sciendo
Acta hort regiotec, 24, 2021(2): 134–140

DOI: 10.2478/ahr-2021-0035

Research Article

The impact of apple preparation on the content of chlorpyrifos pesticide residues in the final products

Aleksandar Dimoski*, Vezirka Jankuloska, Gorica Pavlovska, Anka Trajkovska-Petkoska
"St. Kliment Ohridski" University – Bitola, Republic of North Macedonia

Article Details: Received: 2021-06-26 | Accepted: 2021-09-24 | Available online: 2021-11-30

The apples of Red Delicious are round fruits that have red colour when ripe according to which they are named. They can be eaten fresh, but also can be processed into a variety of processed products including apple juice. In order to grow or obtain better yield of a good quality apples, pesticides are usually used to protect apple trees, but they can adversely affect human health, therefore, some precautions should be taken when these chemicals are used as food contact materials. This study summarizes the presence of chlorpyrifos pesticide residues in apples that are prepared by different methods including mechanical treatments, fresh, washed, peeled as well as heat treatment of apples when prepared into an apple juice. For this purpose, the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used for residue extraction in apple samples after different methods of preparation, and their analyses were performed by liquid chromatography-mass spectrometry (LC-MS/MS). The concentration of the pesticide chlorpyrifos in different samples of apples was in the range of less than 0.0005 mg.kg^{-1} to 0.00348 mg.kg^{-1}. This study provides a conclusion that all samples of apples are safe for consumption while the peeling method and heat treatment are the most efficient in reduction of chlorpyrifos content in the final products.

Keywords: apple, chlorpyrifos, apple juice, processing factor, food safety

1 Introduction

Pesticides are chemicals usually used to protect plants from diseases and pests in the period before and after harvesting the fruits and vegetables, in order to reduce their impact on the quantity and quality of plants (Singh et al., 2014; Lozowicka et al., 2015; Suárez-Jacoboa et al., 2017). They could be used in the cultivation of apples as well, but their concentration should be restricted by national/regional laws in order not to overcome the maximum level; otherwise it will cause adverse effects on human health and environment (Simon et al., 2011). According to Badr et al. (2019), pesticides have consequent long-term effects on the national income, the ecosystem, and public health. High concentrations of pesticide residues (active substances (a.s), metabolites or decomposition products), which exceed the maximum allowed limits (Official Journal of the European Union, 2005; Koch et al., 2017; Official Newspaper of the Republic of Macedonia, 2018), can accumulate in human body and have a negative impact on human health; therefore, it is necessary to monitor their concentration in the fruits (Sabarwal et al., 2018). In many countries, monitoring of pesticide residues in fruits and vegetables is one of the most important procedures to reduce potential health hazards. In this context, according to Mebdou et al. (2017), in good agricultural practice (GAP), pesticide residues in food items should not exceed the maximum residue limits (MRLs) and detected concentrations of pesticide residues should be within the prescribed values. The continuous usage of organophosphorus pesticides increases the possibility of these pesticides to be found in horticultures and thus affecting their safety and quality; this is the reason for the commitment of public health and food safety institutions (Quintero et al., 2008). Organophosphate pesticides are toxic to insects and mammals, including humans; they have the ability to affect the central nervous system by inhibiting some important enzymes such as acetylcholine. Consuming unsafe food that is a source of toxic substances (pesticides and their metabolites), increases exposure to pesticides

***Corresponding Author:** Aleksandar Dimoski, "St. Kliment Ohridski" University-Bitola, Faculty of Technology and Technical Sciences – Veles, Dimitar Vlahov bb, 1400 Veles, Republic of North Macedonia, phone: 00 389 77 558 796, e-mail: aleksandar.dimoski74@gmail.com; http://ttfv.uklo.edu.mk/index.php/mk/

