



Hygienic-sanitary quality of lettuce (*Lactuca sativa*) and arugula (*Eruca sativa*) produced in an organic farm system in Cuiabá, MT, Brazil

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Submitted on 03/25/2022; Accepted on 02/03/2023; Published on 03/30/2023

ABSTRACT: Demand for organic food has increased as a result of awareness of the health risks posed by the presence of chemical residues. In this context, this study aimed to verify the safety of lettuce (*Lactuca sativa*) and arugula (*Eruca sativa*) produced organically in the metropolitan region of Cuiabá, MT, Brazil, and identify the main microbiological contamination sources of this production system. Fifty-five samples, 10 lettuces and 10 arugula, were collected in a farm and supermarket, in addition to five samples of each agricultural adjuvant used in the production system (irrigation water, vegetable and animal fertilizers). No *Salmonella* spp. was detected, although thermotolerant coliforms above the maximum acceptable limit established by the Commission on Microbiological Specifications for Foods (ICMSF) were observed in 90% (9/10) and 50% (5/10) of the farm arugula and lettuce samples, 20% (2/10) and 10% (1/10) of the supermarket arugula and lettuce samples, 60% of animal and vegetable fertilizers (6/10) and 40% (2/5) of irrigation water samples. Over half of the vegetable samples analyzed herein were, thus, unfit for consumption, indicating the relatively high influence of system inputs on the hygienic-sanitary quality of the arugula and lettuce produced in the investigated organic farm.

Keywords: fertilization; organic farming; sanitary quality; contamination.

Qualidade higiênico-sanitária de alface (*Lactuca sativa*) e rúcula (*Eruca sativa*) produzidas em sistema de produção orgânica em Cuiabá, MT, Brasil

RESUMO: A procura por alimentos orgânicos tem aumentado devido a conscientização sobre os riscos à saúde decorrentes da presença de resíduos químicos. Nesse contexto, este estudo teve como objetivo verificar a segurança de alface (*Lactuca sativa*) e rúcula (*Eruca sativa*) produzidas organicamente na região metropolitana de Cuiabá, MT, Brasil, e identificar as principais fontes de contaminação microbiológica desse sistema de produção. Cinquenta e cinco amostras, sendo 10 de alfaces e 10 de rúculas foram coletadas em uma fazenda e supermercado, além de quinze amostras de adjuvantes agrícola utilizados no sistema de produção. Nenhuma *Salmonella* spp. foi detectada, embora coliformes termotolerantes acima do limite máximo aceitável estabelecido pela Comissão de Especificações Microbiológicas para Alimentos (ICMSF) tenham sido observados em 90% (9/10) e 50% (5/10) das amostras de rúcula e alface da fazenda, 20% (2/10) e 10% (1/10) das amostras de rúcula e alface de supermercado, 60% de fertilizantes animais e vegetais (6/10) e 40% (2/5) de amostras de água de irrigação. Mais da metade das amostras de hortaliças aqui analisadas estavam, portanto, impróprias para consumo, indicando a influência relativamente alta dos insumos do sistema na qualidade higiênico-sanitária da rúcula e alface produzidas na fazenda orgânica investigada.

Palavras-chave: fertilização; agricultura orgânica; qualidade sanitária; contaminação.

1. INTRODUCTION

Consumers are becoming increasingly attentive to the vegetable cultivation origin and socio-environmental aspects instead of only availability, appearance, and economic value (ECHER et al., 2016). An 18.5% increase in the demand for organic products, considered significantly healthier, was noted, for example, in Brazil in 2017 (BRASIL, 2017). In the same year, the Brazilian vegetable market generated US\$ 5,084.05 million. Lettuce is the most important vegetable in Brazil (ECHER et al., 2016), and the last agricultural census carried out by the Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística - IBGE*) indicated a total of 108,382 t year⁻¹ of lettuce produced in the

country, with 7,062 t year⁻¹ produced in the Midwest region and 2,114 t year⁻¹ in the state of Mato Grosso alone (BRASIL 2017; IBGE, 2017).

Leafy vegetables can be produced by both conventional and organic production systems, with the latter employing no phytosanitary products for pest and disease control. However, the concept of organic products is very complex, covering management, ecology and social aspects involved in product marketing (RODRIGUES et al., 2009; BRASIL, 2017).

