci. 2006;42(3):455-60. https://doi.org/10.1590/S1516-93322006000300015
37. Falavigna LM, Freitas CBR, Melo GC, Nishi L, Araújo SM, Falavigna-Guilherme AL. Qualidade de hortaliças comercializadas no noroeste do Paraná, Brasil. Parasitol Latinoam. 2005;60(3-4):144-9. https://doi.org/10.4067/S0717-77122005000200007
38. Colli CM, Bezagio RC, Nishi L, Bignotto TS, Ferreira EC, Falavigna-Guilherme AL et al. Identical assemblage of *Giardia duodenalis* in humans, animals and vegetables in an urban area in southern Brazil indicates a relationship among them. PLoS One. 2015;10(3):1-12. https://doi.org/10.1371/journal.pone.0118065
39. Lellis JR, Rosa NCE, Branco Jr AC. Frequência de protozoários e helmintos entéricos em hortaliças produzidas e comercializadas em Bauru, no centro-oeste paulista. RBAC. 2019;51(4):335-41. https://doi.org/10.21877/2448-3877.201900843
40. Lopes MER, Santos HF. Caracterização de contaminação por enteroparasitas em amostragem de alfaces comercializadas no interior paulista. Nucleus. 2019;16(2):501-8. https://doi.org/10.3738/1982.2278.2913
41. Pacífico BB, Bastos OMP, Uchôa CMA. Contaminação parasitária em alfaces crespas (*Lactuca sativa* var. crispa), de cultivos tradicional e hidropônico, comercializadas em feiras livres do Rio de Janeiro (RJ). Rev Inst Adolfo Lutz. 2013;72(3):219-25.
42. Esteves FAM, Figueirôa EO. Detecção de enteroparasitas em hortaliças comercializadas em feiras livres do município de Caruaru (PE). Rev Baiana Saude Publica. 2009;33(2):38-47. https://doi.org/10.22278/2318-2660.2009.v33.n2.a204
43. Mesquita DR, Silva JP, Monte NDP, Sousa RLT, Silva RVS, Oliveira SS et al. Ocorrência de parasitos em alface-crespa (*Lactuca sativa* l.) em hortas comunitárias de Teresina, Piauí, Brasil. Rev Patol Trop. 2015;44(1):67-76. https://doi.org/10.5216/rpt.v44i1.34802
44. Ferro JJB, Costa-Cruz JM, Barcelos ISC. Avaliação parasitológica de alfaces (*Lactuca sativa*) comercializadas no município de Tangará da Serra, Mato Grosso, Brasil. Rev Patol Trop. 2012;41(1):47-54. https://doi.org/10.5216/rpt.v41i1.17745
45. Medeiros FA, Oliveira TR, Málaga SMR. Segurança dos alimentos: influência sazonal na contaminação parasitária em alface (*Lactuca sativa* L.) comercializada em feiras livres de Belém, Pará. Braz J Food Technol. 2019;22:1-8. https://doi.org/10.1590/1981-6723.20518
46. Montanher CC, Coradin DC, Fontoura-da-Silva SE. Avaliação parasitológica em alfaces (*Lactuca sativa*) comercializadas em restaurantes self-service por quilo, da cidade de Curitiba, Paraná, Brasil. Estud Biol. 2007;29(66):63-71. https://doi.org/10.7213/reb.v29i66.22749

47. Taylor MA, Coop RL, Wall RL. Parasitologia veterinária. 4a ed. Rio de Janeiro: Guanabara Koogan; 2017.

48. Cook N, Paton CA, Wilkinson N, Nichols RAB, Barker K, Smith HV. Towards standard methods for the detection of *Cryptosporidium parvum* on lettuce and raspberries: part 1: development and optimization of methods. Int J Food Microbiol. 2006;109(3):215-21. https://doi.org/10.1016/j.ijfoodmicro.2005.12.015

49. Huamanchay O, Genzlinger L, Iglesias M, Ortega YR. Ingestion of *Cryptosporidium oocysts* by *Caenorhabditis elegans*. J Parasitol. 2004;90(5):1176-8. https://doi.org/10.1645/GE-253R

50. Agência Nacional de Vigilância Sanitária - Anvisa. Cartilha sobre boas práticas para serviços de alimentação. Brasília: Agência Nacional de Vigilância Sanitária; 2004[acesso 12 jul 2020]. Disponível em: http://www.anvisa.gov.br/divulga/public/alimentos/cartilha_gicra_final.pdf

Author's Contributions

Mattos EC, Prado SPT – Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the work Santana JP, Sgarbosa NO – Study planning, acquisition, data analysis, and writing of the work. Sgarbosa NO – Study planning, acquisition, data analysis, and writing of the work. Dal Col R, Daros VMSG – Conception, planning (study design), data analysis, and writing of the work. All authors approved the final version of the work.

Conflict of Interests

The authors inform that there is no potential conflict of interest with peers and institutions, politicians, or financial in this study.