as a potential health risk (Sharma et al., 2010; Drouillet-Pinard et al., 2011). In general, the risk of pesticides is related to their accumulation in the food chain which can lead to human exposure to increased levels of pesticides in food (Badr et al., 2019; Jankuloska et al., 2020), and the main health effects are associated conditions such as cancer, birth defects, neurological and endocrine disorders, and reproductive changes (Khan et al., 2020). Chlorpyrifos is a representative of toxic organophosphate insecticides, the second most detected pesticide in water and food (Mackay et al., 2014); it has been detected as the most common pesticide found in apples (Badr et al., 2019). In general, chlorpyrifos is applied for protection of pome fruits (apples and pears) against San Jose scale, Rosy apple aphid, Pandemispyrusana, Obliquebanded leafroller, Climbing cutworms, and American plum borer. The widespread use of chlorpyrifos in agriculture and its persistence in the environment have raised public safety awareness and concerns; therefore, novel technologies are proposed that will overcome pollution and toxicity problems with this chemical (John & Shaike, 2015). According to the Official Journal of the European Union (2020)b, this pesticide has shown negative effects on eventual genotoxic and neurological effects in child development. Due to health issues caused by this pesticide, the EU countries have forbidden the import of fruits that contain this pesticide as well as the use of it (deadline of adjustment is set in the Official Journal of the European Union (2020)a, Official Journal of the European Union (2020)b; and Official Journal of the European Union (2021)). Among different food categories, fruits and vegetables are recognized as a group that could contain higher levels of pesticide residues compared to other food groups, because they are mainly consumed as raw (Stachniuk et al., 2017). Apples can be eaten fresh, but also can be processed into an apple juice, apple compote, apple cider vinegar, jam or other products (Hancock et al., 2008), but also can be prepared as apple pulp used as a raw material to produce other products (Paz et al., 2017).

There are several varieties of apples cultivated in R.N. Macedonia; but in the region of Resen, the most often cultivated variety is Red Delicious. This variety is very lush, diploid, and usually flowers late. The fruits are round, large, usually ripen in months of September/ October (Icka& Damo, 2014). The apple juice is the second most consumed fruit juice worldwide (Rupasinghe & Thilakarathna, 2016) and its consumption has many positive effects on human health due to its nutritional value and bioactive components, such as antioxidant and antimicrobial activity (Paz et al., 2015). Various scientific data have showed that mechanical processing of the fruit removes some of the present pesticide residues, while heat treatment of the fruits further reduces their amount depending on the nature of the pesticide and the nature of fruits/vegetables (Chavarri et al., 2005; Štěpán et al., 2005; Balinova et al., 2006; Kaushik et al., 2009; Ling et al., 2011; Satpathy et al., 2012). Therefore, it is expected that in apples, by washing, peeling and processing them into apple juice, the amount of pesticide residues will be reduced (Keikotlhaile et al., 2009). Dasika et al. (2012) have presented the level of chlorpyrifos in apples before or after washing step with warm water or salt water, but chlorpyrifos was still present in certain amounts. Washing the fruits and vegetables allowed decreasing the concentration of endosulfan (54.24%), imidacloprid (59.24%), diafentiuron (20.96%) and emamectin benzoate (9.09%), while washing with detergent followed by sun drying reduced the concentration of pesticide residues by up to 95% of the peel (Satpathy et al., 2012; Sheikh et al., 2015; Yang et al., 2017).

In this study, samples of fresh apples of Red Delicious variety as well as in its processed forms – washed, peeled and converted into apple juice – were examined in terms of the presence of pesticide residues. The pesticide was extracted by using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method (Anastassiades et al., 2003; Bruzzoniti et al., 2014). The main advantages of the QuEChERS method over the traditional sampling methods include recoveries for a high number of pesticides, good accuracy and precision, reducing the volume of used organic solvent, preparation of a high number of samples for short time and ease of implementation. Modern instrumental methods, including chromatographic techniques, are commonly used to analyze pesticides. More particularly, liquid chromatography is the most widely used separation technique including analysis of pesticides in fruits (Radišićet al., 2009).

