

Proceeding Paper

Effects of Cleaning Procedures on the Concentration of Pesticide Residues on Crisp Fresh-Cut Lettuce (cv. Vera) [†]

Noel Alonzo, Hugo do Carmo, Ana Paula Paullier, Inés Santos, Brian de Mattos, Magdalena Irazoqui and Lucía Pareja *

Departamento de Química del Litoral, Cenur Litoral Norte, Universidad de la República, Estación Experimental Mario A. Cassinoni, Ruta 3, km 363, Paysandú, Uruguay; nalonzo@fq.edu.uy (N.A.); hugodocarmo@fq.edu.uy (H.d.C.); appaullier@gmail.com (A.P.P.); mariaines.8226@gmail.com (I.S.); brian_12_acontraluz@hotmail.com (B.d.M.); mirazoqui@fing.edu.uy (M.I.)

* Correspondence: lpareja@fq.edu.uy

Abstract: Decontamination procedures are a need when talking about ready-to-eat foods, especially vegetables. In this work particularly, we are focusing on the effects of four cleaning solutions and ultrasound baths on the amount of pesticide residues left on lettuces. Five pesticides were applied to lettuces grown in controlled conditions. The residues were analyzed with an acetate QuEChERS method and a HPLC-MS/MS system. All tested methods shown diminution of residues without significant differences among each other. Out of 16 pesticides analyzed in commercial samples, only five were found on seven of them, without exceeding MRLs.

Keywords: pesticide residues; lettuce; QuEChERS; decontamination processes

Citation: Alonzo, N.; do Carmo, H.; Paullier, A.P.; Santos, I.; de Mattos, B.; Irazoqui, M. Effects of Cleaning Procedures on the Concentration of Pesticide Residues on Crisp Fresh-Cut Lettuce (cv. Vera). **2021**, *68*, x. <https://doi.org/10.3390/xxxxx>

Published: 15 October 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Consumption of fresh fruits and vegetables are part of a healthy lifestyle as they are a source of vitamins, fibers and many nutrients. Food safety is a major issue when talking about fresh produce as many are eaten raw to maintain more of their benefits, but this may lead to foodborne illnesses if not washed properly [1]. Regarding raw vegetables, ready-to-eat foods have increased in popularity as they present an easier way to get already washed and cut produce.

To prepare vegetables for consumption several parameters need to be taken into consideration, this work will focus on pesticide residues, which, for Uruguay, the maximum residue limits (MRL) are set by the Codex Alimentarius [2]. During production, farmers use pesticides to avoid plagues, to improve production and to prevent diseases that could affect their crop [3]. When applied using good agricultural practices, pesticides should leave residues beneath the established MRLs, furthermore, these residues can be lowered by different cleaning procedures.

Ultrasound baths are an environmentally friendly decontamination method used widely to reduce microorganisms and residues in different industries [4]. Cavitation bubbles formed by the ultrasonic waves can detach residues from surfaces and when they burst, they have the ability to break molecules [5]. This last capacity has proven to be effective to affect pesticides [6], therefore the intention is to study whether or not the different frequencies applied and different lengths of application have different effects on the residues, without damaging the leaves as to preserve the fresh look of the lettuces.

Comparing and contrasting some of the methods used in the industries nowadays in vegetables is the main objective of this paper.

2. Materials and Methods

2.1. Materials and Equipment

Work mixes were prepared from pesticide stock solutions that had been made by dissolving high purity standards in the appropriate solvent and were kept at -40° . HPLC grade acetonitrile (MeCN) and methanol (MeOH) as well as deionized water from a MilliQ system were used. Sodium acetate (NaOAc), ammonium formate, formic acid 88%, sodium chloride (NaCl) and glacial acetic acid (HAc) were also employed during this work.