Several factors can contribute to the origin of pathogens in fresh vegetables, such as fertilizers from plant compost and animal, which waste are commonly used in organic

production systems, and may contribute to the presence of intestinal parasites (helminths and protozoa), viruses and bacteria, which may, in turn, contaminate foodstuffs and lead to foodborne illnesses in consumers (BARKER-REID et al., 2009; JOHANNESSEN et al., 2004). Other factors include the use of animal or human manure, the presence of insects, soil dust contaminated with animals and human feces (BARKER-REID et al., 2009; POMA et al., 2016), rain, irrigation and post-harvest washing water, ice, equipment and, finally, handler hygiene during the harvesting, processing and transportation steps (JOHANNESSEN et al., 2002; JUNG et al., 2014).

A total of 83 foodborne disease outbreaks associated to fresh products have been reported in the United States of America from 2000 to 2017, ten triggered by the Shiga-toxin producing *Escherichia coli* (STEC) serotype and tree by the Enteritidis, Newport and Paratyphi B serotypes *Salmonella*, both microorganisms found in contaminated lettuce (CARSTENS et al., 2019). Foodborne outbreaks triggered by the presence of these microorganisms in leafy vegetables have also been reported from 1973 to 2016 (HERMAN et al., 2015; WADAMORI et al., 2017; JOHNSON, 2019; TURNER et al., 2019). Studies evaluating data associated to foodborne outbreaks from 2000 to 2018 in Brazil report the presence of both *Salmonella* spp. coliforms and *E. coli* in fruits and vegetables (ELIAS et al., 2018; FINGER et al., 2019). These microorganisms can cause conditions ranging from enteritis to systemic diseases, mainly affecting low-income populations in developing countries and resulting in public health and economy impacts (BENNETT et al., 2018). Monitoring the hygienic conditions of vegetable production is, thus, recommended, which can be carried out indirectly by microbiological evaluations (BERGER et al., 2010).

Contamination in organic production systems can occur due to the use of contaminated irrigation water and fertilizers, as well as in the harvest, transport, and handling stages and during marketing. Previous microbiological studies have, in fact, indicated the presence of *E. coli* in Brazil even in organic vegetables (MAFFEI et al., 2013), Norway (LONCAREVIC et al., 2005), and Zambia (NGUZ et al., 2005). In this context, this study aimed to verify the microbial safety of organic lettuce (*Lactuca sativa*) and arugula (*ErUCA sativa*) produced in Cuiabá, in the state of Mato Grosso, Brazil, by assessing the main microbiological contamination sources in organic production systems.

2. MATERIAL AND METHODS

2.1. Sample collection

Samplings were performed in an organic leafy vegetable farm that produces lettuce, arugula, chives, parsley, cilantro, and vegetables such as carrots, cherry tomatoes, beets and broccoli, located in the rural area of the city of Cuiabá, approximately 12 km from the capital. The farm is certified for organic production by an accredited Brazilian National Institute of Metrology, Quality and Technology (*Instituto Nacional de Metrologia, Qualidade e Tecnologia* - INMETRO) company. A total of 10 lettuces and 10 arugulas packaged for sale directly on the farm, as well as 10 samples of each vegetable sold in supermarkets and five samples of irrigation water, vegetable fertilizer and animal fertilizer (chicken waste) each were obtained, totaling fifty-five samples. The samples were packed in first-use plastic bags, placed on ice in an isothermal box and transported to the Laboratory of Molecular Microbiology of Food (LabMMA) belonging to

the Faculty of Nutrition at the Federal University of Mato Grosso (UFMT).

2.2. Microbiological analyses

Microbiological analyses were performed according to American Public Health Association (APHA), these methodologies were applied for total and thermotolerant coliform determinations (SILVA et al., 2017). The International Organization for Standardization (ISO) 6579 methodology was applied for *Salmonella* spp. determinations (ISO, 2002). For coliforms, the same amount was diluted in 225 mL of 0.1% peptone saline solution followed by subsequent dilutions up to 10^{-3} . Total (35 °C) and thermotolerant (45 °C) coliforms were determined by the multiple tube method, through the determination of the most probable number (MPN g^{-1}). Briefly, 1 mL aliquot dilutions were inoculated in a series of three tubes containing 9 mL of Lauryl Sulfate Tryptose (LST) broth (Himedia®, Mumbai, India) and incubated at 35 ± 1 °C for 24 - 48 hours. Samples displaying turbidity and gas formation were considered positive (Silva, Junqueira, Silveira, Taniwaki, & Gomes, 2017) and subsequently inoculated in Brilliant Green Bile (VB) broth (Himedia®, Mumbai, India) and incubated at 35 ± 1 °C for 24 - 48 h, and inoculated in *Escherichia coli* broth (EC) at 44.5 ± 0.5 °C for 24 - 48 h.

