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Test setup and first report of the removal efficacy of a fruit and vegetable industrial cleaner on fresh-cut vegetables contaminated with *Taenia* sp. eggs

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ABSTRACT

Food-borne disease outbreaks associated with fresh vegetables represent a growing problem for public health worldwide. In Argentina, *Cryptosporidium* spp., *Toxoplasma gondii, Giardia* spp. and *Norovirus* were reported in a risk ranking of foodborne pathogens transmitted by leafy green vegetables. In that sense, disinfection is a critical step in the processing of fresh vegetables to ensure their sanitization. The use of sodium hypochlorite solution as a disinfectant is very extended, but disadvantages like the generation of chlorine gas must be considered harmful for industry workers and consumers. The present study aimed to evaluate the parasite removal efficacy of two formulations of an industrial cleaner on fresh-cut vegetables contaminated with *Taenia* sp. eggs. Lettuce and broccoli were contaminated with *Taenia* sp. inocula and washed with ECOPAMPA® industrial cleaners (L1 and L2) through different assays, using distilled water as a control test. ECOPAMPA® (patent N° AR 119444 B1) was compounded by a non-ionic tensioactive and other ingredients of the Argentine Food Code. Comparison of the egg recovery between treatments showed that cleaners L1 and L2 resulted in a higher removal efficacy than distilled water in the washing of lettuce. Regarding broccoli, the superhydrophobicity of its cuticle could have affected the contamination with parasites. In that way, the recovery of parasite structures was low after washing with both cleaners and water. This study proposed the potential use of this industrial cleaner as an alternative treatment to remove parasite structures on fresh-cut vegetables.

Keywords: industrial cleaner, fruits and vegetables, parasites.

RESUMEN

Las enfermedades transmitidas por alimentos asociadas al consumo de verduras frescas representan un problema de importancia para la salud pública en todo el mundo. En Argentina, se han reportado ciertos parásitos como Cryptosporidium spp., Toxoplasma gondii, Giardia spp., y el virus digestivo Norovirus, en un ranking de riesgo de patógenos transmitidos por vegetales de hojas verdes. En este sentido, la desinfección es un paso crítico en el procesamiento de verduras frescas para garantizar su sanitización. El uso de hipoclorito de sodio como desinfectante está muy extendido, pero deben considerarse desventajas como la generación de cloro gaseoso, lo cual puede ser perjudicial para los trabajadores de la industria y los consumidores. El objetivo del presente estudio fue evaluar la eficacia de remoción de parásitos de dos formulaciones de un limpiador industrial sobre verduras frescas contaminadas con huevos de Taenia sp. Los vegetales frescos lechuga y brócoli fueron contaminados con inóculos de huevos de Taenia

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sp. y lavados con los limpiadores industriales ECOPAMPA® (L1 y L2) mediante diferentes ensayos, utilizando agua destilada como prueba de control. Luego se concentraron los líquidos de lavado para cuantificar los parásitos encontrados. ECOPAMPA® (patente N° AR 119444 B1) se compone de un tensioactivo no iónico y otros ingredientes del Código Alimentario Argentino. La recuperación de estructuras parasitarias en los ensayos con lechuga evidenció que los limpiadores L1 y L2 tuvieron mayor eficacia de remoción de parásitos en comparación con el agua destilada. En cuanto al brócoli, la superhidrofobicidad de su cutícula pudo haber afectado la contaminación con parásitos; de esta manera, la recuperación de las estructuras parasitarias fue baja después de lavar con ambos limpiadores, así como con agua. Este estudio propone al limpiador ECOPAMPA® como una alternativa en el tratamiento de verduras frescas para remover estructuras parasitarias.