2 Materials and methods

Detection of the pesticide chlorpyrifos in apples of the Red Delicious variety and its processed forms is monitored in this study. The samples of apples were taken from two locations in the region of Resen, i.e. location 1 (Evla) and location 2 (Kriveni), after their harvest (October 2020). Table 1 presents the samples used in this study. Samples 1 and 2 are unwashed apples, while samples 3 and 4 are washed (washed with cold tap water at 12 °C for 30 s). Samples 5 and 6 are samples that were initially washed and then peeled off with a sharp knife (the peel thickness was 1 ±0.3 mm). Peeled apples were homogenized and then analyzed for the purpose of this study. Samples 7 and 8 represent juice prepared of collected apples for both locations (preparation occurred at in-house conditions according to the traditional recipe). Fig. 1 shows a diagram for apple juice preparation.

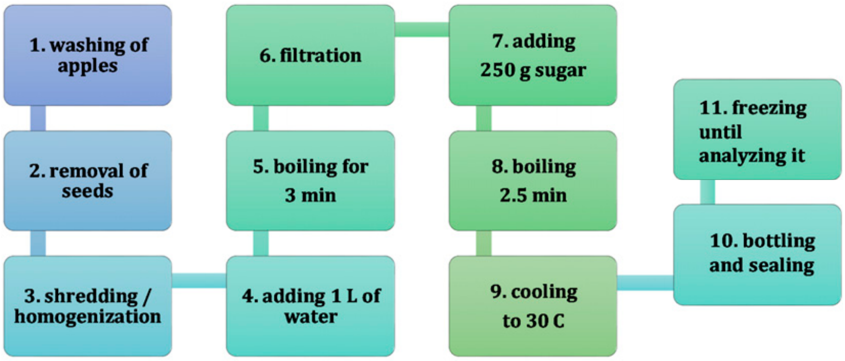

Figure 1 Steps for apple juice preparation

Extraction of chlorpyrifos pesticide residues was performed using the QuEChERS method according to the standard MKS EN 15662:2018 LC-MS/MS. The QuEChERS method involves liquid extraction of pesticide residues from fruits using a solvent acetonitrile (Anastassiades et al., 2003; Bruzzoniti et al., 2014). This method consists of using gas or liquid chromatography (Standardization Institute of the Republic of Macedonia, 2011). The procedure for extracting residues was as follows: 10 ±0.1 g homogenized fruit sample was weighed in a tube of 50 mL, then was added 10 mL acetonitrile and the sample was extracted with ultra turax for 2 min at 4,000 rpm. Then a mixture of 4 g of magnesium sulfate anhydride ($MgSO_4$), 1 g sodium chloride, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate hexahydrate were added to the extract. The sample was vigorously mixed by hand for 1 min and centrifuged at 4,000 rpm for 10 min. A 6 mL extract was transferred to a 15 mL plastic tube which already contained 150 mg PSA (primary secondary amine) and 900 mg $MgSO_4$. The mixture was mixed vigorously by hand or vortex for 30 s and centrifuge at 3,000 rpm for 5 min. When necessary, the extract was filtered into an autosampler vial through a 0.45 µm filter; then 0.1% formic acid was added and applied to the UPLC-MS/MS system. The dilution factor was 1. The samples were analyzed by liquid chromatography (Waters, UPLC-MS/MS), mass spectrometer with triple quadrupole (XEVO TQ-S micro, Waters), analytical column (Acquity UPLC BEH C18 1.7 µm, 2.1 × 100 mm, Waters), mobile phase A – LC/MS water with 0.1% formic acid and 5 mM ammonium formate, mobile phase B – methanol with 0.1% formic acid and 5 mM ammonium formate. Processing factors (PF) were calculated for all transformation steps by a ratio between the pesticide residue concentration (mg.kg^{-1}) in the processed commodity and the pesticide residue concentration (mg.kg^{-1}) in the raw, non-processed commodity (Scholz et al., 2018; El-Sayedet al., 2021).

