Revista de Ciências Farmacêuticas Básica e Aplicada
Journal of Basic and Applied Pharmaceutical Sciences

Rev Ciênc Farm Básica Apl., 2016;37(1)
ISSN 1808-4532

Leafy Vegetables Free Of Coliforms And Parasites After Washing In Chlorine Treated Water

Camila Luchesi[1]; Bibiana Paula Dambrós[1*]; Jane Mary Lafayette Neves Gelinski[1]; Monica Frighetto[1]; César Milton Baratto[1]

[1] Universidade do Oeste de Santa Catarina, UNOESC, Departamento de Farmácia, Núcleo Biotecnológico, Videira, SC, Brasil.

ABSTRACT

The production and preparation of vegetables for consumption involve procedures that pose health risks to consumers. In this context the water plays an important role in the quality of minimally processed vegetables. We assessed the hygienic-sanitary quality of leafy vegetables and obtained data of interest to health surveillance agencies and public health. They were tested for the presence of total and faecal coliforms, and also parasitic protozoa and helminths. The vegetable samples were purchased in supermarkets of the Midwest of the State of Santa Catarina, Brazil. We performed bacteriological analyses of wash water samples using chromogenic substrates to obtain the most probable number of the coliform group at 35 °C and Escherichia coli. We used Hoffman's spontaneous sedimentation technique for the parasitological analysis in order to check the occurrence of heavy and light helminths eggs and protozoan cysts. The analyses indicated the presence of coliforms, nematodes and free-living protozoa, except for the last wash. We concluded that the washing process using running water and no active chlorine was not efficient in reducing the load of microorganisms in the vegetables. Therefore, intensive educational programmes should be implemented by health authorities to encourage hygienic-sanitary practices and risk reduction of food-borne diseases.

Keywords: Public Health. Vegetables. Parasitic nematodes. Faeces.

Autor correspondente: Bibiana Paula Dambrós, Universidade do Oeste de Santa Catarina, Departamento de Farmácia, Núcleo Biotecnológico, Videira, SC, Brasil. E-mail: bibiana.paula@unoesc.edu.br

INTRODUCTION

Currently, the consumption of vegetables is one of the nutritional recommendations of the World Health Organization targeted at preventing and controlling chronic non-communicable diseases, such as obesity, cancer, diabetes mellitus, and cardiovascular diseases (Nascimento & Alencar 2014). However, besides contributing to the prevention of diseases, vegetables are important for maintenance and development of human beings, provided that they feature suitable hygienic-sanitary conditions for consumption.

This fact is crucial, because the production and consumption of vegetables involves several steps that encompass potential risk factors for food safety (Neres et al., 2011). The transmission of diseases through food and water results predominantly from the hygienic-sanitary conditions in which individuals live. These conditions exert great influence on the transmission of parasites through the faecal-oral contamination cycle and their control has gained considerable attention throughout the world (Duedu et al., 2014). The main form of enteroparasites contamination in vegetables is associated with the use of water contaminated by human faecal material during the irrigation of vegetable gardens. Other forms of contamination are those arising from soils contaminated with organic fertilizers made from animal faeces (Rangel et al., 2005; Seo & Matthews, 2012) and the contact between vegetables and animals, such as birds, mice, or insects such as flies, in addition to inadequate handling and transport of the vegetables (Robertson & Gjerd, 2001). The places where vegetables are sold also contribute significantly to the spread of pathogens related to food-borne diseases (Nascimento &Alencar, 2014). Therefore the quality of minimally processed vegetables depends on various factors. The water quality suitable for human use can also change in households becoming a risk factor for human healthy (Espinosa-García et al., 2014).

Raw vegetables are widely consumed by the populations; however, due to lack of minimum sanitary conditions, they may contain protozoan cysts and/or

helminths eggs and larvae, which constitute an important route for the transmission of enteroparasites (Robertson & Gjerde, 2001). Studies have shown that there is a prevalence of vegetables contaminated with enteroparasites and the coliform group (Abougrain et al., 2010; Santos et al., 2012; Silva & Gontijo 2012).