Palabras clave: *limpiador industrial, frutas y verduras, parásitos.*

INTRODUCTION

A healthy and protective diet against chronic diseases includes fresh fruits and vegetables (FAO, 2003). Food-borne disease outbreaks associated with fresh vegetables represent a growing problem for public health worldwide, with repercussions that affect the economic and social spheres (FAO, 2003). Pathogenic microorganisms can contaminate these products during the pre-harvest or post-harvest stages. The main contributing factors to this situation are the use of untreated wastewater for irrigation, the application of human and animal excreta as natural fertilizer during cultivation, and/or poor food handling practices (Beuchat, 2002; Idahosa, 2011; Kirk *et al*., 2015). In Argentina, microbiological criteria were established in 2012 for *Escherichia coli*, *Salmonella* spp., *E. coli* O157:H7/NM, and Shiga toxin-producing *E. coli* in fresh vegetables, fresh fruits, and minimally processed vegetables (Argentine Food Code (CAA for its acronym in Spanish), 2021). Although virus and parasite detection are not a mandatory regulation in Argentina, *Cryptosporidium* spp., *Toxoplasma gondii, Giardia* spp. and *Norovirus* were reported in a risk ranking of foodborne pathogens transmitted by leafy green vegetables (Brusa *et al*., 2023). In that sense, disinfection is a critical step in the processing of fresh vegetables to ensure their sanitization (Olmez and Kretzschmar, 2009). Among chemical products, sodium hypochlorite solution is the most widely used water disinfectant applied in the fresh-cut produce industry. However, several disadvantages such as the formation of carcinogenic halogenated disinfection by-products (DBP) and the generation of chlorine gas must be considered harmful for industry workers and consumers (Amy *et al*., 2000; Gil *et al*., 2009). Non-ionic tensioactive compounds are essential raw materials for producing various industrial cleaning agents. Their structure, which combines a hydrophilic non-ionic group with a long hydrophobic hydrocarbon chain, creates a surfactant effect that concentrates the agent at the interface. This composition makes them effective as detergents, wetting agents, and emulsifiers (Salager, 2002). ECOPAMPA®, an industrial cleaner provided by Productos Alimenticios Harmony S.A., fulfills all these functions by removing particles through a drag effect. Due to above mentioned issues, the present study aimed to evaluate the parasite removal efficacy of two formulations of the industrial cleaner ECOPAMPA® on fresh-cut vegetables contaminated with *Taenia* sp. eggs.

MATERIALS AND METHODS

Generation of parasitic inocula

Eggs of *Taenia* sp. were obtained from adult specimens from deworming rounds with arecoline, carried out in Cushamen (Esquel) in April 2023 by the Zooanthroponosis Department of the Health Secretary of Chubut. The eggs were concentrated and preserved in PBS 1X. Parasite inocula was formed by a suspension of PBS 1X with approximately 11,66 eggs/µL.

Test setup

The cleaners were provided by Productos Alimenticios Harmony S.A. They included two different formulations (cleaners L1 and L2) of an industrial solution specifically designed to remove residues or contaminants from the surface of vegetables and fruits (ECOPAMPA®, patent N° AR 119444 B1). This product comprises a non-ionic surfactant and other components approved by the CAA, which work synergistically with the primary ingredient to enhance cleaning efficacy.

The removal efficacy was tested by emulating the washing conditions and the recovery of the parasitic structures without vegetables. Three different treatments (cleaner L1, cleaner L2 and water) were done using the inocula previously mentioned in duplicates. Glass flasks of 350 mL were contaminated with 120 µL of egg suspension, sprayed with 2 mL of the industrial cleaners (L1 and L2) and vigorously shaken with 100 mL of distilled water (DW) during a minute. The washing liquid was passed through a 300 µm sieve, collected in 50 mL tubes, and finally centrifuged at 3500 rpm for 10 min. The supernatant was removed, and the pellet was conserved. The recovered eggs were observed under a light microscope and the recovery efficacy was calculated.

Washing assay

Lettuce and broccoli were selected for the assays. A total of three assays (A) were conducted using different volumes of washing liquids: A1) lettuce in 100 mL of DW, A2) lettuce in 50 mL of DW, and A3) broccoli in 50 mL of DW. The different treatments were done in triplicates, along with an internal control without vegetables, using one of the vegetable samples for each repetition. The vegetables were inoculated on the surface with 120 µL of egg suspension, air-dried for 30 minutes,

and then placed into the glass flasks. They were sprayed on the surface with 2 mL of the industrial cleaners (L1 and L2) for a contact period of two minutes. During the washing step, each sample was immersed in DW at a vegetable/DW ratio of 1:11 for A1 and 1:6 for A2 and A3, and was shaken vigorously for one minute. The washing liquids were processed following the previously established steps for the egg recovery. Finally, twenty samples for each replicate were observed under a light microscope to quantify the concentration of *Taenia* sp. eggs recovered in the washing liquids.