The analysis was performed in an Agilent 1200 series HPLC system, coupled to a 4000 QTRAP[®] mass spectrometer system from Applied Biosystems SCIEX[™]. The column employed was a ZORBAX Eclipse XDB-RP-C18 (150 mm \times 4.6 mm, 5 μ m) from Agilent. The spectrometer was working in Multi Reaction Monitoring (MRM) mode with nitrogen as a collision gas. Mobile phase solutions were A: deionized water with 2% MeOH, 0.1 % formic acid and 5 mM ammonium formate and B: MeOH with 2% deionized water, 0.1% formic acid and 5 mM ammonium formate. The gradient started with 30% of mobile phase B for a minute, then increased to 100% after 11 min, it was kept constant for two minutes and then reduced back to 30% in two minutes. It remained there until the 21 min of run-time were up. A flow of 0.6 mL/min was kept throughout the analysis. The software employed was the Analyst Software (version 1.8).

2.2. Lettuce Acquisition

This study was performed using two groups of lettuces. On one hand, commercial lettuces were purchased from local points. On the other hand, lettuces were grown in a greenhouse in controlled conditions and obtained at ripe stage. Both groups were analyzed for pesticide residues with their own blanks associated.

2.2.1. Commercial Samples and Processing

All in all, 22 lettuces were bought from different local producers and shops in a two months period. Three of those lettuces were obtained from an organic producer, which were used as blank samples for the validation procedure and for calibration curves.

Each head of lettuce was cut into stripes and milled with liquid nitrogen to a fine dust as soon as it got to the laboratory, and was kept frozen until analysis.

2.2.2. Lettuce Production and Pre-Harvest Treatments

In order to obtain lettuces with similar levels of pesticides, an experiment in controlled conditions was carried out. Crisp lettuce (*Lactuca sativa*, cv Vera) was grown in a greenhouse with daily watering in Estación Experimental Dr. M.A. Cassinoni, near Paysandú, Uruguay. A total of 70 lettuces were cultivated following Good Agricultural Practices during September and October 2020.

From the pesticide pool available for use on lettuce in Uruguay, acetamiprid, boscalid, carbendazim, chlorpyrifos ethyl and methyl, cyromazine, dimethoate, fluvalinate, imidacloprid, iprodione, methomyl, pyraclostrobin, pyrimethanil, pirimicarb, propamocarb and spynosad were chosen for this study after consulting with lettuce producers from the north-west region. Five of these products were chosen to be used during the controlled conditions experiment, the selected pesticides, chlorpyrifos ethyl, pirimicarb, imidacloprid, boscalid and pyraclostrobin. The application was performed following each label recommendations, using a hand spray applicator.

2.2.3. Lettuce Harvest and Post-Harvest Processing

Lettuce heads were harvested at ripe stage at four to six-week-old and immediately processed. Applied (A) and non-applied (NA) heads were shredded manually with a stainless-steel knife to obtain homogenous batches.

NA-samples were processed with liquid nitrogen and kept frozen until analysis. Additionally, an A-sample was taken to generate positive controls, while the rest of the batch was used for different decontamination procedures.

2.2.4. Chemical Treatments

Three disinfectant solutions were tested simulating domestic conditions, 100 ppm of sodium hypochlorite, 80 ppm of peracetic acid and 40,000 ppm acetic acid (4%), together with tap water. For this purpose, six liters of each solution were placed in ten-liter tubs with leaves of the equivalent of half a lettuce from the A-samples. They were kept submerged for ten minutes and stirred mid-way. Once the time was up, the excess liquid was removed with a domestic salad spinner, each sample was milled into a fine dust with liquid nitrogen and kept frozen until analysis.

2.2.5. Ultrasound Treatments

Two stainless steel ultrasound units (Elma, Germany) Transsonic TI-H (25 kHz, 45 kHz) and Elmasonic P (37 kHz, 80 kHz) with operating powers of 100–120 W were used. Ultrasound power dissipation (P) was determined calorimetrically, recording temperature of the bath throughout the different procedures.

For each of the four frequencies, 70 g of chopped lettuce and three liters of cold distilled water were placed in the ultrasound baths to be treated for two time periods, ten or fifteen minutes. Each combination was tested twice.

After the application of treatments, excess of water was eliminated using a domestic salad spinner, then, each sample was milled into a fine dust with liquid nitrogen and kept frozen until analysis.