Bacteriological contamination of vegetables indicates the necessity of prophylactic measures designed to reduce the risk of food-borne diseases caused by the consumption of these products (Nascimento et al., 2003). The presence of coliforms is often associated with foods grown close to the ground or human handling during harvesting and processing. Escherichia coli is commonly present in the gastrointestinal tract of warm-blooded animals and is used as an indicator of faecal contamination (Oliver et al., 2012). Consumers, in turn, cannot assess visually all safety aspects when they purchase food. Bacteriological contamination levels are invisible and can only be determined by laboratory testing (Henrique et al., 2014).

Bleach or sodium hypochlorite-based aqueous solutions that feature active chlorine content between 2.0 and 2.5% and that can only contain sodium hydroxide as a stabilizer—is considered the most widely used sanitizing agent for food hygiene in Brazilian homes (Santos et al., 2012). However, the variation in the concentration of this agent during the processing of vegetables may harm consumers' health due to the presence of these chlorinated derivatives (Nascimento et al., 2003). Therefore, continuous guidance provided by health surveillance agencies can assist in reducing errors caused by misuse.

Safe food with zero risk does not exist, but the reduction of risks should be a constant goal during production (Forsythe, 2002). In this sense, consumers can only contribute to this reduction when they are fully aware about the risks and the measures that must be taken so that food intake does not represent health risk.

Therefore, with the goal of providing data of interest to health surveillance agencies and public health the present study assessed hygienic-sanitary quality through the analysis of bacterial and parasitological contamination of the following vegetable species: Lactuca sativa L., cichoraceae (lettuce); Nasturtium officinale (watercress); Eruca sativa (rocket); and Cichorium endivia (chicory).

METHODS

Samples

Four varieties of vegetables were purchased randomly from August to November 2014 in different supermarket chains of the Midwest of the State of Santa Catarina, Brazil. The samples consisted of crisphead lettuce heads (Lactuca sativa L., cichoraceae), rocket bunches (Eruca sativa), watercress bunches (Nasturtium officinale), and chicory heads (Cichorium endivia). Twenty-four vegetable samples were analysed in duplicate.

Heads or bunches were established as analytical units, regardless of their weight or size, with a minimum amount of 15 leaves per head or bunch. The criterion adopted was that every sample should exhibit good quality and organoleptic characteristics (healthy, fresh, and non-wilted leaves, without bruising or deterioration).

Preparation of samples

Samples were prepared through the following procedure: each vegetable was wrapped in a plastic bag; 400 mL of residential potable water containing 0.2-5.0 mg/L Cl2 (Santa Catarina Company of Waters and Sanitation 2014) were added; then, they were agitated manually for 30 seconds and filtrated using filter paper (Mellita® No. 102); 300 mL were placed in sedimentation flasks (Flask 1), in order to conduct the parasitological analysis, and 100 mL (first wash water) were transferred to perform the bacteriological analyses in order to identify the microorganisms present in the vegetables before the washing process. Subsequently, we washed each head or bunch of vegetables by hand, leaf by leaf, with running water, mimicking the procedures carried out in the consumers' homes.

In order to detect the presence of other microorganisms in the leaves of each head or bunch of vegetables, we washed them manually again with 800 mL of water in a basin. The leaves of each vegetable were washed again one by one rubbing with a No. 16 flat brush five times on each side of the leaves. The 800 mL of water were distributed in two flasks in equal volumes, and 4 mL of sodium hypochlorite solution (200 ppm of active chlorine) were added to one of the flasks and the two flasks were left to stand for 30 minutes. Subsequently, 100 mL from flask 2 (without active chlorine) were considered second wash water, and 100 mL from flask 3 (with 200 ppm of active chlorine) were considered third wash water. The wash water samples were subjected to bacteriological and parasitological analysis.