Data analysis

The removal efficacy of the treatments was calculated taking into account the ratio between the final concentration of *Taenia* sp. eggs recovered after the washing steps (eggs/µL) and the initial concentration of *Taenia* sp. eggs in the inocula (eggs/µL) x 100, for each replicate. All analyses were performed using the software InfoStat, version 2008 (InfoStat Group, FCA, Universidad Nacional de Córdoba, Argentina). An analysis of variance (F test) and the one-way ANOVA test were used to compare the mean values of the treatments for each assay. The level of statistical significance was $p<0.05$.

RESULTS AND DISCUSSION

The present study evidenced the preliminary results of the removal efficacy of the ECOPAMPA® industrial cleaners on vegetables contaminated with *Taenia* sp. eggs. The test setup allowed the evaluation of the parasites' recovery to select the most appropriate assay. Assays 1 and 2 with lettuce showed different egg recovery within the treatments. Cleaners L1 and L2 showed a significantly higher mean removal efficacy than water in assay 2, evidenced by the higher concentration of *Taenia* sp. eggs recovered after the washing of the lettuce (table 1). In assay 1, only the treatment with cleaner L1 recovered a higher concentration of eggs, with similar removal efficacy between cleaner L2 and water. The higher volume of water used in this assay (100 mL) for washing could have increased the loss of structures at the moment of discarding the supernatant (fig. 1).

The current study presents preliminary findings on the removal efficacy of the ECOPAMPA® industrial cleaners for vegetables contaminated with *Taenia* sp. eggs. The experimental setup enabled the evaluation of parasite recovery, helping to identify the most effective vegetable washing method. Assays 1 and 2 with lettuce showed varying egg recovery across treatments. In assay 2, cleaners L1 and L2 exhibited a significantly higher mean removal efficacy compared to water, as indicated by the higher concentration of *Taenia* sp. eggs recovered after washing the lettuce (table 1). In assay 1, only the treatment with cleaner L1 resulted in a high concentration of recovered eggs, with removal efficacy being similar between cleaner L2 and water. The large volume of distilled water used in this assay (100 mL) for washing may have led to a greater loss of structures during the discarding of the supernatant (fig. 1).

Assay 3 with broccoli showed a similar low mean removal efficacy among the three treatments (fig. 1). The contamination of this vegetable with eggs of *Taenia* sp. could be affected by the superhydrophobicity of its surface. This vegetable (*Brassica oleracea*) has a cuticle of cutin, a multilayer structure that is composed of a dense structure of wax crystals (Lallana *et al*., 2006; Koch *et al*., 2006), which could have repelled the drops of egg suspension, avoiding its contamination and modifying the final egg recovery, also explained by the high dispersion between the values, especially in the treatment where cleaner L1 was used (fig. 1).

Several researches about the sanitation of fresh vegetables have reported the use of mechanical or chemical techniques to eliminate microorganisms such as bacteria and fungi. A study carried out using tomato juice showed that sonication treatments decreased the microbial loads by raising the localized pressure and temperature, which destroyed the biological species (Faisal Manzoor *et al*., 2023). Rahman *et al*. (2021) evaluated different low-cost disinfectants on fresh fruits and vegetables, reporting that vinegar solution was more efficient than sterile water, blanching, and salt solution treatments in reducing bacterial and fungi loads. Regarding parasites, there were few reports about sanitation techniques for fresh produce. A study carried out in Iran observed a 99.25% reduction in the contamination of vegetables with parasitic structures using a sanitizer of 0.95% $Ca(ClO)_2$ solution for fresh produce sanitation in food processing systems. For domestic use, the addition of lemon juice or vinegar to water used for washing could decrease the number of contaminated vegetables to 83% and 70% respectively, compared to washing vegetables with dishwashing liquid which reduced only 60% (Hajipour *et al*., 2021).

The technical report developed by the National Institute of Industrial Technology (INTI for its acronym in Spanish) (OT 86-20920, 2019) showed a removal effect of 99.9% on *Salmonella* spp., *E. coli* O157, *Lysteria monocytogenes* and *Pseudomona aeruginosa* from the surface of lettuce. In addition, an in vitro assay with this cleaner evidenced growth inhibition of the bacteria *Xanthomonas citri* subsp. citri, which causes the disease citrus canker, suggesting a potentially direct-action bactericidal effect of the cleaner to be applied on citrus plants (Conte *et al*., 2023). The present study achieved the first results of the re-

Washing treatment	A1 Lettuce (%)	A2 Lettuce (%)	A3 Broccoli (%)
Water	26 0.10 A	39 0.09 A	56 0.18 A
L1	80 0.17 B	83 0.18 B	37 0.44 A
L2	30 0.06 A	82 0.08 B	48 0.33 A

Different letters within the same column indicate significant differences (ANOVA followed by LSD Fisher's test, $p<0.05$).

