Avaliação microbiológica e eficiência de sanitizantes convencionais em hortaliças (*Lactuca sativa* e *Nasturtium officinale*) de cultivo convencional e hidropônico

Microbiological evaluation and efficiency of conventional sanitizers in vegetables (*Lactuca sativa* and *Nasturtium officinale*) of conventional and hydroponic cultivation

Evaluación microbiológica y eficiencia de desinfectantes convencionales en hortalizas (*Lactuca sativa* y *Nasturtium officinale)* de cultivo convencional e hidropónico

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Resumo

Este artigo objetiva avaliar a qualidade higiênico-sanitária de 60 amostras de hortaliças (Lactuca sativa e Nasturtium officinale) de cultivo convencional e hidropônico comercializadas em São Luís (MA) e avaliar a eficiência de sanitizantes convencionais. As análises microbiológicas foram realizadas com base na metodologia descrita no Compendium of Methods for the Microbiological Examination of Foods – APHA. Das 60 amostras analisadas, 95,00% (n=57) estiveram em desacordo com a legislação vigente para coliformes a 45°C, foi identificada a presença de *Escherichia coli* nas duas hortaliças. Todos os sanitizantes apresentaram potencial inibitório. Portanto, os resultados satisfatórios obtidos neste estudo asseguram a utilização de sanitizantes, enfatizando seu custo viável, eficiente e rápida aplicação.

Palavras-chave: Sanitizante; Alface; Agrião.

Abstract

This article aims to evaluate the hygienic and sanitary quality of 60 samples of vegetables (*Lactuca sativa* and *Nasturtium officinale*) of conventional and hydroponic cultivation commercialized in São Luís (MA) and to evaluate the efficiency of conventional sanitizers. Microbiological analyses were performed based on the methodology described in the Compendium of Methods for the Microbiological Examination of Foods - APHA. Of the 60 samples analyzed, 95.00% (n=57) were in disagreement with the current legislation for coliforms at 45°C, and the presence of *Escherichia coli* was identified in both vegetables. All sanitizers presented inhibitory potential. Therefore, the satisfactory results obtained in this study ensure the use of sanitizers, emphasizing their viable cost, efficient and fast application.

Keywords: Sanitizer; Lettuce; Watercress.

Resumen

Este artículo tiene como objetivo evaluar la calidad higiénica y sanitaria de 60 muestras de verduras (Lactuca sativa y Nasturtium officinale) de cultivo convencional e hidropónico comercializados en San Luís (MA) y evaluar la eficiencia de los desinfectantes convencionales. Los análisis microbiológicos se realizaron sobre la base de la metodología descrita en el Compendio de Métodos para el Examen Microbiológico de Foods - APHA. De las 60 muestras analizadas, el 95,00%(no57) estaban en desacuerdo con la legislación vigente para coliformes a 45 ^{o}C, y se identificó la presencia de *Escherichia coli* en ambas hortalizas. Todos los desinfectantes presentaban potencial inhibitorio. Por lo tanto, los resultados satisfactorios obtenidos en este estudio aseguran el uso de desinfectantes, haciendo hincapié en su costo viable, eficiente y rápida aplicación.

Palabras clave: Desinfectante; Lechuga; Berros.

1. Introduction

The U.S. Centers for Disease Control and Prevention (CDC) has indicated that fruits and vegetables pose the same risk of contamination of foods such as meat and chickens, foods

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that are largely associated with outbreaks of food poisoning. This risk is due to the conditions from cultivation to the distribution phase, and may allow the growth of pathogenic microorganisms, capable of promoting food diseases, and bacteria are the most responsible in terms of serious diseases and infections worldwide. These diseases transmitted by contaminated foods date back to raw material or even post processing, constituting the presence of living or non-living elements, adventitiousness of their nature and may cause such damage to health when consumed (Neres et al., 2011).

Negligence in the necessary precautions throughout the process results in contamination by *Escherichia coli* and other thermotolerant coliforms, in addition to *Shigela*, *Salmonella*, *Bacillus cereus* and *Clostridium botulinum* (Thiney, 2001; SCHERER, 2016). These risks during this process of production of food of plant origin include sources by microbial contamination in hardwood vegetables such as the use of contaminated irrigation water, handling and use of poorly processed organic fertilizers (Lotto, 2008). The ingestion of these contaminated foods poses a risk to the health of the consumer, and can cause several infections, being confined to the gastrointestinal tract or else, starting in the intestine and spreading to other parts of the body. Food can act as a vehicle for the pathogen or provide conditions for multiplication of it in large quantities, capable of causing infectious diseases (Scherer, 2016).