Table 1 Designation of apple samples used for this study

Sample number	Type of processing	Location
1	unwashed apples	Evla
2	unwashed apples	Kriveni
3	washed apples	Evla
4	washed apples	Kriveni
5	peeled apples	Evla
6	peeled apples	Kriveni
7	apple juice	Evla
8	apple juice	Kriveni

3 Results and discussion

The analysis of the pesticide chlorpyrifos was performed in 8 samples; fresh (unwashed), washed and peeled apples as well as apple juice were analyzed. The results are presented in Table 2.

From the results it can be seen that the highest concentration of the pesticide chlorpyrifos was observed in the fresh (unwashed) apples (samples 1 and 2). Namely, in sample 2, chlorpyrifos is detected at a concentration of 0.00348 mg.kg^{-1}, while in sample 1 it is 0.002895 mg.kg^{-1}. The concentration of chlorpyrifos in samples 3 and 4 was decreased compared to samples 1 and 2; sample 3 showed 0.001048 mg.kg^{-1}, that is 63.8% less than in sample 1, while the concentration of chlorpyrifos in sample 4 was 0.001419 mg.kg^{-1} or 59.2% less than in sample 2. Peeled samples of apples showed further decrease in pesticide concentration; namely, in samples 5 and 6 there were detected <0.0005 mg.kg^{-1} of chlorpyrifos. [Since the range of detection of the instrument for the analysis is 0.0005 mg.kg^{-1}, it could be concluded that the pesticide residues are less than this limit or even no presence of it in the tested samples]. The amount of present chlorpyrifos in sample 5 was decreased by 82.7% compared to sample 1, while in sample 6, the amount of chlorpyrifos was decreased up to <0.0005 mg.kg^{-1}, i.e. was decreased by 85.6% compared to sample 1. The concentration

Table 2 Presence of chlorpyrifos in various apple samples

Sample	1	2	3	4	5	6	7	8
Chlorpyrifos (mg.kg^{-1})	0.002895	0.00348	0.001048	0.001419	<0.0005	<0.0005	<0.0005	<0.0005

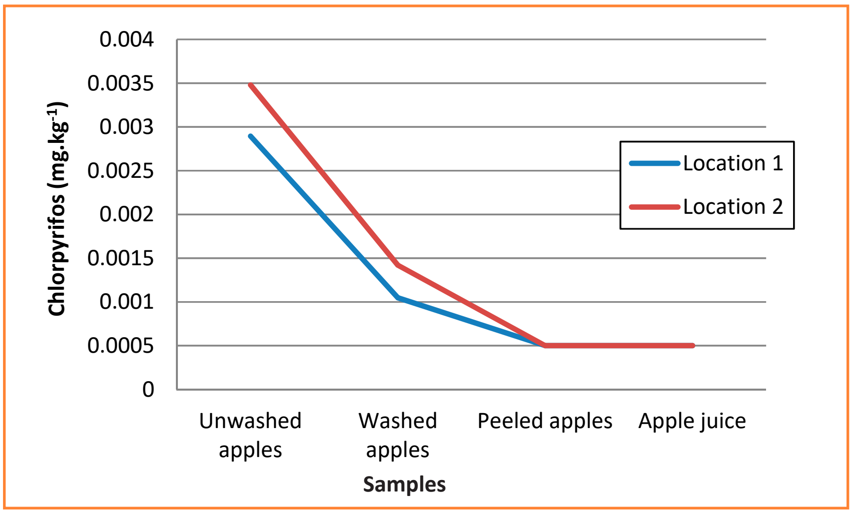

Figure 2 Effect of apple processing on the concentration of chlorpyrifos (mg chlorpyrifos.kg^{-1} sample) in all tested samples from locations 1 and 2