Bacteriological and parasitological analysis

Bacteriological analyses assessed bacteria of the coliform group, including assessment of E. coli performed with the wash water samples. We placed 100 mL of the first, second and third wash water samples into sterile flasks, and treated the third sample with active chlorine. Bacteriological analyses were carried using chromogenic substrates (Colilert®, IDEXX Laboratories Inc.) validated by AOAC International (Association of Official Analytical Chemists) method 991.15 (AOAC, 2007), whose culture medium contains the compound 4-ethylumbelliferyl-β-D-glucuronide (MUG). The indicator of contamination was the most probable number (MPN) of total coliforms (35 °C/100 mL) and the presence of E. coli for 24 hours/100 mL.

This technique is accurate, because it identifies the presence of E. coli specifically, without false-positive results for Klebsiella pneumoniae, thus eliminating the subjective interpretation of traditional methods by detecting a single

coliform or E. coli per sample. To that end, a Colilert® single-unit dose was diluted for every 100 mL of the first, second, and third wash water samples, homogenised and transferred into a Quant-Tray®/2000 that provides the maximum MPN count of 2,419/100 mL without dilution. The panels were sealed and incubated for 24 hours at 35°C. The reading was performed counting the yellow cavities relating to the number of total coliforms, and the yellow and fluorescent under UV light (365 nm) relating to the number of E. coli to obtain the MPN according to the reading in the table provided by the manufacturer.

In order to perform the parasitological analysis—which aimed at observing the occurrence of heavy and light helminths eggs and protozoan cysts—we used the remaining 300 mL of water from the three flasks. We used Hoffman's spontaneous sedimentation technique (Neves, 2005), according to which the flasks containing the samples were left to stand for 24 hours. After 24 hours, we transferred aseptically 10 mL of each sample settled in the flasks to be centrifuged at 2500 rpm for three minutes. The supernatant was discarded and an aliquot of sediment (50 µL) was transferred to a glass slide, adding a drop of Lugol's solution and covering with cover slip for subsequent microscopic analysis. Each sediment sample was analysed in duplicate using an optical microscope (Nikon®) at 100 to 400x magnification in all fields of the slides. The average number of organisms was obtained by the area visible under the maximum magnification power (per high power field, HPF). We took photographs (Motorola®) and sent them via email to be assessed by the CDC - DPD x - Centers for Disease Control and Prevention (USA) (CDC, 2014).

Statistical analysis

The average values obtained with the results for the MPN of total coliforms and E. coli were assessed statistically through analysis of variance (ANOVA) (type of vegetable x washing medium) followed by Tukey's method of multiple comparisons. We used a significance level of 0.05 in all tests (Sigmastat 3.5).

RESULTS AND DISCUSSION

Bacteriological analyses

Considering the average values obtained (MPN/100 mL) for total coliforms, no statistically significant difference was observed in the results between the wash waters of the different types of vegetables ($p = 0.119$). Statistically significant difference was observed between washing phases ($p \leq 0.001$) (Table 1); however, there was no significant interaction between type of vegetable and medium ($p = 0.148$). The presence of coliform group bacteria includes microorganisms associated with inadequate hygienic-sanitary conditions, since the presence of these agents is indicative of possible faecal contamination (Cheeptham & Lal, 2010). Therefore, this contamination can be associated with pathogens that cause direct health risk to consumers (Franco, 2005).

Table 1. Average values of total coliforms count (MPN*/100 mL).

Water samples	Vegetables			
	Rocket	Chicory	Watercress	Lettuce
1st wash	1.48x10³ A	1.48x. 10³ A	1.48 x10³ A	1.25x 10³ A
2nd wash	1.10 x. 10³ B	0.21x10³ B	1.40x10³ B	0.54 x10³ B
3rdwash	0 C	0 C	0 C	0 C

Note. * = most probable number; different capital letters in the same column indicate statistically significant differences between the different washing steps. Significance level a=0.05.