There are several systems of cultivation of vegetables, but all have a high potential for growth of pathogenic microorganisms, establishing the role of these systems as a means of transmission of various infectious diseases. These cultivation systems remain due to the Brazilian market, including vegetables undergoing changes over the years to follow the increasing pace of globalization, since the population prioritizes healthy foods of easy and fast preparation (Buckley et al., 2007; Ragaert et al., 2004; Korhonen, 2002).

One of these systems is hydroponic cultivation, and hydroponics consists of a system where plants light adherents in substrates, or allocated in cultivation channels, where circulates an aqueous nutrient solution with nutrients essential to the development and need of the respective plant (Costa, 2001). On the other hand, conventional cultivation consists of seven basic practices, including intensive soil cultivation, monoculture, irrigation application, use of highly soluble synthetic fertilizers, chemical control of pests, diseases and weeds, in addition to genetic manipulation of cultivated plants (Cerveira, 2002). This system aims at high production, not worrying about the conservation of the environment (Cerveira, 2002).

Lactuca sativa (lettuce) is classified as leafy vegetables, belonging to the Family *Asteraceae,* for its contribution of vitamins, minerals, dietary fibers and low caloric value, being widely recommended in diets, because they present potential antioxidant activities (Montanher et al., 2017). Lettuce production reached 1.27 million tons in Brazil in 2011, still the largest consumer in South America, since it is stipulated the cultivation of 35,000 hectares of lettuce in the country (Pinto et al., 2005; Reetz et al., 2014; Lopes et al., 2010). As lettuce comes into contact with contaminants present in the soil, water, natural inputs, these provide the development and survival of pathogenic microorganisms, characterizing it as a possible transmitter of these.

This also fits the *Nasturtium officinale* (watercress), belonging to the Brassicaceae family *Brassicaceae* (Freitas, 2012; Barker, 2009). Its leaves have pharmacological potentials, being also used against hypertension and prevention of cardiovascular diseases (Freitas, 2012). It stores vitamins such as A, C and group B (B1, B2, B6 and B12) as well as elements such as iron, folic acid, sulfur, potassium, calcium, phosphorus, iodine, beta carotene and fibers (Carvalho et al. 2009).

Thus, aiming at the importance of food safety and seeking a solution of the methods employed by the consumer himself in the hygiene of his food, this focus study was developed with the objective of evaluating microbiological quality by determining the Most Probable Number (MPN) of coliforms at 45°C (thermotolerant), identification of species of the Enterobacteriaceae family in 60 samples of conventional and hydroponic vegetables (lettuce and watercress) marketed in the municipality of São Luís-MA and verify the efficiency of hygiene methods conventionally adopted by consumers through microbiological evaluation of post-treatment vegetables.

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2. Methodology

This study is an experimental research and the results obtained were presented in qualitative and quantitative data.

2.1.Collection of samples

Sixty vegetable samples were collected, 15 from *Lactuca sativa* (lettuce) of hydroponic cultivation, 15 of *Nasturtium officinale* (watercress) of hydroponic cultivation, 15 of *Lactuca sativa* (lettuce) of conventional cultivation and 15 of *Nasturtium officinale* (watercress) of conventional cultivation commercialized in the city of São Luís, MA.

After collection, the samples were aseptically packed in isothermal boxes and immediately transported to the Food and Water Microbiology Laboratory of the Federal University of Maranhão (UFMA) for the beginning of the analyses.

All microbiological analyses were performed according to the techniques recommended by the *Compendium of Methods for the Microbiological Examination of Foods* (APHA, 2001).

2.2.Research and quantification of the Most Likely Number (MPN/g) of thermotolerant coliforms (45°C)

Coliforms at 45°C were determined using the multiple tube technique. Where 25g of each sample was diluted in 225 mL of sterile saline solution at 0.85% NaCl, in order to obtain dilution 10^{-1}, successive dilutions were subsequently performed, 10^{-2} to 10^{-3}.

Aliquots of 1000μL of each dilution were inoculated in a triple series of three tubes containing Tryless Lauryl Sulfate broth and inverted Durham tubes, being incubated at 35°C for 24-48 hours. From the tubes where the consumption of the medium and gas trapping in the Durham tube were evidenced, a confirmatory test was performed.

Aliquots of 100 μL of the EC broth were inoculated, and subsequently incubated in a water bath at 45°C for 24-48 hours. The values for MPN/g were determined with the aid of the Hoskis table (APHA, 2001).

2.3.Isolation and Biochemical Identification of Species of the Enterobactericeae Family

The analyses performed for the identification of Enterobactericeae were made from the positive tubes from the EC Broth, with its inoculum sowing using the technique of depletion with platinum handle plate with Methylene Blue Eosin Agar (EMB) and MacConckey Agar (MC) incubating at 35°C for 24 hours.