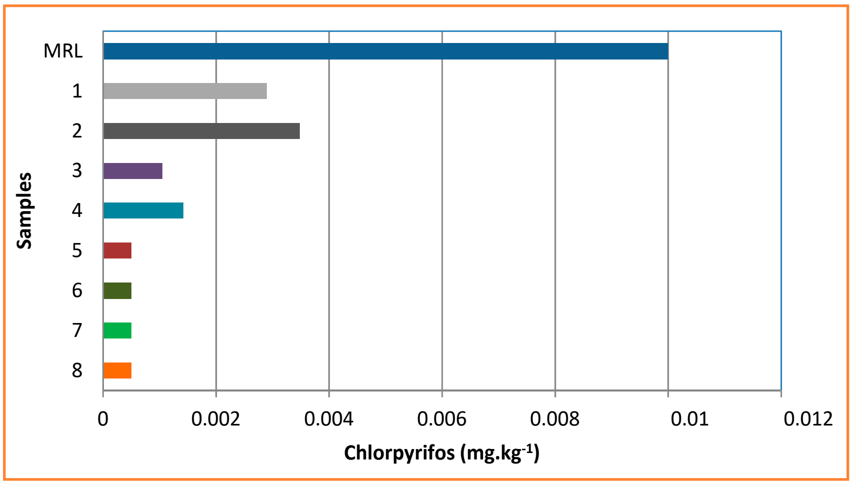

Figure 3 Concentration of chlorpyrifos (mg chlorpyrifos.kg^{-1} sample) in all tested samples compared to MRL

of present chlorpyrifos in sample 5 was decreased compared to sample 3 to <0.0005 mg.kg^{-1}, or was decreased by 52.3%, and in sample 6, the amount of the pesticide decreased to <0.0005 mg.kg^{-1}, or the concentration was decreased by 64.7% in relation to sample 4. The concentration of the pesticide chlorpyrifos in apple juice from both locations 1 and 2 was less than <0.0005 mg.kg^{-1}. In sample 7, the pesticide concentration was decreased by 82.7% compared to sample 1, and in sample 8, the amount of present pesticide, compared to sample 2, was decreased by 85.6%. The concentration of the pesticide chlorpyrifos in sample 7 was decreased by 52.3% compared to sample 3. The concentration of pesticide in sample 8 was decreased by 64.7% compared to sample 4. These results are in a good agreement with the results of the research conducted by Ling et al. (2011) and Sheikh et al. (2015). Fig. 2 shows the decrease in chlorpyrifos concentration in the analyzed samples.

Processing factors (PF) were calculated after each heat or mechanical treatment of the examined samples and the values are as follows: for sample 3 it is 0.41 while for sample 4 it is 0.36 while PF for peeled and heat treated apples (samples 5to 8) is 0.14. According to Bonnechère et al. (2012) and Scholz (2018), if a PF is lower than 1, it indicates the reduction of a pesticide, while if higher than 1, it indicates a concentration in regulatory practice, regardless of changes in volume or weight for the processed food (due to dilution, removal or degradation). According to the Official Journal of the European Union (2020)a and the Official Newspaper of the Republic of Macedonia (2018), the maximum residue levels (MRL) for chlorpyrifos in apples is 0.01 mg.kg^{-1}. Fig. 3 shows the concentration of the pesticide chlorpyrifos in all analyzed samples compared to maximum residue level.

Fresh (unwashed) apples from location 1 have the chlorpyrifos concentration that is 3.45 times lower than MRL, while unwashed apples from location 2 have the chlorpyrifos concentration of 2.87 times lower than MRL. Washed apples from locations 1 and 2 have the chlorpyrifos concentration lower by 9.54 and 7.04 times than MRL, respectively. Peeled apples from both locations as well as apple juice were found to contain less than 0.0005 mg.kg^{-1} of chlorpyrifos that is 20 or more times less than MRL.

In all tested samples, the concentration of the pesticide chlorpyrifos are within the MRL which means that apples/apple products are safe for consumption. These observations are in good agreement with other studies conducted (Dömötörová et al., 2006; Kovalczuk et al., 2008; Dasika et al., 2012; Yang et al., 2017; Rahman et al., 2021).

