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Study of the efficacy of domestic use sanitizers in the reduction of microbial load on *in natura* crisp lettuce (*Lactuca sativa*)

Estudo da eficácia de saneantes comerciais de uso doméstico na redução da carga microbiana em alface (*Lactuca sativa*) crespa *in natura*

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ABSTRACT

Introduction: Raw hardwood vegetables are possible sources of microbiological contamination and need to be sanitized before consumption. The different sanitizers vary in their ability to reduce microorganisms, and chlorine, in its different forms, is widely used in food for this purpose. **Objective:** To evaluate the efficacy of three different types of commercial domestic sanitizers in the reduction of microbial load on in natura crisp lettuce from conventional cultivation. **Method:** Five lots of three different chlorine-based sanitizers used for lettuce sanitization were studied. Concentration of free chlorine and the existence of thermotolerant coliforms and *Salmonella*spp were investigated in the lettuce samples after sanitization. **Results:** Only one of the evaluated sanitizers obtained free chlorine concentration between 100 and 200 ppm. All lettuce samples showed an absence of *Salmonella* sp./25 g and 60% of them had no reduction of coliform at 45°C to acceptable levels for the product to be suitable for consumption. **Conclusions:** The tested products were not effective in reducing the microbial load of lettuce to safe levels, which may be putting the health of the consumer at risk. However, more studies are needed to elucidate issues related to the food hygiene process.

KEYWORDS: Sanitizing Products; Lettuce; Coliforms; *Salmonella*

RESUMO

Introdução: Hortaliças folhosas cruas são possíveis fontes de contaminação microbiológica e precisam ser higienizadas antes do consumo. Os diferentes saneantes variam quanto à sua capacidade de redução de microrganismos, e o cloro, em suas diferentes formas, é amplamente utilizado em alimentos para este fim. **Objetivo:** Avaliar a eficácia de três diferentes tipos de saneantes comerciais de uso doméstico na redução de carga microbiana em alface crespa *in natura* de cultivo convencional. **Método:** Foram estudados cinco lotes de três diferentes saneantes comerciais à base de cloro utilizados para sanitização de alface; avaliada a sua concentração de cloro livre e pesquisados coliformes termotolerantes e *Salmonella* spp. nas amostras de alface após a sanitização. **Resultados:** Apenas um dos saneantes avaliados obteve concentração de cloro livre entre 100 e 200 ppm. Todas as amostras de alface apresentaram ausência de *Salmonella* sp./25 g e 60% delas não tiveram redução de coliformes a 45°C a níveis aceitáveis para que o produto estivesse próprio para o consumo. **Conclusões:** Os produtos testados não foram eficazes para reduzir a carga microbiana da alface a níveis seguros, o que pode estar colocando em risco a saúde do consumidor. Contudo, mais estudos são necessários para elucidar questões relativas ao processo de higienização de alimentos.

PALAVRAS-CHAVE: Saneantes; Alface; Coliformes; *Salmonella*

INTRODUCTION

As street food consumption increases, the concern of the population with its quality also increases, especially when it comes to vegetables. Raw leafy vegetables are possible sources of microbiological contamination mainly caused by *Salmonella* sp. and *Escherichia coli*, but also by viruses, protozoans and helminths, which may lead to Foodborne Diseases (FBD). Approximately 182 individuals died in Brazil from 2000 to 2017 because of FBD outbreaks[1].

The regular consumption of leafy vegetables is important and recommended by government health agencies because these vegetables are rich in dietary fiber, vitamins and minerals and reduce the risk of chronic noncommunicable diseases like diabetes, cardiovascular diseases, hypertension and some types of cancer[2,3].

Poor sanitary conditions in rural and urban production areas increase the contamination of vegetables and turn them into pathogen transmission vehicles. This microbiological contamination can occur in several ways: through the use of water contaminated by fecal matter to irrigate the vegetable garden, untreated manure for fertilization, inadequate transport and lack of hygiene of handlers throughout the production chain. Therefore, vegetable contamination can occur at pre-harvest, harvest and post-harvest and requires great care from growers[4,5]. In order to ensure hygienic and sanitary quality, it is important that these vegetables undergo an adequate cleaning process, which should be efficient to decrease the microbial load to safe levels for consumption according to the legislation[6,7].

According to Collegiate Board Resolution (RDC) n. 216 of September 15, 2004[7], sanitizers are, by definition, "substances or preparations intended for sanitization, disinfection or home disinfestation, in collective and/or public environments, in places of common use and in water treatment". It is therefore important that a product with an antimicrobial agent can reduce pathogenic microorganisms from the environment, food and the hands of the handlers, without causing harm to human health due to, for example, the ingestion of toxic substances. In other words, after direct contact with the food, the product must not pose risks of toxicity or affect the food's sensory characteristics[8].