Conventional biochemical tests of the colonies with characteristics of lactose-fermenting bacteria were subsequently submitted. Previously isolated on TSA Agar surface and incubated at 35°C for 24h

2.4.Evaluation of the efficiency of sanitizers

For the evaluation of the efficiency of sanitizers, the following sanitization processes and their respective proportions were used: (1) acetic acid diluted in 500 mL of water; (2) 10 drops hydrosteril solution in 500 mL of water; (3) 2.5 mL of sodium hypochlorite diluted 500 ml in water; (4) running water for 5 minutes; (5) Sample without washing. The sanitization treatments consisted of immersion for 10 minutes of lettuce and watercress samples to be treated in the respective sanitizer solutions. After the sanitization process, 10g of the sample was diluted in 90 mL of sterile saline solution at 0.85% NaCl, in order to obtain dilution $10^{-1,}$ successive dilutions up to 10-5 were subsequently performed^{-5} for further analysis. To evaluate the efficiency rate of sanitizers, the mesophilic bacteria count was used using the depth inoculation technique. They were sown 1000 µL of dilutions performed in item 2.2 in Petri dishes containing Plate Count Agar (PCA) followed by incubation of the plates in greenhouse B.O.D. (35°C) standardizing the reading of the plates in 24h.

3. Results and Discussion

3.1.Coliforms at 45°C and biochemical identification]

Of the 60 samples of conventional and hydroponic vegetables analyzed, 95% (n = 57) presented coliform values at 45°C higher than those allowed by current legislation RDC no. 12, January 2, 2001, according to Table 1, which establishes maximum standards of 100 NMP/g.

Table 1. Contamination of samples by thermotolerant coliforms (45°C).

Vegetables (Cultivation)	n	% PA	Media NMP g^{-1}	APL
***L. sativa* (Hydroponic)**	**15**	**100% (15)**	**1579,0**	**100% (15)**
***N. officinale* (Hydroponic)**	15	100% (15)	1564,0	93,3 % (14)
***L. sativa* (Conventional)**	15	100 % (15)	1910,0	86,6% (13)
***N. officinale* (Conventional)**	**15**	**100 % (15)**	**2097,0**	**100,00 % (15)**
Total	**60**	**100% (60)**	1787,5	95% (57)

Where: n= number of samples analyzed; BP = presence in samples of thermotolerant coliforms; APL = Above legislative standards (10^2 NMP g^{-1}); Source: Authors.

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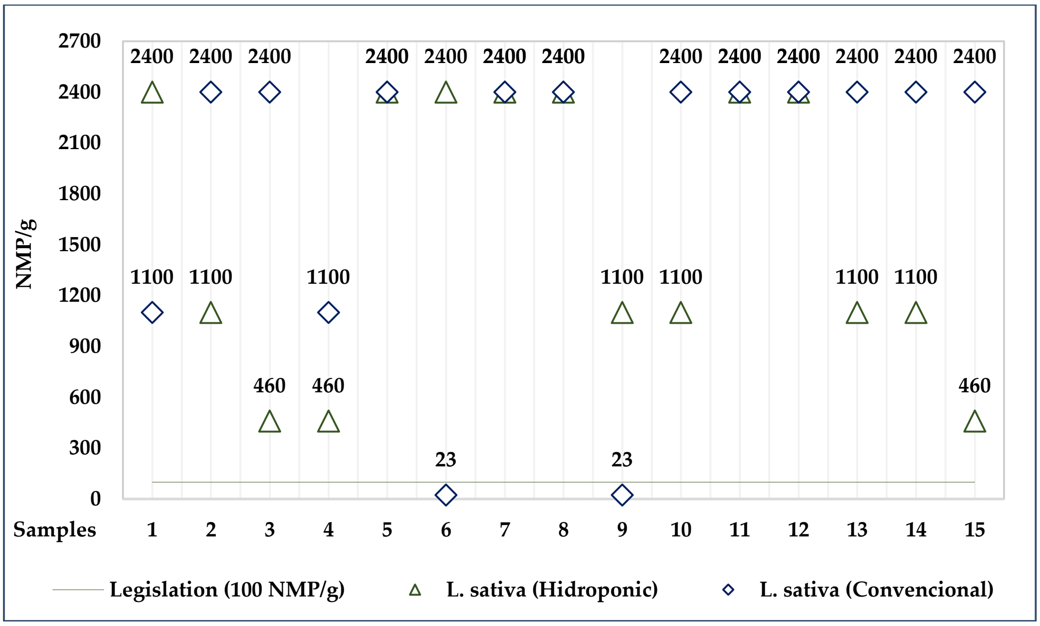
The presence of these microorganisms is justified by Almeida&Resende (2012) where they state that coliforms thermotolerant may be present in these foods by numerous factors such as contamination of irrigation water, inadequate sanitization, food handlers, utensils and contaminated equipment.