Concerning *Salmonella* sp., 25 g of each sample were inoculated in 225 mL of 1% Buffered Peptone Water (Oxoid®, Basingstoke, United Kingdom), incubated at 35 ± 1 °C for 24 h. Subsequently, 1 mL and 0.1 mL aliquots were mixed with Tetrathionate and Rappaport-Vassiliadis broths (Oxoid®, Basingstoke, United Kingdom), and incubated at 35 ± 1 °C and 42 °C for 24 h respectively. Aliquots were then plated in (BGA) Brilliant Green Agar (Himedia®, Mumbai, India) and (XLD) Xylose Lysine Deoxycholate (Himedia®, Mumbai, India) Agar and incubated at 35 ± 1 °C for 24 h. Typical translucent colonies with a red halo in BGA and a black center in XLD agar were subjected to biochemical tests (TSI-Triple Sugar Iron Agar, LIA-Lysine Iron Agar and Urea Agar [Christensen] - Himedia®, Mumbai, India) for the presumptive confirmation of *Salmonella* spp. (ISO, 2002).

Regarding reference standards, 1 Log MPN/100 mL was set as the maximum acceptable limit for thermotolerant coliforms in irrigation water, 2.30 Log MPN g^{-1} for the evaluated fresh leafy vegetables, and 2 Log MPN g^{-1} for fertilizer samples. The absence of *Salmonella* spp. is indicative of satisfactory quality according to the International Commission for Microbiological Specifications for Food (ICMSF, 2015).

3. RESULTS

No *Salmonella* spp. was detected in any of the 55 vegetable, water or fertilizer samples obtained from the organic leafy vegetable production farm located in the metropolitan region of Cuiabá, MT, Brazil (Tables 1 and 2).

Acceptable coliform counts were detected in the lettuce, arugula and irrigation water samples (ICMSF, 2015). These counts were the highest in 90% of the arugula farm samples, followed by 50% of the lettuce farm samples (Table 1), in contrast with 10% and 20% supermarket lettuce and arugula samples (Table 1) and 40% irrigation water samples obtained on days 1 and 3. On the other hand, 60% of the organic fertilizers exhibited thermotolerant coliforms above the maximum recommended limit established by the ICMSF (2015) (Table 2).

Table 1. Total coliforms (35 °C), thermotolerant coliforms (45 °C) and *Salmonella* spp. in arugula (*Eruca sativa*) and lettuce (*Lactuca sativa*) samples produced in an organic system in the metropolitan region of Cuiabá, Mato Grosso, Brazil, obtained directly from the vegetable farm and from supermarkets.

 Tabela 1. Coliformes totais (35 °C), coliformes termotolerantes (45 °C) e *Salmonella* spp. em amostras de rúcula (*Eruca sativa*) e alface (*Lactuca sativa*) produzidas em sistema orgânico na região metropolitana de Cuiabá, Mato Grosso, Brasil, obtidas diretamente da horta e de supermercados.