4 Conclusions

In this study, the presence of pesticide chlorpyrifos was analyzed in fresh and processed apples. Namely, the influence of the mechanical and thermal treatment of the apples of the Red Delicious variety on the concentration of the pesticide was monitored. Chlorpyrifos was detected in all samples before any treatment, but in an allowed limit concentration. After processing of the apples, the concentration of chlorpyrifos decreases to a value that is in the detection range of the instrumental technique, from which one can conclude that any processing method of apples has an impact on the content of chlorpyrifos. The greatest decrease in the concentration of chlorpyrifos was observed during the heat treatment of the apples, i.e. in the prepared apple juice. Also, the mechanical preparation of apple samples affects the reduction of the pesticide content while the washing of the apples has the least impact. Additional research is needed on this topic or by expanding the analysis to other types of pesticides used for protection on apple trees.

References

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *Journal of AOAC International*, 86(2), 412–431. https://doi.org/10.1093/jaoac/86.2.412

Badr, A. N., Ahmed, M. B. M., Amer, M. M., Thang, N. V., & Fouzy, S. M. A. (2019). Pesticides Evaluation in Egyptian Fruits and Vegetables: A Safety Assessment Study. *Journal of Environmental Science and Technology*, 12(2), 81–91. https://dx.doi.org/10.3923/jest.2019.81.91

Balinova, A. M., Mladenova, R. I., & Shtereva, D. nD. (2006). Effects of processing on pesticide residues in peaches intended for baby food. *Food Additives Contaminants*, 23(9), 895–901. https://doi.org/10.1080/02652030600771715

Bonnechère, A., Hanot, V., Jolie, R., Hendrickx, M., Bragard, C., Bedoret, T., & Van Loco, J. (2012). Processing Factors of Several Pesticides and Degradation Products in Carrots by Household and Industrial Processing. *Journal of Food Research*, 1(3), 68–83. https://doi.org/10.5539/jfr.v1n3p68

Bruzzoniti, M. C., Checchini, L., De Carlo, R. M., Orlandini, S., Rivoira, L., & Del Bubba, M. (2014). QuEChERS sample preparation for the determination of pesticides and other organic residues in environmental matrices: a critical review. *Anal Bioanal Chemistry*, 406(17), 4089–4116. https://doi.org/10.1007/s00216-014-7798-4

Chavarri, M. J., Herrera, A., & Arino, A. (2005). The decrease in pesticides in fruit and vegetables during commercial processing. *International Journal of Food Science & Technology*, 40(2), 205–211.
https://doi.org/10.1111/j.1365-2621.2004.00932.x

Dasika, R., Tangirala, S., & Naishadham, P. (2012). Pesticide residue analysis of fruits and vegetables. *Journal of Environmental Chemistry and Ecotoxicology*, 4, 19–28. DOI: 10.5897/JECE11.072 Retrieved September 23 2021 from https://africaneditors.org/journal/JECE/full-text-pdf/77998-122308

Dömötörová, M., Hercegová, A., & Matisová, E. (2006). Monitoring of pesticide residues in apples from Slovakia for baby food production. *Czech Journal of Food Sciences*, 24(2), 84–92. https://doi.org/10.17221/3303-CJFS

Drouillet-Pinard, P., Boisset, M., Periquet, A., Lecerf, J. M., Casse, F., Catteau, M., & Barnat, S. (2011). Realistic approach of pesticide residues and French consumer exposure within fruit & vegetable intake. *Journal of Environmental Science and Health*, Part B, 46(1), 84–91.
https://doi.org/10.1080/03601234.2011.534413

El-Sayed, E., Hassan, H., Abd El-Raouf, A., & Salman, S. N. (2021). Investigation of the effects of household processing on the reduction rate of chlorpyrifos, metalaxyl and diazinon residues in orange fruit. *Hellenic Plant Protection Journal*, 14, 64–75. https://doi.org/10.2478/hppj-2021-0007

Hancock, F. J., Luby, J. J., Brown, S. K., & Lobos, G. A. (2008). Apples. In J. F. Hancock (Ed.), *Temperate fruit crop breeding* (pp. 1–37). Kluwer Academic Publishers.