Through Figure 1, it was observed that all samples of *L. sativa* presented coliform contamination at 45°C (thermotolerant), and the hydroponic lettuce presented similar results to conventional lettuce. However, only 3.3% (n = 2) of lettuce samples by conventional cultivation were outside legislative standards.

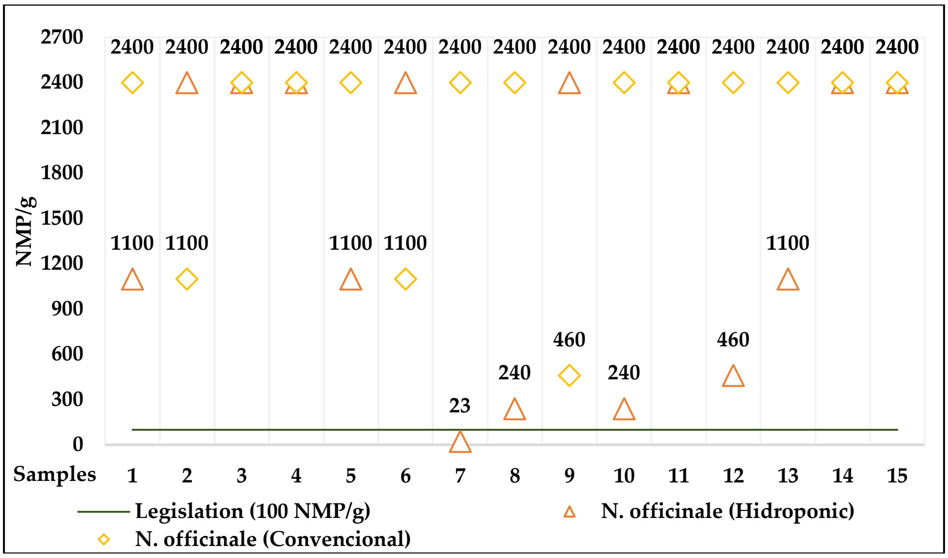
Figure 1. NMP/g of coliforms at 45°C of *L. sativa* samples (lettuce).



Source: Authors.

Through Figure 2, it was verified that all samples of *N. officinale* also presented contamination by coliforms termtolerantes, with watercress of hydroponic cultivation showing results similar to conventional.

Figure 2. NMP/g of coliforms at 45°C of N. *officinale*(watercress) samples.



Source: Authors.

However, only 1.7% (n = 1) samples of watercress from hydroponic cultivation were outside legislative standards.

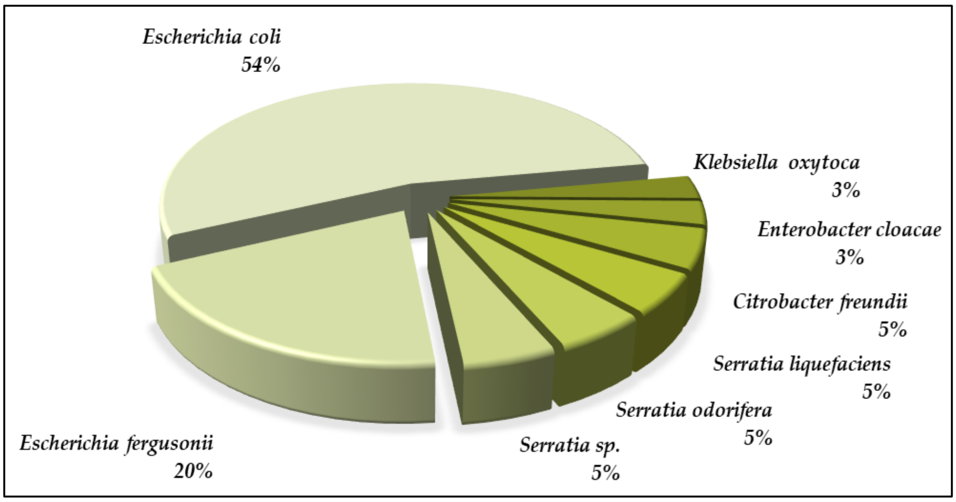
Pereira (2017) evaluating the microbiological quality of conventional lettuce in two fairs in the city of Parintins-AM found in its results the presence of thermotolerant coliforms, revealing the lack of hygienic and sanitary conditions, stating the need for intervention in the process of production, handling, transportation and methods of food conservation by the presence of them

Barbosa et al. (2016) compared whether there was a significant difference in terms of contamination (parasitological and microbiological) in lettuce samples from two forms of cultivation: conventional production and hydroponic production, marketed in supermarkets in the city of Teresina-PI and as well as in this study, no significant differences in contamination were evidenced in relation to the types of lettuce cultivation, still verifying a high incidence of microbiological and parasitological contaminants in lettuces , from three types of cultivation. The authors warn of the need to comply with current legislation and the detection of public health risk conditions in relation to the vegetable production chain from production to sale.