2.3. Development and Validation of Extraction Method

After testing the three traditional QuEChERS methods (original, acetate and citrate), the acetate version without clean-up was validated according to the SANTE guidelines [7] for the 16 pesticides selected. It begun with 5 g of lettuce in 50 mL Falcon tubes in which 10 mL of 1% HAc in MeCN were added. The mixture was shaken manually for a minute and then 4 g of MgSO₄ and 1 g of AcONa were added, after three minutes of manual shaking, the tube was centrifuged for 5 min at 3500 rpm. The extract was filtered through a 0.45 µm pore syringe filter into 2 mL glass vials for HPLC-MS/MS analysis.

3. Results

3.1. Method Validation

The acetate buffered QuEChERS method without clean-up was chosen for this analysis after testing its performance against the original and citrate buffered methods [8–10], and then against itself with a clean-up procedure. Once testing was done, SANTE guidelines were followed in order to validate the selected methodology [7]. Three levels of concentration with five replicates each were put to the test, achieving limits of quantitation of 10 µg/kg for 12 pesticides, 25 µg/kg for carbendazim, chlorpyrifos methyl and pyraclostrobin, and 50 µg/kg for iprodione. This implies recoveries between 70–120% and standard deviations below 20%. The matrix effect was below 20% for all pesticides except for carbendazim, therefore there's the advantage that this method can be used without a matrix-matched calibration curve. Details of the validation parameters are shown in Table 1.

Table 1. Recoveries, relative standard deviations (RSD), limits of quantitation (LOQ), linear ranges and matrix effects obtained for each pesticide.

Pesticide	10 µg/kg		25 µg/kg		50 µg/kg		LOQ (µg/kg)	Lineal Range (µg/kg)	Matrix Effect (%)
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)			
Acetamiprid	87	1	96	3	98	2	10	5–100	-14
Boscalid	92	10	90	9	108	9	10	5–50	1
Carbendazim	---	---	82	3	83	4	25	5–100	-29
Chlorpyrifos ethyl	112	5	100	5	100	3	10	5–100	1
Chlorpyrifos methyl	---	---	92	8	117	14	25	5–50	-8
Cyromazine	94	4	88	1	85	1	10	5–100	1
Dimethoate	97	3	97	1	98	1	10	5–100	-7
Fluvalinate	93	20	96	17	101	15	10	5–100	1
Imidacloprid	97	11	94	4	94	8	10	5–100	2
Iprodione	---	---	---	---	91	8	50	25–100	-7
Methomyl	104	4	96	2	96	3	10	5–100	0
Pirimicarb	101	2	96	2	96	1	10	5–100	0
Propamocarb	95	2	91	2	90	2	10	5–100	4
Pyraclostrobin	---	---	117	3	109	4	25	10–100	-3
Pyrimethanil	95	7	96	7	99	4	10	5–100	-4
Spynosad	102	2	100	3	101	3	10	5–100	-11

3.2. Commercial Lettuce Samples

Once the method was validated, it was challenged through the analysis of 22 commercial samples. Only seven of those lettuces showed pesticides residues at quantifiable levels for five pesticides (acetamiprid, boscalid, iprodione, propamocarb and pyraclostrobin). These results are presented in Table 2 together with the maximum residue levels (MRL) from the Codex Alimentarius [2] and the European Union [11]. As it stands, all the samples were below the established regulation for Uruguay but iprodione failed EU [11] standards.

Table 2. Maximum residue limits (MRL) from Codex Alimentarius (CA) and European Union (EU), results of positive samples.