With respect to faecal coliforms including E. coli, we observed statistically significant interaction between types of vegetables and washing phases ($p \leq 0.001$). Jay (2000) conducted a study using two-year data relating to the presence of coliforms and faecal coliforms and found that some ready-to-eat vegetables, including lettuce, contained from 103 to almost 104 UFC/100g in each group, 10% of the coliforms were faecal type and the four genera of the coliform group were present. On the other hand, based on several research records, Brandl and Amundson (2008) found that lettuce had already been among the green leafy vegetables (not including coriander or parsley) most often associated with epidemic outbreaks in the United States, and E. coli O157: H7 had been the most common etiological agent.

In the present study, we found that the vegetables with smaller leaves, such as rocket, watercress, and chicory, exhibited a greater amount of total and faecal coliforms in the first two wash waters compared with lettuce. This way, it can be affirmed that vegetables with less complex morphologies, such as chicory and lettuce, have less adhesion of faecal coliforms—including E. coli—than vegetables like rocket and watercress. All vegetable samples exhibited lower total coliforms count after the manual washing procedure, with the following ascending order: chicory < lettuce < rocket < watercress. As observed in waters with 200 ppm of active chlorine (third wash), this contamination was eliminated; however, it was not possible to determine whether the depletion of bacteria occurred after 30-minute action of chlorine or within the 24 hours, i.e., the period determined by the methodology used.

The assessment of hygienic-sanitary quality of vegetables, including salad vegetables served in some restaurants in the State of Santa Catarina, Brazil, indicated the presence of total and faecal coliforms, even though these vegetables had already been sanitised and were ready for consumption (Zanoni & Gelinski, 2013). Despite the lack of federal standards for levels of total coliforms in vegetables, contamination occurs and represents deficient hygienic-sanitary conditions (Takayanagui et al., 2001).

According to Beuchat et al. (2001), washing of lettuce leaves reduces mechanically up to 90% of the microbial load. In the present study, the vegetables were

Table 2. Values of faecal coliform count (MPN*/100 mLμμ) during washing steps - positive for Escherichia coli

Water samples	Vegetables			
	Rocket	Chicory	Watercress	Lettuce
1st wash	2.51x10² Ab	0.73x10² Abc	5.71x10² Aa	0.26x10² Ac
2nd wash	0.15x10² Ba	0.01x10² Aa	0.48x10² Ba	0.08x10² Aa
3rd wash	0 Ba	0 Aa	0 Ba	0 Aa

Note. *NMP = most probable number; different capital letters in the same column indicate statistically significant differences between washing steps; different lowercase letters in the same line indicate statistically significant differences between different leafy veetables in each washe. Significance level α=0.05.

submitted to manual washing in running water, but only the chicory wash water samples exhibited 90% of microbial population reduction (total coliforms). The lettuce wash water samples exhibited reduction of 56% and watercress and rocket wash water samples exhibited reduction of approximately 30%.

The average values obtained for faecal coliforms with presence of E. coli (Table 2) exhibited statistically significant interaction between types of vegetables and wash waters ($p \leq 0.001$). There was also significant decrease in the MPN/100 mL of faecal coliforms with the presence of E. coli between the first, second, and third wash waters. This is best seen in Figure 1 that shows the Log reduction of coliforms after 1st and 2nd water washes and total elimination after washing with chlorinated water (3rd wash). Contamination was not detected in waters with 200 ppm of active chlorine (third wash); however, it was not possible to determine whether the depletion of bacteria occurred after 30-minute action of chlorine or within the 34 hours, i.e., the period determined by the methodology used.