Harvest days	Samples	Farm samples			Supermarket samples		
		Coliforms (Log ₁₀ MPN g ⁻¹)		<i>Salmonella</i> spp. in 25 g ⁻¹	Coliforms (Log ₁₀ MPN g ⁻¹)		<i>Salmonella</i> spp. in 25 g ⁻¹
		35 °C	45 °C		35 °C	45 °C	
1	Lettuce 1	≥3.04	≥3.04	Absent	1.60	0	Absent
	Lettuce 2	≥3.04	≥3.04	Absent	0.093	0	Absent
	Arugula 1	≥3.04	≥3.04	Absent	2.30	0.22	Absent
	Arugula 2	≥3.04	≥3.04	Absent	1.90	0.091	Absent
2	Lettuce 1	≥3.04	2.04	Absent	3.00	0	Absent
	Lettuce 2	≥3.04	≥3.04	Absent	2.70	0.40	Absent
	Arugula 1	2.04	≥3.04	Absent	≥3.04	0	Absent
	Arugula 2	≥3.04	≥3.04	Absent	≥3.04	0.48	Absent
3	Lettuce 1	≥3.04	2.04	Absent	2.30	1.60	Absent
	Lettuce 2	≥3.04	≥3.04	Absent	0.51	0.032	Absent
	Arugula 1	≥3.04	≥3.04	Absent	0.51	0.09	Absent
	Arugula 2	≥3.04	2.32	Absent	2.20	0.51	Absent
4	Lettuce 1	2.04	2.32	Absent	2.70	0.66	Absent
	Lettuce 2	2.04	2.17	Absent	2.20	0	Absent
	Arugula 1	2.04	≥3.04	Absent	≥3.04	0.091	Absent
	Arugula 2	≥3.04	≥3.04	Absent	≥3.04	≥3.04	Absent
5	Lettuce 1	2.66	<0.3	Absent	2.10	0.32	Absent
	Lettuce 2	≥3.04	2.17	Absent	≥3.04	3.00	Absent
	Arugula 1	≥3.04	≥3.04	Absent	≥3.04	2.30	Absent
	Arugula	2.04	2.17	Absent	≥3.04	0.51	Absent

MPN = Most Probable Number. NMP = Número Mais Provável.

 Table 2. Total coliforms (35 °C), thermotolerant coliforms (45 °C) and detection of *Salmonella* spp. in vegetable and animal origin (chicken waste) fertilizers employed in an organic vegetable farm in the metropolitan region of Cuiabá, Mato Grosso, Brazil.

 Tabela 2. Coliformes totais (35 °C), coliformes termotolerantes (45 °C) e detecção de *Salmonella* spp. em fertilizantes de origem vegetal e animal (resíduos de frango) empregados em horta orgânica na região metropolitana de Cuiabá, Mato Grosso, Brasil.

Fertilizer products / Days	Coliforms Log ₁₀ MPN g ⁻¹		<i>Salmonella</i> spp. in 25 g ⁻¹
	35 °C	45 °C	
Vegetable fertilizer (Day 1)	≥3.04	2.04	Absent
Animal fertilizer (Day 1)	≥3.04	2.04	Absent
Vegetable fertilizer (Day 2)	≥3.04	≥3.04	Absent
Animal fertilizer (Day 2)	≥3.04	2.17	Absent
Vegetable fertilizer (Day 3)	≥3.04	2.17	Absent
Animal fertilizer (Day 3)	≥3.04	1.63	Absent
Vegetable fertilizer (Day 4)	2.04	1.87	Absent
Animal fertilizer (Day 4)	≥3.04	2.66	Absent
Vegetable fertilizer (Day 5)	2.04	1.17	Absent
Animal fertilizer (Day 5)	2.66	1.44	Absent

MPN = Most Probable Number. NMP = Número Mais Provável.

4. DISCUSSION

Organic vegetable production systems fertilized with improperly composted animal manure can transmit *E. coli*, a thermotolerant coliform, to the soil and to leafy vegetables (LUNA-GUEVARA et al., 2019). In the present study, 60% of the samples were positive for thermotolerant coliforms. This corroborates other studies performed in Brazil, for example, in one study, 40% of control lettuce samples and 20% of lettuce samples fertilized with chicken and bovine manure animals tested positive for thermotolerant coliforms (ABREU et al., 2010). In another assessment, Arbos et al. (2010) reported 20% (1/5) of contaminated organic lettuce in the city of Curitiba, in the state of Paraná, while Niguma et al. (2017) reported 12.5% (5/40) of organic samples

positive for thermotolerant coliforms in the city of Londrina, also in the state of Paraná. This group of coliforms was also reported in 19.3% (12/62) of lettuce samples and 35.7% (5/14) of arugula samples produced in the region of Campinas, in the state of São Paulo, in conventional farms (SIMÕES et al., 2001), as well as in 32.5% of lettuce and coriander samples produced in conventional farms marketed in Santo Antônio de Jesus, in the state of Bahia (SILVA et al., 2016). Thus, it is clear that contaminated fertilizers can contribute to fecal vegetable contamination, confirmed by our findings and in accordance with previous studies.