Moreira et al. (2018) analyzed 50 lettuce samples and 50 watercress samples acquired in several establishments in baixada Fluminense-RJ, with the objective of researching the parasitic and microbial contamination of these vegetables, verifying in their results that in 95% of their samples they presented contamination by enterobacteria and 56% by enteroparasites.

Forty-one strains isolated from watercress samples were identified, among the bacteria that were identified are: *Klebsiella oxytoca* (3%), *Enterobacter cloacae* (3%), *Citrobacter* *freundii* (5%), *Serratia liquefaciens, Serratia odorifera e Serratia sp.* (5%), *Escherichia fergusoni* (20%) e *Escherichia coli* (54%), as shown in Figure 3.

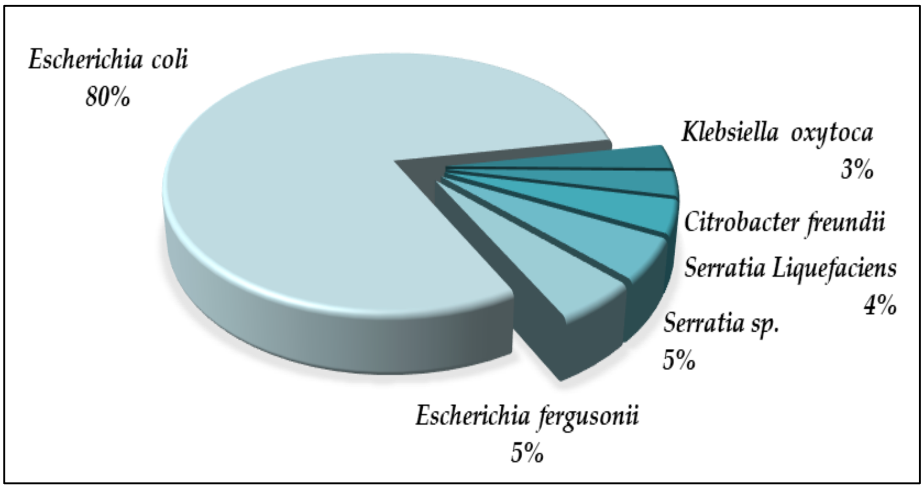
Figure 3. Identification of species of the *Enterobacteriaceae* family from samples of *N. officinale*(watercress).



Source: Authors.

Forty-one strains isolated from lettuce samples were identified among the bacteria that were identified are *Klebsiella oxytoca* (3%), *Citrobacter freundii* (3%), *Serratia liquefaciens (4%), Serratia sp.* (5%), *Escherichia fergusoni* (5%) and *Escherichia coli* (80%), as shown in Figure 4.

Figure 4. Identification of species of the Enterobacteriaceae family from samples of *L. sativa* (lettuce).



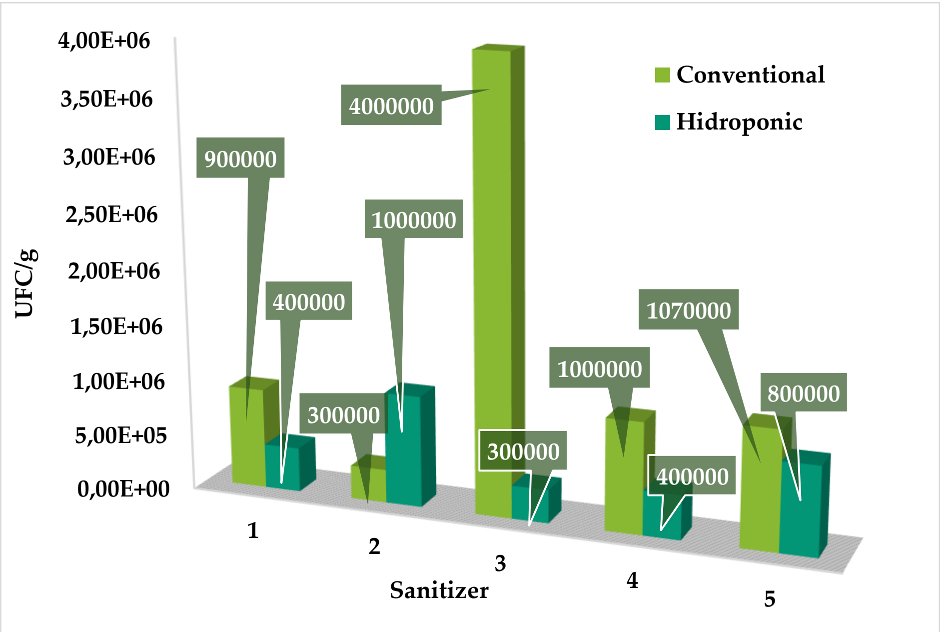
Source: Authors.