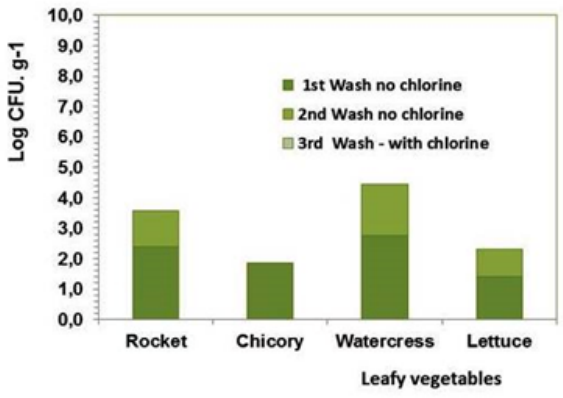

Figure 1. Log reduction of faecal coliforms in leaf vegetables during different washing steps (active chlorine =0,5 ppm).

According to Guimarães et al. (2003) vegetables contaminated with faecal coliforms indicate the fundamental need of performing irrigation carefully. In addition, the contamination also suggests that there has been contact with human and/or animal faeces at some point—i.e., during harvesting, production, transport, storage, and handling.

Parasitological analysis

The results obtained revealed that all wash water samples from flasks 1 and 2 exhibited free-living nematodes and protozoa (Figure 2). However, the average number of nematodes from flask 2 (4/HPF) was 50% lower compared with flask 1 (2/HPF). Based on esophageal morphology of nematodes, they were included in Rhabditida and Desmodorida Order. Thus, free-living organisms. We also identify as free-living the main protozoa specimens that were found: Ciliata Class. These results were confirmed by the team of CDC - DPD x - Centers for Disease Control and Prevention (CD 2014). On the other hand, no parasitic form was found in the wash water sample from flask 3.

Figure 2. Free-living nematodes and protozoa: (A) free-living protozoa; (B) free-living protozoa; (C) free-living nematode larva. Stained with Lugol's solution and displayed at 400x magnification.

Several authors have observed parasitological contamination of medical importance in vegetables consumed by the population (Soares & Cantos, 2005; Belinelo, 2009; Duedu et al., 2014). In contrast, the present study did not find parasitic forms of medical importance in the water used to wash the heads and bunches of the four vegetable varieties assessed. However, the presence of free-living nematodes and protozoa was not in accordance with the standards established by the National Health Surveillance Agency- ANVISA (Brazil, 1978) in Resolution CNNPA No. 12 of 1978 of the Brazil, which determines that, according to microscopic characteristics, vegetables should lack dirt, parasites, and larvae.

Although the free-living parasites found in the parasitological analysis of the vegetables assessed in the present study did not have medical importance, their presence indicate that parasitic forms find suitable conditions in the structure of vegetables to stay in their leaves, even after conventional washing. The interference of the structure of vegetables in the adhesion of parasitic forms had been previously suggested in a study conducted by Soares and Cantos (2006). These authors had considered watercress as the most propitious vegetable for the adhesion of protozoan cysts and helminths eggs due to its multiple and separate leaves with large contact area.

Prophylactic measures are necessary in order to avoid this type of contamination. Thus, the Health Surveillance

Centre of the State of São Paulo-CVS establishes that pre-washing should be performed to sanitize fruits and vegetables using running water and in an appropriate place. On the other hand, for complete sanitation, fruits and vegetables should be immersed in chlorinated solution (2.0 to 2.5% sodium hypochlorite and/or organic chlorine, both at a concentration of 100 to 250 ppm) for 15 to 30 minutes and rinsed in clean water (São Paulo, 1999).

The absence of any kind of parasitic form in the samples of water from flask 3 may have resulted from the action of 200 ppm of active chlorine for 24 hours. This result is corroborated by a study conducted by Baruffaldi et al. (1984). Who early demonstrated that exposure of lettuce leaves to sodium hypochlorite disinfectant solution, at the concentration of 40 ppm free chlorine for 10 minutes, had been efficient from the parasitological point of view.