Table 1. Mean removal efficacy (%) of *Taenia* sp. eggs in the three assays A1) lettuce in 100 mL of DW, A2) lettuce in 50 mL of DW, and A3) broccoli in 50 mL of DW under different treatments: Water, Cleaner L1 and Cleaner L2.

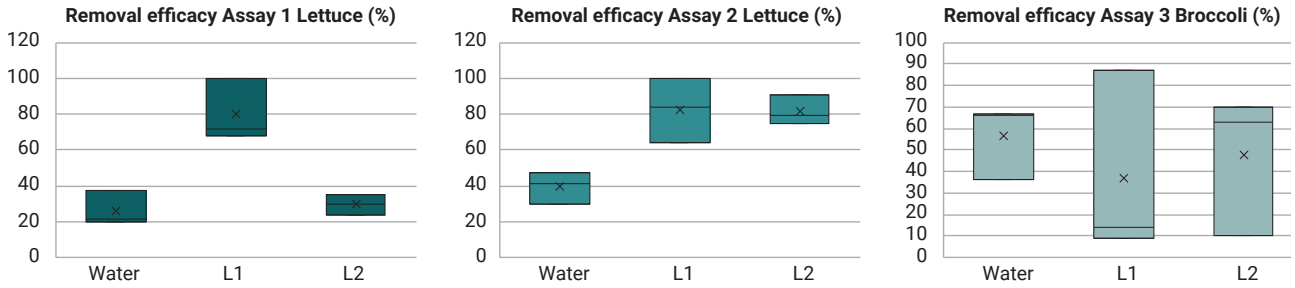

Figure 1. Distribution of the removal efficacy (%) of the three treatments (Water, Cleaner L1 and Cleaner L2) on *Taenia* sp. eggs observed in the three assays (Assay 1: Lettuce in 100 mL of DW; Assay 2: Lettuce in 50 mL of DW; Assay 3: Broccoli in 50 mL of DW). The bars of the boxplot indicate the distribution of *Taenia* sp. eggs recovered after each treatment with the mean (cross), median (line) percentages, and atypical values.

moval efficacy of parasite structures using ECOPAMPA®. Added to its bacterial removal effect, this product could be used as an alternative cleaning treatment for fresh-cut vegetables to drag parasites.

CONCLUSION

The present study developed the first assays for cleaning parasite structures on fresh-cut vegetables, showing the important efficacy of the ECOPAMPA® cleaner in removing *Taenia* sp. eggs from the surface of lettuce. Further assays are necessary to increase the evidence of the removal efficacy of this cleaner on different fruits and vegetables contaminated with other parasites.

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REFERENCES

AMY, G.; BULL, R.; CRAUN, G.F.; PEGRAM, R.A.; SIDDIQUI, M.; WORLD HEALTH ORGANIZATION. 2000. Disinfectants and disinfectant by-products. World Health Organization. 27-33 pp. (Available at: https://iris.who.int/handle/10665/42274 verified on March 10, 2024).

BEUCHAT, L.R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infection, 40, 413-423.

BRUSA, V.; COSTA, M.; OTEIZA, J. M.; GALLI, L.; BARRIL, P.A.; LEOTTA, G.A.; SIGNORINI, M. 2023. Prioritization of vegetable-borne biological hazards in Argentina using a multicriteria decision analysis tool. Food Science and Technology International, 0 (0), 1-17. https://journals.sagepub.com/doi/pdf/10.1177/10820132231180640

CÓDIGO ALIMENTARIO ARGENTINO. 2023. Alimentos Vegetales, Cap. XI, art. 925 bis, ter and quáter. (Available at: https://www.argentina.gob.ar/anmat/codigoalimentario verified on April 15, 2024).