Species such as *Escherichia coli*, found in a majority percentage in this study, are responsible for the appearance of diseases of food origin, used as an indicator of contamination in hygiene and sanitization practices, because their presence in food is essentially due to problems of contamination of soil, water and the hands of handlers (CHUA et al.,2008). This bacterium is an inhabitant of the gastrointestinal tract of animals, including man, with beneficial effect, suppressing the multiplication of harmful bacteria and synthesizing some vitamins, however, some strains of *E. coli* can cause diseases in individuals, the so-called enteropathogenic *E. coli* (Silva et al., 2003).

3.2.Efficiency of sanitizers

Through Figure 5, it was observed that the use of sanitization process 2 (hydrostéril solution) presented the lowest contamination index for conventional watercress, while process 3 (sodium hypochlorite) inhibited the higher growth of bacteria for watercress from hydroponic cultivation. However, using sodium hypochlorite in conventional watercress samples, it was verified that it did not suffer a reduction of microorganisms, with an inverse situation occurring, favoring their growth (Figure 5). The same was observed in the sanitization of watercress of hydroponic cultivation with the hydrosteril solution.

Figure 5. Count of mesophilic bacteria from N. *officinale* (watercress) samples after use of sanitization processes.



Source: Authors.

Table 2 showed that for conventional watercress, the processes presented the following efficiencies: 15.9% for the use of acetic acid resulting in a decrease of 1.7 x 10^5 CFU/g of the colonies of mesophilic bacteria, 72% for the sanitization process using hydrosteril solution, inhibiting the growth of 7.7 x 10^5 CFU/g.

Table 2. Percentage of reduction of mesophilic bacteria after use of sanitization processes in samples of *N. officinale* (watercress).

		N. officinale **(% reduction of mesophilics at 35°C)**	
	Sanitization	**Conventional**	**Hydroponic**
1	**Acetic Acid**	15,89 %	50,00 %
2	**Hydrosteril Solution**	**71,96 %**	00,00 %
3	**Sodium hypochlorite**	00,00 %	**62,50%**
4	**Running water**	06,54 %	50,00%

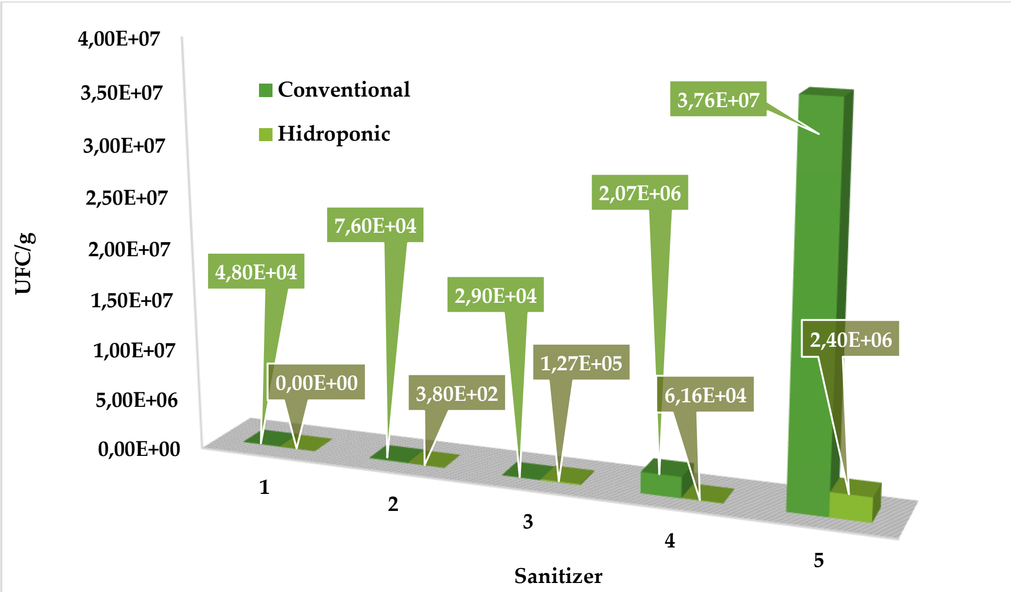
Source: Authors.

The washing process with running water conventionally used by the large population presented the second lowest efficiency of 6.5% followed by the null efficiency of the sodium hypochlorite sanitizer that had an inverse effect in the conventional *N. officinale* samples.