Sanitation measures should be effectively implemented, because several studies reinforce the existence of parasites in vegetables widely sold and consumed in different regions of the country. The results of a study conducted with vegetables purchased in supermarkets of Cuiabá, State of Mato Grosso, found that 67% of the lettuce was contaminated by helminths and protozoa (Alves et al., 2013).

In fact, fresh vegetables can be contaminated from production, when they are irrigated, harvested, transported, stored, and sold (Quadros et al., 2008). Horticulturists can establish hygienic production practices using alternative methods for the control of parasites in vegetables. These measures include: use of gloves during harvesting; pre-washing of vegetables after harvesting; packaging and transport in closed trucks; continuing and guided educational activities; and parasitological tests performed in the individuals involved in every stage, from producers to consumers (Belinelo et al., 2009).

The present study assessed the occurrence of microbiological contamination in vegetables washed in running water without treatment and with treatment (sodium hypochlorite). The vegetables assessed are leafy and widely consumed in Brazil, since they are associated with a healthy and balanced diet. However, they are also recognized as vehicles of pathogens throughout the world (Berger et al., 2010).

CONCLUSIONS

In Brazil, the indication of using sodium hypochlorite in the homes or restaurants does not ensure that this measure is followed. Therefore, educational practices based on sanitation measures that provide guidance on safe production and consumption can be effective and important in reducing diseases caused by contaminated vegetables.

Manual washing of the vegetables in running water reduced the initial microbial load of total coliform, faecal, and E. coli bacteria present in the leaves. However, this washing procedure is not efficient to properly sanitize vegetables to prevent contamination with bacteria and parasites. Therefore, it is not able to reduce the load of microorganisms to minimum and safe levels for consumption. In this way, we suggest that health surveillance agencies implement measures in order to provide guidance on good sanitation practices (from production to marketing) aimed at sanitation quality of minimally processed vegetables, which are widely consumed and appreciated by the population.

ACKNOWLEDGEMENTS

We are thankful to the Government of the State of Santa Catarina for the support and fellowship, and to CDC - DPD x - Centers for Disease Control and Prevention (USA) for the participation in the identification of the parasites.

REFERENCES

Abougrain AK, Nahaisi MH, Madi NS, Saied MM, Ghenghesh KS. Parasitological contamination in salad vegetables in Tripoli-Libya. Food Control. 2009;21:760-2. doi:10.1016/j.foodcont.2009.11.005.

Alves AS, Cunha Neto A. Rossignoli PA. Parasitos em alface-crespa (Lactuca sativa), de plantio convencional, comercializada em supermercados de Cuiabá, Mato Grosso, Brasil. Rev Patol Trop. 2013;42, 217-229; available from: http://www.revistas.ufg.br/index.php/iptsp/article/viewFile/25529/14678. doi: 10.5216/rpt.v42i2.25529.

Association of Official Analytical Chemists. AOAC. Inclusion of Colilert® as an approved method (991.15) for the detection and enumeration of total coliforms and E coli in water. EUA; 2007. available from: https://www.idexx.com/pdf/en_us/water/096223000L.pdf .

Baruffaldi R, Penna TCV, Machoshvili IA, Abe E. Tratamento químico de hortaliças poluídas. Rev Saúde Pública. 1984;18:225-34. Available from: http://www.scielo.br/pdf/rsp/v18n3/04.pdf. doi:10.1590/S0034-89101984000300004.

Belinelo VJ, Gouvêia MI, Coelho MP, Zamprogno AC, Fianco BA, Oliveira LGA. Enteroparasitas em hortaliças comercializadas na cidade de São Mateus, ES, Brasil. Arq Ciênc Saúde. 2009; 13: 33-6. Available from: http://revistas.unipar.br/saude/article/view/2794/2080

Berger CN, Sodha SV, Shaw RK, Griffin PM, Pink D, Hand P. et al. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environm Microbiol. 2010;12:2385-97. doi:10.1111/j.1462-2920.2010.02297.x.