Pesticide	MRL		Sample 12	Sample 13	Sample 14	Sample 15	Sample 16	Sample 17	Sample 18
	CA (mg/kg)	EU (mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)	(mg/kg)
Acetamiprid	---	1.5	ND	ND	ND	ND	0.115	0.414	ND
Boscalid	---	50	ND	ND	ND	ND	0.315	0.985	ND
Carbendazim	5	0.1	ND	ND	ND	ND	<0.010	<0.010	ND
Iprodione	10	0.01	ND	ND	ND	ND	0.053	0.131	ND
Propamocarb	100	40	2,5	4,2	0,033	<0.010	ND	ND	10
Pyraclostrobin	40	2	ND	ND	ND	ND	0.125	0.308	ND
Pyrimethanil	---	20	ND	ND	ND	ND	<0.025	<0.010	ND
Spynosad	---	10	ND	<0.010	ND	ND	<0.025	<0.010	ND

3.3. Decontamination Procedures

Regarding the cleaning solutions used, all four showed a reduction in concentration of the applied pesticides when comparing with the positive control. Chlorpyrifos proved to be the most resilient with reductions as little as 5% while pyraclostrobin was the easiest to remove with a maximum decrease of 90%. Still, no significant difference was found between the selected solutions according to the ANAVA test Pillai Bartlett with alfa 0.05.

Ultrasound baths on their part also demonstrated to be an effective method of pesticide residue reduction. Each combination time-frequency managed to lower the selected pesticides where, again, chlorpyrifos was a though contestant with an average diminution of just 20%. On the other hand, pyraclostrobin had an average of 58%, with a maximum of 70%. Once more, despite the differences among pesticides, the combinations did not showed differences when analyzed with the ANAVA test Pillai Bartlett with alfa 0.05.

All in all, though vastly different in mode of action and cost, both the ultrasound and the cleaning solutions managed to achieve what was expected and, amazingly, there was no significant differences among the 12 methodologies when using the ANAVA test Pillai Bartlett with alfa 0.05 as the *p*/value was 0.28. All results are on Figure 1.

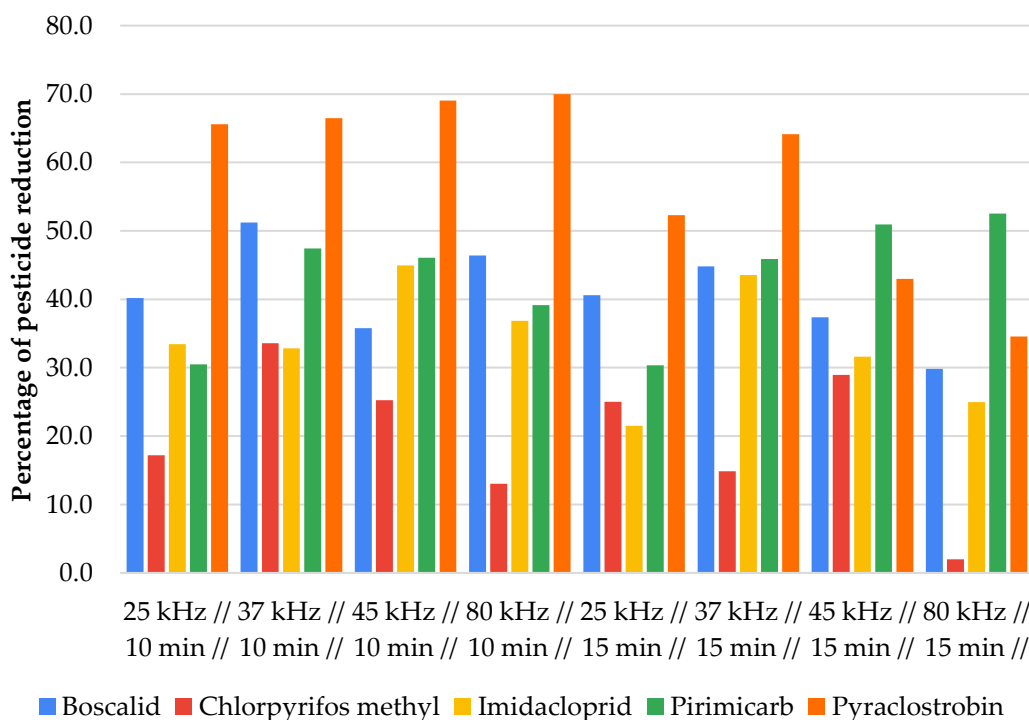


Figure 1. Results for each treatment expressed as percentage of pesticide reduction.