CONTE, M.; DOMANICO, R.; HIRSCH, D.; CONTI, G. 2023. Determinación de la capacidad antimicrobiana del "Producto limpiador de frutas y hortalizas" para el manejo de patógenos cítricos como *Xanthomonas citri supsp. citri*. 10.° Congreso Argentino de Citricultura, Concordia, Entre Ríos, Argentina.

FAISAL MANZOOR, M.; ALI, M.; AADIL, R.M.; ALI, A.; GOKSEN, G.; LI, J.; ZENG, X.A.; PROESTOS, C. 2023. Sustainable emerging sonication processing: Impact on fungicide reduction and the overall quality characteristics of tomato juice. Ultrasonics Sonochemistry, 94, 106313. https://doi.org/10.1016/j.ultsonch.2023.106313

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). 2003. Prevention and control of microbiological hazards in fresh fruits and vegetables. Part 4: Specific commodities. Meeting report. 5-15 pp. World Health Organization, Rome, Italy.

GIL, M.I.; SELMA, M.V.; LÓPEZ-GÁLVEZ, F.; ALLENDE, A. 2009. Fresh-cut product sanitation and wash water disinfection: problems and solutions. International Journal of Food Microbiology, 134, 37-45. https://doi.org/10.1016/j.ijfoodmicro.2009.05.021

HAJIPOUR, N.; SOLTANI, M.; KETZIS, J.; HASSANZADEH, P. 2021. Zoonotic parasitic organisms on vegetables: Impact of production system characteristics on presence, prevalence on vegetables in northwestern Iran and washing methods for removal. Food Microbiology, 95, 103704. https://doi.org/10.1016/j.fm.2020.103704

IDAHOSA, O.T. 2011. Parasitic contamination of fresh vegetables sold in Jos markets. Global Journal of Medical Research 11 (1), 21-25.

INTI. 2019. OT 86-20920 (10/09/19). Informe Técnico, Laboratorio de Microbiología, INTI Agroalimentos.

KIRK, M.D.; PIRES, S. M.; BLACK, R. E.; CAIPO, M.; CRUMP, J. A.; DEVLEESSCHAUWER, B.; DÖPFER, D.; FAZIL, A.; FISCHER-WALKER, C. L. HALD, T.; HALL, A. J.; KEDDY, K. H.; LAKE, R. J.; LANATA, C.F.; TORGERSON, P. R.; HAVELAAR, A. H.; ANGULO, F. J. 2015. World Health Organization Estimates of the Global and Regional Disease Burden of 22 Foodborne Bacterial,

Protozoal, and Viral Diseases, 2010: A Data Synthesis. PLOS Medicine, 12 (12), e1001921. https://doi.org/10.1371/journal.pmed.1001921

KOCH, K.; HARTMANN, K.D.; SCHREIBER, L.; BARTHLOTT, W.; NEINHUIS, C. 2006. Influences of air humidity during the cultivation of plants on wax chemical composition, morphology and leaf surface wettability. Environmental and Experimental Botany, 56, 1-9. https://doi.org/10.1016/j.envexpbot.2004.09.013

LALLANA, M.C.; BILLARD, C.E.; ELIZALDE, J.H.; LALLANA, V.H. 2006. Breve revisión sobre características de la cutícula vegetal y penetración de herbicidas. Ciencias, Docencia y Tecnología, 33 (17), 229-241.

ÖLMEZ, H.; KRETZSCHMAR, U. 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. LWT Food Science and Technology, 42, 686-693. https://doi.org/10.1016/j.lwt.2008.08.001

RAHMAN, M.M.; AZAD, M.O.K.; UDDAIN, J.; ADNAN, M.; ALI, M.C.; AL-MUJAHIDY, S.M.J.; RONI, M.Z.K.; RAHMAN, M.S.; ISLAM, M.J.; RAHMAN, M.H.; CHOI, K.Y.; NAZNIN, M.T. 2021. Microbial Quality Assessment and Efficacy of Low-Cost Disinfectants on Fresh Fruits and Vegetables Collected from Urban Areas of Dhaka, Bangladesh. Foods, 10, 1325. https://doi.org/10.3390/foods10061325

SALAGER, J.L. 2002. Surfactantes. Tipo y usos. Cuaderno FIRP S300 A. Módulo de enseñanza en fenómenos interfaciales. Escuela de Ingeniería Química, Facultad de Ingeniería, Universidad de los Andes (Eds.). 54 p.