Beuchat LR, Farber JM, Garrett EH, Harris LJ, Parish ME, Suslow T. et al. Standardization of a method to determine the efficacy of sanitizers in inactivating human pathogenic microorganisms on raw fruit and vegetables. J Food Protect. 2001;64:1079-84.

Brandl MT, Amundson R. Leaf Age as a Risk Factor in Contamination of Lettuce with Escherichia coli O157:H7 and Salmonella enterica. Appl Env Microbiol.

2008;74:2298-2306. Available from: http://aem.asm.org/content/74/8/2298.full.pdf+html. doi: 0.1128/AEM.02459-07.

Brasil. Anvisa. Ministério da Saúde. Resolução da Comissão Nacional de Normas e Padrões para Alimentos n° 12, de 24 de julho de 1978. Brasília: Ministério da Saúde; 1978. Available from: http://www.anvisa.gov.br/anvisalegis/resol/12_78.pdf.

Companhia Catarinense de Águas e Saneamento - CASAN. Relatório Anual De Qualidade Da Água Distribuída. Videira, Santa Catarina, Brazil; 2014. Available from: http://www.casan.com.br/ckfinder/userfiles/files/rel_anu_qual_agua_2014/sro/videira/videira.pdf#3726

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;7:836. doi:10.1186/1756-0500-7-836.

Center for Disease Control and Prevention – CDC/DPDx Team. 2014 [Electronic mail on the Internet]. Message to: Dambrós BP. (Universidade do Oeste de Santa Catarina, Brazil). 2014 Aug 19 [Information about morphology of clinically relevant species from images]. [cited 2014 Aug 19].

Cheeptham N, LAL A. Use of EC-MUG media to Confirm Escherichia coli contamination in water samples protocol. ASM-American Society of Micobiology; 2013. Available from: http://www.microbelibrary.org/component/resource/laboratory-test/3201-use-of-ec-mug-media-to-confirm-escherichia-coli-contamination-in-water-samples-protocol.

Espinosa-García AC, Díaz-Ávalos C, Solano-Ortiz R, Tapia-Palacios MA, Vázquez-Salvador N, Espinosa-García S, Sarmiento-Silva RE, Mazari-Hiriart M. Removal of bacteria, protozoa and viruses through a multiple-barrier household water disinfection system J Water Health. 2014;12:94-104. doi:10.216/wh.2013.080.

Forsythe SJ. The microbiology of safe food London: Blackwell Science; 2002.

Franco BDGM, Landgraf M. Microbiologia de Alimentos. São Paulo: Atheneu; 2005.

Guimarães AM, Alves EGL, Figueiredo HCP, Costa GM, Rodrigues LS. Frequency of intestinal parasites in samples of lettuce (Lactuca sativa) commercialized in Lavras, Minas Gerais State. Rev Soc Bras Med Trop. 2003;5: 621-3. Available from: http://dx.doi.org/10.1590/S0037-86822003000500014

Henrique C, Parisi MCM, Prati P. Contaminação Bacteriológica Pós-colheita. Pesq Tecnol. 2014.Available from: http://www.aptaregional.sp.gov.br/acesse-os-artigos-pesquisa-e-tecnologia/2014/janeiro-junho/1545-contaminacao-microbiologica-pos-colheita/file.html.

Jay JM. Modern Food Microbiology. 6th. ed. Gaithersburg, MD: Aspen Publishers; 2000.

Nascimento ED, Alencar FLS. Antimicrobial and antiparasitic efficiency of

desinfectants in the sanitation of vegetables in natal city–RN. Ciênc Natura. 2014; 36:93- 106. doi: http://dx.doi.org/10.5902/2179460X12755

Nascimento, M.S., Silva, N. & Catanozi, M.P.L.M. 2003 Emprego de sanitizantes na desinfecção de vegetais. Revista Higiene Alimentar 12, 42–47.