4. Conclusions

All in all, a fit for purpose methodology based on the QuEChERS approach, for the determination of pesticides residues in lettuce was developed and validated for 16 compounds. Said method had its applicability tested in the analysis of 22 commercial samples, where seven showed pesticide residues, below the corresponding MRL.

Both the ultrasound and the cleaning solutions managed to reduce pesticide residues without significant differences among each other. Chlorpyrifos proved to be the most resilient while pyraclostrobin was the easiest to remove.

Author Contributions:

Funding: This research was funded by CSIC through the PAIE program.

Institutional Review Board Statement:**Informed Consent Statement:****Data Availability Statement:**

Acknowledgments: We would like to thank Horacio Silva for his assessment on plague control, as well as producer Marcelo Chrispens for his support, for providing us with the plants used and his knowledge regarding crop management.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Castro-Ibañez, I.; Gil, M.; Allende, A. Ready-to-eat vegetables: Current problems and potential solutions to reduce microbial risk in the production chain. *LWT-Food Sci. Technol.* **2017**, *85*, 284–292, doi:10.1016/j.lwt.2016.11.073.
2. Codex Alimentarius Commission. Codex Pesticides Residues in Food Online Database. Available online: <http://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/pestres/pesticides/en/> (accessed on 15 September 2021).
3. Arienzo, M.; Cataldo, D.; Ferrara, L. Pesticide residues in fresh-cut vegetables from integrated pest management by ultra-performance liquid chromatography coupled to tandem mass spectrometry. *Food Control* **2013**, *31*, 108–115, <https://doi.org/10.1016/j.foodcont.2012.09.021>.
4. Khandpur, P.; Gogate, P.R. Effect of novel ultrasound-based processing on the nutrition quality of different fruit and vegetable juices. *Ultrason. Sonochemistry* **2015**, *27*, 125–136, <https://doi.org/10.1016/j.ultsonch.2015.05.008>.
5. Fu, X.; Belwal, T.; Cravotto, G.; Luo, Z. Sono-physical and sono-chemical effects of ultrasound: Primary applications in extraction and freezing operations and influence on food components. *Ultrason. Sonochemistry* **2019**, *60*, 104726, <https://doi.org/10.1016/j.ultsonch.2019.104726>.
6. Rohnul Azam, S.M.; Ma, H.; Xu, B.; Devi, S.; Stanley, S.L.; Md Siddique, A.B.; Mujumdar, A.S.; Zhu, J. Multi-frequency multi-mode ultrasound treatment for removing pesticides from lettuce (*Lactuca sativa* L.) and effects on product quality. *LWT-Food Sci. Technol.* **2021**, *143*, 111147, <https://doi.org/10.1016/j.lwt.2021.111147>.
7. European Commission DG-SANTE. Document No. SANTE 12682/2019. In *Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed*.
8. Lehotay, S.J.; Matovská, K.; Lightfield, A.R. Use of Buffering and Other Means to Improve Results of Problematic Pesticides in a Fast and Easy Method for Residue Analysis of Fruits and Vegetables. *J. AOAC Int.* **2005**, *88*, 615–629, doi:10.1093/jaoac/88.2.615.
9. Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D.; Schenck, F.J. Fast and easy multiresidue method employing acetonitrile extraction/partitioning and “dispersive solid-phase extraction” for the determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431.
10. Anastassiades, M.; Scherbaum, E.; Taşdelen, B.; Štajnbaher, D. Recent Developments in QuEChERS Methodology for Pesticide Multiresidue Analysis. In *Pesticide Chemistry: Crop Protection, Public Health, Environmental Safety, Chapter 46*, 1st ed.; Ohkawa, H., Miyagawa, H., Lee, P.W., Eds.; 2007; <https://doi.org/10.1002/9783527611249.ch46>.
11. European Commission. Regulation (EC) No.299/2008 of the European Parliament and of the Council of 11 March 2008 amending Regulation (EC) No. 396/2005 on maximum residue levels of pesticides in or on food and feed of Plant and animal origin. *Off. J. Eur. Commun.* **L9**.