Furthermore, irrigation water may have also influenced arugula and lettuce sample contamination from days 1 and 3 (Table 1), while the applied vegetable fertilizer significantly

affected a higher number of samples, i.e., two arugula samples from days 1, 2 and 3 and two lettuce samples from day 1 and one from days 2 to 3 the most noteworthy (Table 1). Animal fertilizer, however, contributed to the contamination of the two arugula samples collected on days 1, 2 and 4, and two lettuce samples obtained on day 1 and one sample obtained on days 2 and 4 (Table 1). Both fertilizers contained thermotolerant coliform contamination on days 2 and 4 (Tables 1 and 2). These data refer to the samples collected on the farm. However, the limited data reported herein does not allow for a conclusive relationship between positive thermotolerant coliform vegetable samples and fertilizer and irrigation water contamination, in contrast to that reported by Loncarevic et al. (2005) in Norway. However, the number of contaminated irrigation water samples was lower than that reported by Abreu et al. (2010), of 100%, perhaps due to the low presence of humans and domestic animals near the irrigation water collection point, similarly to that reported by Araújo et al. (2015) for irrigation water collection points in a stream denominated *Córrego Sujo*, in the state of Rio de Janeiro, Brazil. The inadequate management of manure employed as fertilizer can also influence thermotolerant coliform soil and water contamination, subsequently affecting vegetable production (CADONA et al., 2016).

Thermotolerant coliform counts in the investigated water samples were mostly below the maximum acceptable limits, while most fertilizer samples (animal and vegetable) presented counts above the maximum established limits (ICMSF, 2015). Thus, good agricultural practices in the handling and use of water and fertilizer inputs seem to not have been followed on the investigated property, as following the Good Agricultural Practices (GAP) program results in low or absent counts of this group of organisms (NIGUMA et al., 2017). At the assessed property, vegetables are harvested and sold directly to the final consumer. Thus, if good practices are not applied in the local vegetable production chain, comprising production, harvest, hygiene, and distribution steps, the produced vegetables may represent consumer health risks (SOTO et al., 2018).

Low thermotolerant coliform counts were observed in the supermarket lettuce and arugula samples (Table 1), lower than those detected in the previous farm planting, harvesting, and distribution stages. Leafy vegetable storage and transport under well-controlled temperature conditions (4 °C) may control total and thermotolerant coliform counts (ALLEN et al., 2013; FAOUR-KLINGBEIL et al., 2016), which may account for this finding. Furthermore, the arugula supermarket samples contained roots, although no visible dirt was verified, demonstrating thorough cleaning of this product.

Organic product production has increased in recent years, alongside challenges associated to better production and good quality (BRASIL, 2017). This can be achieved by controlling factors that can contribute to pathogen transmission to fresh vegetables, such as the presence of insects, environmental soil and water quality, and, finally, adequate equipment and product handling and marketing (JOHANNESSEN et al., 2002; JOHANNESSEN et al., 2004; BARKER-REID et al., 2009; JUNG et al., 2014; POMA et al., 2016).

5. CONCLUSIONS

The microbiological quality of organic lettuce and arugula marketed in the metropolitan region of Cuiabá, Brazil, is not entirely satisfactory, as high thermotolerant coliform counts were detected. Therefore, it seems that good practices for the production of agricultural products are not being followed, and vegetable contamination by potential pathogens may consequently represent a risk to consumer health, even though *Salmonella* spp. was absent from all samples, including animal fertilizer products.

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Author Contributions:

E.C.N.B – conceptualization, methodology, research and data collection, writing (original draft); A.J.Q - conceptualization, methodology, investigation and data collection, writing (original draft); A.C.N - methodology, research supervision, writing (proofreading and editing); V.S.C - methodology, statistical treatment, writing (critical review of the work), translation; E.E.S.F – conceptualization, acquisition of funding, coordination, research planning, writing (critical review of the work).

Institutional Review Board Statement:

Not applicable.

Informed Consent Statement:

Not applicable.

Data Availability Statement:

The research data will be made available via e-mail to the corresponding author or responsible for the research project.

Conflicts of Interest:

We inform that there was no scientific, economic, social (among others) conflict of interest during the development of the research and preparation of the article.