It is also verified through Table 2, that for watercress of hydroponic cultivation, the process that used sodium hypochlorite had the best efficiency preventing microbial growth quantified in 62.5%, followed by acetic acid and running water presenting efficiency of 50% both. Only the hydroseryl solution did not present sanitizing efficiency.

Figure 6 shows that the use of sanitization process 3 (sodium hypochlorite) showed the lowest contamination rate for conventional and hydroponic lettuce. All sanitizers employed showed efficiency.

Figure 6. Count of mesophilic bacteria from *L. sativa* (lettuce) samples after use of sanitization processes.



Source: Authors.

Table 3 shows that for conventional lettuce, the sanitization processes presented the following efficiencies: 99.80% for the use of acetic acid resulting in a decrease of approximately 3.8 x 10^7 CFU/g of the colonies, 99.8% for the sanitization process using hydroserril solution, inhibiting 3.75 x 10^7 CFU/g, 99.92% for sodium hypochlorite and 94.49% for running water. Thus, we obtained similar results in the face of all these sanitizers employed.

Table 3. Percentage of reduction of mesophilic bacteria after use of sanitization processes in L. *sativa*(lettuce) samples.

		L. sativa **(% reduction of aerobic mesophilics 35°C)**	
	Sanitization	**Conventional**	**Hydroponic**
1	**Acetic Acid**	99,87%	**100,00%**
2	**Hydrosteril Solution**	99,80%	99.98%%
3	**Sodium hypochlorite**	**99,92%**	94.71%%
4	**Running water**	94,49%	97.43%

Source: Authors.

In Table 3 for watercress of hydroponic cultivation, the process that used acetic acid had the best efficiency preventing microbial growth quantified in 100%, followed by hydroserril solution, running water and sodium hypochlorite, presenting respectively the efficiencies of 99.98%, 97.43% and 94.71%.

The critical points that address the washing efficiency of food contaminated with microorganisms occur at some point during production. Because the earlier contamination occurs, the more difficult disinfection will become, so the effectiveness will depend on the time interval between contamination and washing (Sapers, 2001).

It is emphasized that the use of disinfectants in food may not completely eliminate pathogenic microorganisms (Maistro, 2001), so in this phase it is important to choose a sanitizer, besides being effective it is important to be nontoxic. Agents such as vinegar, peracetic acid, which have been accepted in world trade (Nascimento&Silva, 2003) are used on a larger scale. Sodium hypochlorite is widely used because it has fast action, easy application and complete dissociation in water (FDA, 2001; Antoniolli et al., 2005). The agent's vinegar, acetic acid, peracetic acid, mentioned above, aroused interest from companies, but there have always been controversies regarding the toxicity of chlorine in food (Nascimento et al., 2003).

Thus, it was possibleto say that the sanitizers used have efficiency that implies the incentive of their use for hygiene of vegetables analyzed in this study.

4. Final Considerations

Through the results obtained, it was observed that there is antimicrobial inhibitory action due to the use of sanitizers used in the vegetables under study. Therefore, the results obtained for microbiological evaluation revealed the lack of hygienic and sanitary conditions of the vegetables of both crops by the high indexes above the legislative standards verified, thus being necessary intervention in the production process of these up to the distribution.

Furthermore, the satisfactory results obtained for the sanitizers used ensure the use of sanitizers, emphasizing their viable, efficient and rapidly applicable cost. This study suggests for future studies the studyof the inhibitory action of sanitizers in bacteria isolated from the analyzed vegetables.

References

Antoniolli, L. R., Benedetti, B. C., Souza Filho, M. D. S. M., & Borges, M. D. F. (2005). Efeito do hipoclorito de sódio sobre a microbiota de abacaxi'Pérola'minimamente processado. *Revista Brasileira de Fruticultura*, *27*(1), 157-160.

Barbosa, V. A. A., das Chagas Cardoso Filho, F., da Silva, A. X. D. L., Oliveira, D. G. S., de Albuquerque, W. F., & Barros, V. C. (2016). Comparação da contaminação de alface (Lactuca sativa) proveniente de dois tipos de cultivo. *Revista Brasileira de Higiene e Sanidade Animal*, *10*(2), 231-242.

Barker, D. (2009). Nasturium officinale (Watercress). *Pacific Northwest Aquatic Invansive. FSH: Washington*.

Buckley, M., Cowan, C., & McCarthy, M. (2007). The convenience food market in Great Britain: Convenience food lifestyle (CFL) segments. *Appetite*, *49*(3), 600-617.

Carvalho, J. L. D. S., Cunico, M. M., Dias, J. D. F. G., Miguel, M. D., & Miguel, O. G. (2009). Termoestabilidade de processos extrativos de Nasturtium officinale R. Br., Brassicaceae por sistema Soxhlet modificado. *Química Nova*, *32*(4), 1031-1035.