Sanitizing agents vary in their ability to reduce microorganisms depending on the physical and chemical characteristics of the vegetable in question, the temperature and concentration of the sanitizing solution, the amount of time they stay in contact with the food and the type of target microorganism[9]. In this context, it is fundamental to use antimicrobial agents generally recognized as safe (GRAS)[10] with proven efficacy against microorganisms like *E. coli* when following the label instructions about contact time and dilution[11].

Chlorine, in its different forms, like hypochlorite and sodium dichloroisocyanurate, is widely used for food because it has a broad spectrum of action and reacts and destroys the microbial cell membrane proteins. Sodium hypochlorite is the most commonly used sanitizing agent because it acts fast, is easy to use, completely dissociates in water and is cheap. Sodium dichloroisocyanurate (NaDCC) is an organic chlorinated compound marketed as an effervescent tablet or powder; it is safe and important for food because it does not release heavy metals, trihalomethanes or carcinogenic byproducts[9].

Given this context, the present study aimed to evaluate the efficacy of three different types of commercial sanitizers for reducing microbial load in fresh crisp lettuce of conventional cultivation.

METHODS

Material

We evaluated five batches of three commercial domestic chlorine-based sanitizers registered in the Brazilian Health Surveillance Agency (Anvisa). We used five random samples of fresh crisp lettuce to test the efficacy of the sanitizers as a control parameter of the initial microbial load. We acquired these samples between September and October 2018 in stores in the city of Rio de Janeiro, state of Rio de Janeiro (RJ), Brazil. They were packed in sterile bags, refrigerated and immediately processed at the Food Microbiology Laboratory of the Basic and Experimental Nutrition Department of the Nutrition Institute of the Rio de Janeiro State University.

Sanitizing the samples

The lettuce samples were selected one by one. We discarded the damaged ones and washed them in running drinking water to remove surface impurities. Then, we separated them into three different beakers. We treated each sample (following the manufacturer's instruction - Table 1) with commercial sanitizers, that were named A, B and C. A and B were composed primarily of sodium hypochlorite and C was composed of sodium

Table 1. Information on the labels of commercial sanitizing products purchased at different stores in Rio de Janeiro, RJ.

Product	Active ingredient	Dilution	Immersion time (minutes)	Washing
A	Sodium hypochlorite	15 mL/L	10	Yes
B	Sodium hypochlorite	1 mL/L	15	No
C	Sodium dichloroisocyanurate	100 mg/2L	15	No

A, B and C: Sanitizers of trademarks A, B and C.

dichloroisocyanurate. As a control sample, we also performed microbiological analyses on the samples of unwashed lettuce to determine the baseline microbial load.

Chlorine concentration check

Chlorine concentration was determined in all batches of the sanitizers with free chlorine indicator strips (Ecolab) to evaluate the adequacy of the products after the dilution indicated on label[11].

Microbiological analysis

We performed the microbiological analysis of the unwashed (control) and sanitized lettuce samples. We looked for *Salmonella* sp./25 g and coliforms at 45° C/g as recommended by RDC n. 12 of January 2, 2001[12], following the protocol described by the American Public Health Association (APHA)[13].

Salmonella spp. detection

Salmonella spp. detection was performed by the classical culture method of presence/absence. This qualitative method consisted of three steps: pre-enrichment in 1% peptone water (Bacterial Pepton - Oxoid, LTD., Basingstoke, Hampshire, England), selective enrichment in Rapapport Vassiliadis broth (Oxoid) and differential selective plating on XLD Agar (Oxoid) for detection of typical colonies.

We pre-enriched twenty-five grams of the sample in 225 mL of 1% peptone water (Oxoid) and incubated it at 35 ± 2° C/24 h. We inoculated the aliquots of the pre-enriched incubated culture into Rapapport Vassiliadis broth (Oxoid) and incubated it at 42-43° C/18 to 24 h. A cutoff of this broth was streaked on XLD Agar (Oxoid) and incubated at 35° C ± 2° C/18 at 24 h.

Coliform determination at 45° C

We performed coliform determination at 45° C by the Most Probable Number (MPN) technique. We performed the assay in three sets of three test tubes each (3 x 3) containing culture medium in inverted Durhan tubes. We weighed 10 g of analytical unit from each sample and homogenized it in 90 mL of 0.1% peptone water (Oxoid) and obtained 10^{-1} dilution. We then obtained subsequent serial decimal dilutions until the dilution of 10^{-3}. The Confirmatory Coliform Test at 45° C consisted of inoculating a 1 mL aliquot of the dilutions of 10^{-1}, 10^{-2} and 10^{-3} in *E. coli* broth (EC broth - Oxoid) and incubating it at 44.5 ± 0.2° C/24 for 48 hours. We considered the tubes positive when they presented turbidity and gas production.