Neres AC, Nascimento AH, Lemos KRM, Ribeiro EL, Leitão VL, Pacheco JBP. et al. Enteroparasitos em amostras de alface (Lactuva sativa var. crispa), no município de Anápolis, Goiás, Brasil. Biosci J. 2011;27:336-41.

Neves DP. Parasitologia Humana. São Paulo: Atheneu; 2005.

Oliver JC, Germano JL, Veiga SMOM. Eficiência de sanificantes alternativos sobre frutos contaminados artificialmente com Escherichia coli. Rev Univ Vale do Rio Verde. 2012;10:351-9 doi: http://dx.doi.org/10.5892/719.

Quadros RM, Marques SMT, Favaro DA, Pessoa VB, Arruda AAR, Santini J. Parasitos em alfaces (Lactuca sativa) de mercados e feiras livres de Lages - Santa Catarina. Rev Ciênc Saúde. 2008; 1:78-84.

Rangel JM, Sparling PH, Crowe C, Griffin PM, Swerdlow DL. Epidemiology of Escherichia coli O157:H7 outbreaks, United States, 1982-2002. Emerg. Infect. Dis. 2005;11:603-9. doi:10.3201/eid1104.040739.

Robertson LJ, Gjerde BK. Ocurrence of parasites on fruits and vegetables in Norway. J Food Protect. 2001;64:1793-8.

Santos HS, Muratori MCS, Marques ALA, Alves VC, Cardoso Filho FC, Costa APR. et al. Avaliação da eficácia da água sanitária na sanitização de alfaces (Lactuca sativa). Rev Inst Adolfo Lutz. 2012;71:56-60; available from: http://www.ufpi.br/subsiteFiles/cca/arquivos/files/1433_56-60_Hugo_Sousa_Santos.pdf

São Paulo. Centro de Vigilância Sanitária do Estado de São Paulo. Portaria CVS-6/99 10 de março de 1999. São Paulo. Available from: http://www.cvs.saude.sp.gov.br/zip/E_PT-CVS-06_100399.pdf.

Seo S, Matthews KR. Influence of the plant defense response to escherichia coli O157:H7 cell surface structures on survival of that enteric pathogen on plant surfaces. Appl Env Microbiol. 2012;78:5882-9. doi:10.1128/AEM.01095-12.

Silva MG, Gontijo EEL. Avaliação parasitológica de alfaces (Lactuca sativa) comercializadas em supermercados e feiras livres do município de Gurupi, Tocantins. Rev Cient ITPAC. 2012;5:4. Available from: http://www.itpac.br/arquivos/Revista/54/6.pdf

Soares B, Cantos GA. Qualidade parasitológica e condições higiênico-sanitárias de hortaliças comercializadas na cidade de Florianópolis, SC, Brasil. Rev Bras

Epidemiol.2005;8:77-84.doi: http://dx.doi.org/10.1590/S1415-790X2005000400006.

Soares B, Cantos GA. Detecção de estruturas parasitárias em hortaliças comercializadas na cidade de Florianópolis, Santa Catarina, Brasil. Braz J Pharm Sci. 2006; 42:455-60. Available from: http://www.scielo.br/pdf/rbcf/v42n3/a15v42n3.pdf.

Takayanagui OM, Oliveira CD, Bergamini AMM, Capuano DM, Okino MHT, Febrônio LHP et al. Fiscalização de verduras comercializadas no município de Ribeirão Preto, SP. Rev Soc Bras Med Trop. 2001; 34:37-41.

Zanoni K, Gelinski JMLN. Condições higiênico-sanitárias de salada de vegetais servidas em três restaurantes self-service em município do interior de Santa Catarina, Brasil. Rev Eletr Farm. 2013:10:30-42; Available from: http://ww.revistas.ufg.br/index.php/REF/article/view/22023. doi:10.5216/ref.v10i3.22023.

Received on May 11th 2016.

Accepted on July 12th 2016