Cerveira, R. (2002). *Agroecologia & desenvolvimento: estudo de caso do Grupo Curupira, Jaboti-PR* (Doctoral dissertation, Universidade de São Paulo).

Chua, D., Goh, K., Saftner, R. A., & Bhagwat, A. A. (2008). Fresh-cut lettuce in modified atmosphere packages stored at improper temperatures supports enterohemorrhagic E. coli isolates to survive gastric acid challenge. *Journal of food science*, *73*(3), M148-M153.

Costa, E. (2001). Avaliação da produção de alface em função dos parametros climaticos em casas de vegetação com sistema hidroponico nos periodos de outono e inverno.

FDA, U. (2001). Methods to reduce/eliminate pathogens from fresh and fresh-cut produce. *Analysis and Evaluation of Preventive Control Measures for the Control and*

Reduction/Elimination of Microbial Hazards on Fresh and Fresh-Cut Produce. http://www. cfsan. fda. gov/~ comm/ift3-5. html.

Freitas, E. S. D. S. (2014). *Estudo do efeito antimicrobiano de extractos de agrião-de-água (Nasturtium officinale R. Br.) e de isotiocianatos em isolados de origem humana e vegetal* (Master's thesis).

Korhonen, H. (2002). Technology options for new nutritional concepts. *International Journal of Dairy Technology*, *55*(2), 79-88.

Lopes, C. A., Quezado-Duval, A. M., & Reis, A. (2010). *Doenças da alface*. Brasília^ eDF DF: Embrapa Hortaliças.

Lotto, M. D. C. (2008). Avaliação da contaminação de alface (Lactuca sativa) por coliformes termotolerantes e Escherichia coli em sistemas de cultivo orgânico e convencional.

Maistro, L. C. (2001). Alface minimamente processada: uma revisão. *Revista de Nutrição, 14*(3), 219-224.

Montanher, C. C., de Camargo Coradin, D., & Fontoura-da-Silva, S. E. (2007). Avaliação parasitológica em alfaces (Lactuca sativa) comercializadas em restaurantes self-service por quilo, da cidade de Curitiba, Paraná, Brasil. *Estudos de Biologia*, *29*(66).

Moreira, C. C., COSTA, K., Duarte, A. C., Serra-Freire, N. M., & Norberg, A. N. (2018). Avaliação microbiológica e parasitológica de hortaliças comercializadas na Baixada Fluminense, Rio de Janeiro. *Revista Uniabeu*, *10*(26), 234-243.

Nascimento, M. S., Silva, N., Catanozi, M. P. L. M., & Silva, K. C. (2003). Avaliação comparativa de diferentes desinfetantes na sanitização de uva. *Braz J Food Technol*, *6*(1), 63-8.

Nascimento, M. S., Silva, N., & Catanozi, M. P. L. M. (2003). Emprego de sanitizantes na desinfecção de vegetais. *Revista Higiene Alimentar*, *17*, 42-46.

Neres, A. C., Nascimento, A. H., Lemos, K. R. M., Ribeiro, E. L., Leitão, V. O., Pacheco, J. B. P., ... & Aversi-Ferreira, T. A. (2011). Enteroparasitos em amostras de alface (Lactuva sativa var. crispa), no município de Anápolis, Goiás, Brasil. *Bioscience Journal*, *27*(2).

Pereira, A. T. D. S. (2017). Avaliação microbiológica da Lactuca sativa (Alface) comercializada nas feiras da cidade de Parintins-AM.

Pinto, PAC; Santos, NGN; Germino, GFS; Deon, TD; Silva, AJ. Eficiência agronômica de extratos concentrados de algas marinhas na produção da alface em Neossolo Flúvico. 2010. *Horticultura Brasileira* 28: S3980-S3986.

Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food quality and preference*, *15*(3), 259-270.

Reetz. Anuário brasileiro de hortaliças. Editora Gazeta, Santa Cruz do Sul, p. 42, 2014.

Scherer, K., Granada, C. E., Stülp, S., & Sperotto, R. A. (2016). Bacteriological and physico-chemical analysis of irrigation water, soil and lettuce (Lactuca sativa L.). *Ambiente e Agua-An Interdisciplinary Journal of Applied Science*, *11*(3), 665-675.

Silva, N. D., Silveira, N. F. D. A., Yokoya, F., & Okazaki, M. M. (2003). Ocorrência de Escherichia coli 0157: H7 em vegetais e resistência aos agentes de desinfecção de verduras. *Food Science and Technology*, *23*(2), 167-173.

Thiney, P. A. D. O. (2001). Isolamento e caracterização bioquimica e molecular de Escherichia coli em amostras de alfaces e de suas aguas de irrigação.

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