Data analysis

We expressed the data obtained from microbiological analyses in MPN/g for coliforms at 45° C and in presence/absence of *Salmonella* sp. in 25 g of the product. We described the results in percentages and compared them with the standards established by the legislation[12].

RESULTS AND DISCUSSION

Table 2 shows the results of the free chlorine concentration tests of the sanitizers. All batches of each sanitizer presented the same results regarding free chlorine concentration. In addition, only sanitizer A presented free chlorine concentration between 100 and 200 ppm, and therefore within the recommended levels[8,14].

The results of the microbiological analyses have shown that 40% of the control lettuce samples had low initial microbial load and, after sanitization, remained with values in accordance with current legal standards[12]. However, coliforms at 45° C did not decrease to acceptable levels in 60% of the samples. Therefore, they did not reach satisfactory sanitary conditions and were not suitable for consumption (Table 3).

Detection analyses of *Salmonella* sp./25 g have shown that none of the lettuce samples had typical colonies of *Salmonella* sp./25 g and, therefore, were in accordance with current legal standards (Table 3).

There is no standard for thermotolerant coliforms for fresh vegetables that have not been sanitized.

Santos et al.[15] found different results from those of the present study. When they evaluated the efficacy of bleach to sanitize 28 lettuce samples, the microbial load of thermotolerant coliforms decreased after three different immersion times (15, 30 and 45 minutes) and the food was suitable for consumption. These authors also reported that they used a solution of bleach with a concentration of 200 ppm of active chlorine as recommended by Anvisa[15]; however, this concentration was not reached by the sanitizers analyzed in the present study.

A batch of sanitizer A and a batch of sanitizer B decreased the coliforms at 45° C to unsafe levels. Rodrigues et al.[16] found similar results when they evaluated two distinct methods of sanitizing tomatoes, pears, grapes, apples, guava and lettuce. One of the methods they tested consisted of using a 1% sodium hypochlorite solution with a concentration of 100-250 ppm for 15 minutes. In addition to other samples, those of lettuce also did not decreased to acceptable levels according to the legislation. Specifically, in the second test with sanitizer B, the number of coliforms found in lettuce after the sanitation procedure increased. This was also found in one of the analyses done by Rodrigues et al.[16].

A study by Ferreira et al.[17] about the efficacy of lettuce sanitization with 2% sodium hypochlorite for 15 minutes and subsequent

Table 2. Free chlorine concentration test of domestic commercial sanitizers purchased at different stores in Rio de Janeiro, RJ.

Product	Free Chlorine Concentration (ppm)	Reference Standard[8,14] (ppm)
A	> 100 and <200	
B	< 50	100 to 200
C	< 50	

A, B, and C: All the batches of sanitizers A, B, and C.

Table 3. Coliform determination at 45° C and *Salmonella* spp. detection in samples (control and sanitized) of fresh crisp lettuce purchased at different stores in Rio de Janeiro, RJ.

Tests*	Microorganisms	Control Sample	Sanitized samples			Maximum limit defined by RDC n. 12/2001[12]
			Sanitizing A	Sanitizing B	Sanitizing C	
1	Coliforms at 45° C (MPN/g)	> 1,100	240	> 1,100	> 1,100	10^2
	Salmonella sp./25 g	Absent	Absent	Absent	Absent	Absent
2	Coliforms at 45° C (MPN/g)	14	< 3	23	3.6	10^2
	Salmonella sp./25 g	Absent	Absent	Absent	Absent	Absent
3	Coliforms at 45° C (MPN/g)	43	< 3	< 3	< 3	10^2
	Salmonella sp./25 g	Absent	Absent	Absent	Absent	Absent
4	Coliforms at 45° C (MPN/g)	> 1,100	> 1,100	> 1,100	> 1,100	10^2
	Salmonella sp./25 g	Absent	Absent	Absent	Absent	Absent
5	Coliforms at 45° C (MPN/g)	> 1,100	> 1,100	460	> 1,100	10^2
	Salmonella sp./25 g	Absent	Absent	Absent	Absent	Absent

A, B and C: Sanitizers of the trademarks A, B and C.
*Tests of the several batches of each sanitizer.

washing in running water has shown that the thermotolerant coliforms load decreased compared to the samples that did not undergo any sanitization process. This result differed from the present study most likely because the products we used did not reach the recommended free chlorine concentration. Moreover, in the study by Ferreira et al.[17], the initial load of thermotolerant coliforms was already low and one of the samples of fresh lettuce was within the maximum limit for ready-to-eat raw vegetables. This corroborates the results found in our study, once that it was in the samples with low initial microbial load that it decreased after the sanitization procedures.