Icka, P., & Damo, R. (2014). Assessment of harvest time for red delicious cv. trough harvest indexes in Albania. *Bulgarian Journal of Agricultural Science*, 20(3), 628–632. Retrieved September 23 2021 from https://www.researchgate.net/publication/287914349_Assessment_of_harvest_time_for_Red_Delicious_cvthough_harvest_indexes_in_Albania

Jankuloska, V., Pavlovska, G., & Karov, I. (2020). Assessment of adults and children exposure to pesticide residues through apple consumption. *Journal of Hygienic Engineering and Design*, 33, 34–40. Retrieved September 25 2021 from https://keypublishing.org/jhed/jhed-volumes/jhed-volume-33/

John, E., & Shaike, J. (2015). Chlorpyrifos: pollution and remediation. *Environmental Chemistry Letters*, 13, 269–291. https://doi.org/10.1007/s10311-015-0513-7

Kaushik, G., Satya, S., & Naik, S. N. (2009). Food processing a tool to pesticide residue dissipation – A review. *Food Research International*, 42(1), 26–40.
https://doi.org/10.1016/j.foodres.2008.09.009

Keikotlhaile, M. B., Spanoghe, P., & Steurbaut, W. (2009). Effects of food processing on pesticide residues in fruit and vegetables: A meta-analysis approach. *Food and Chemical Toxicology*, 48(1), 1–6. https://doi.org/10.1016/j.fct.2009.10.031

Khan, N., Yaqub, Gh., Hafeez, T., & Tariq, M. (2020). Assessment of Health Risk due to Pesticide Residues in Fruits, Vegetables, Soil, and Water. *Journal of Chemistry,* 2020. https://doi.org/10.1155/2020/5497952

Koch, S., Epp, A., Lohmann, M., & Bol, G. (2017). Pesticide residues in Food: Attitudes, beliefs and misconceptions among conventional and organic consumers. *Journal of Food Protection*, 80(12), 2083–2089. https://doi.org/10.4315/0362-028X.JFP-17-104

Kovalczuk, T., Lacina, O., Jech, M., Poustka, J., &Hajslova, J. (2008). Novel approach to fast determination of multiple pesticide residues using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS). *Food Additives and Contaminants*, 25(4), 444–457. https://doi.org/10.1080/02652030701570156

Ling, Y., Wang, H., Yong, W., Zhang, F., Sun, L., Yang, M. L., Wu, Y. N., & Chu, X. G. (2011). The effects of washing and cooking on chlorpyrifos and its toxic metabolites in vegetables. *Food Control*, 22(1), 54–58. https://doi.org/10.1016/j.foodcont.2010.06.009

Lozowicka, B., Abzeitova, E., Sagitov, A., Kaczynski, P., Toleubayev, K., & Li, A. (2015). Studies of pesticide residues in tomatoes and cucumbers from Kazakhstan and the associated health risks. *Environmental Monitoring and Assessment*, 187(10), 609. https://doi.org/10.1007/s10661-015-4818-6

Mackay, D., Giesy, J. P., & Solomon, K. R. (2014). Fate in the environment and long-range atmospheric transport of the organophosphorus insecticide, chlorpyrifos and its oxon. *Reviews of Environmental Contamination and Toxicology*, 231, 35–76. https://doi.org/10.1007/978-3-319-03865-0_3

Mebdou, S., Lazali, M., Ounane, M. S., Tellah, S., Nabia, F., & Ounane, Gh. (2017). Evaluation of pesticide residues in fruits and vegetables from Algeria. *Food additives & Contaminants: Part B*, 10(2), 91–98. https://doi.org/10.1080/19393210.2016.1278047

Official Journal of the European Union (2005). Regulation (EU) 396/2005. Maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Retrieved May 20, 2021 from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32005R0396&from=EN

Official Journal of the European Union (2020)a. Regulation (EU) 2020/1085. Annexes II and V to Regulation (EC) No 396/2005 of the European Parliament and of the Council as regards maximum residue levels for chlorpyrifos and chlorpyrifos-methyl in or on certain products. Retrieved July 15, 2021 from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R1085&from=EN