The inefficacy of sanitizers to reduce the microbial load to safe levels for consumption in raw vegetables that underwent sanitization procedures has been discussed and largely attributed to the inability of active ingredients to decrease microbial cells[16].

CONCLUSIONS

The commercially available chlorine-based sanitizing products tested in this study were not effective in sanitizing crisp lettuce samples according to manufacturers' instructions, since they did not decrease the microbial load of the fresh lettuce to safe levels for consumption. This result may be violating consumers' rights and posing risks to the health of the population.

On the other hand, the bacteria tested is possibly becoming resistant to the chlorine-based active ingredient or other factors may be interfering and, therefore, further studies are necessary to understand the issues regarding food sanitization processes.

We emphasize that it is extremely important to use effective products in order to properly sanitize raw foods and guarantee the health and safety of consumers.

REFERENCES

1. Ministério da Saúde (BR). Surtos de doenças transmitidas por alimentos no Brasil. Brasília: Ministério da Saúde; 2018[acesso 23 dez 2018]. Disponível em: http://portalarquivos2.saude.gov.br/images/pdf/2018/janeiro/17/Apresentacao-Surtos-DTA-2018.pdf
2. Maham KL, Escott-Stump S, Raimond J, Krause S. Alimentos, nutrição e dietoterapia. 13a ed. Rio de Janeiro: Elsevier; 2010.
3. 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. Environ Microbiol. 2010;12(9):2385-97. https://doi.org/10.1111/j.1462-2920.2010.02297.
4. Abreu IMO, Junqueira AMR, Peixoto JR, Oliveira SA. Qualidade microbiológica e produtividade de alface sob adubação química e orgânica. Cienc Tecnol Aliment. 2010;30(Supl 1):108-18. https://doi.org/10.1590/S0101-20612010000500018
5. 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. Cienc Tecnol Aliment. 2006;26(2):264-9. http://dx.doi.org/10.1590/S0101-20612006000200006
6. Andrade JR, Ribeiro JA, Guimarães RR, Perez PMP, Freitas SML, Miyahira RF. Análise microbiológica de hortaliças *in natura* servidas em uma unidade de alimentação e nutrição de grande porte na cidade do Rio de Janeiro. Braz J Surg Clin Res. 2016;16(1):30-4.
7. Agência Nacional de Vigilância Sanitária – Anvisa. Resolução RDC N° 216, de 15 de setembro de 2004. Dispõe sobre o regulamento técnico de boas práticas para serviços de alimentação. Diário Oficial União. 16 set 2004.
8. Silva Júnior EA. Manual de controle higiênico sanitário em serviços de alimentação. 7a ed. São Paulo: Varela; 2014.

9. Nunes EE, Vilas Boas EVB, Xisto ALRP, Leme SC, Botelho MC. Avaliação de diferentes sanificantes na qualidade microbiológica de mandioquinha-salsa minimamente processada. Cienc Agrotec. 2010;34(4):990-4. https://doi.org/10.1590/S1413-70542010000400027
10. Nascimento MS, Silva N, Catanozi MPLM, Silva KC. Avaliação comparativa de diferentes desinfetantes na sanitização de uva. Braz J Food Technol. 2003;6(1):63-8.
11. Agência Nacional de Vigilância Sanitária – Anvisa. Resolução RDC N° 110, de 6 de setembro de 2016. Dispõe sobre regulamento técnico para produtos saneantes categorizados como água sanitária e dá outras providências. Diário Oficial União. 8 set 2016.
12. Agência Nacional de Vigilância Sanitária – Anvisa. Resolução RDC N° 12, de 2 de janeiro de 2001. Aprova o regulamento técnico sobre padrões microbiológicos para alimentos. Diário Oficial União. 10 jan 2001.
13. American Public Health Association – APHA. APHA committee on microbiological methods for foods: compendium of methods for the microbiological examination of foods. 4a ed. Washington: American Public Health Association; 2001.
14. Centro de Vigilância Sanitária – CVS (SP). Portaria CVS Nº 5, de 9 de abril de 2013. Aprova o regulamento técnico sobre boas práticas para estabelecimentos comerciais de alimentos e para serviços de alimentação, e o roteiro de inspeção, anexo. Diário Oficial Estado. 19 abr 2013.
15. 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(1):56-60.
16. Rodrigues DG, Silva NBM, Rezende C, Jacobucci HB, Fontana EA. Avaliação de dois métodos de higienização alimentar. Saúde Pesq. 2011;4(3):341-50.
17. Ferreira JA, Nenê ARM, Massulo AO, Salamoni RM, Cardoso Filho N. Estudo preliminar da eficácia de sanitização de amostras de alface comercializadas em Campo Grande-MS. Anuário Prod Acad Docente. 2011;5(14):227-36.

Conflict of Interest

The authors report that there are no political or financial conflicts of interest nor conflicts of interest with peers and institutions.