Official Journal of the European Union (2020)b. Regulation (EU) 2020/18. Concerning the non-renewal of the approval of the active substance chlorpyrifos, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market, and amending the Annex to Commission Implementing Regulation (EU) No 540/2011. Retrieved July 15, 2021 from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0018&from=EN

Official Journal of the European Union (2021). CommissionImplementing Regulation (EU) 540/2011. Implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. Retrieved August 27, 2021 from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0540&from=EN

Official Newspaper of the Republic of Macedonia (2018). Rulebook on general food safety requirements regarding the maximum permitted levels of pesticide residues in or on food and animal feed of plant and animal origin, 91. Retrieved June 27, 2021 from https://www.slvesnik.com.mk/Issues/3b17ed621f584062a1c232ce79d2bbfa.pdf

Paz, M., Correia-Sa, L., Vidal, C.B., Becker, H., Longhinotti, E., Domingues, F. V., & Delerue-Matos, C. (2017). Application of the QuEChERS method for the determination of organochlorine pesticide residues in Brazilian fruit pulps by GC-ECD. *Journal of Environmental Science and Health*, part B, 52(1), 48–58. https://doi.org/10.1080/03601234.2016.1229450

Paz, M., Gullon, P., Barroso, M. F., Carvalho, A., Domingues, V. F., Gomes, A. M., Becker, H., Longhinotti, E., & Delerue-Matos, C. (2015). Brazilian fruit pulps as functional foods and additives: evaluation of bioactive compounds. *Food Chemistry*, 172, 462–468. https://doi.org/10.1016/j.foodchem.2014.09.102

Quintero, A., Caselles, M.J., Ettiene, G., de Colmenares, N.G., Ramirez, T., & Medina, D. (2008). Monitoring of organophosphorus pesticide residues in vegetables of agricultural area in Venezuela. *Bulletin of Environmental Contamination and Toxicology*, 81, 393–396. https://doi.org/10.1007/s00128-008-9511-9

Radišić, M., Grujić, S., Vasiljević, T., &Laušević, M. (2009). Determination of selected pesticides in fruit juices by matrix solid-phase dispersion and liquid chromatography-tandem mass spectrometry. *Food chemistry*, 113(2), 712–719. https://doi.org/10.1016/j.foodchem.2008.07.103

Rahman, M., Sazedul Hoque, Md., Bhowmik, Sh., Ferdousi, Sh., Kabiraz, P. M., van Brakel, L. M. (2021). Monitoring of pesticide residues from fish feed, fish and vegetables in Bangladesh by GC-MS using the QuEChERS method. *Heliyon*, 7(3) 1–8. https://doi.org/10.1016/j.heliyon.2021.e06390

Rupasinghe, V. H. P & Thilakarathna, S. (2016). Apple Juice. In Shahidi F., & Alasalvar, C. (Eds.). *Handbook of Functional Beverages and Human Health* (pp. 93–107). CRC Press Taylor & Francis Group.

Sabarwal, A., Kumar, K., & Singh, R. (2018). Hazardous effects of chemical pesticides on human health-cancel and other associated disorders. *Environmental toxicology and pharmacology*, 63, 103–114. https://doi.org/10.1016/j.etap.2018.08.018

Satpathy, G., Tyagi, Y. K., & Gupta, R. K. (2012). Removal of organophosphorus (OP) pesticide residues from vegetables using various washing solutions and boiling. *Journal of Agricultural Science*, 4(2), 69–78. https://doi.org/10.5539/jas.v4n2p69

Scholz, R., van Donkersgoed, G., Herrmann, M., Kittelmann, A., von Schledorn, M., Graven, C., Mahieu, K., van der Velde-Koerts, T., Anagnostopoulos, Ch., Bempelou, E., & Michalski, B. (2018). *European database of processing factors for pesticides*. EFSA supporting publication 2018:EN-1510. https://doi.org/10.2903/sp.efsa.2018.EN-1510

Sharma, D., Nagpal, A., Pakade, Y.B., & Katnoria, J. K. (2010). Analytical methods for estimation of organophosphorus pesticide residues in fruits and vegetables: A review. *Talanta*, 82(4), 1077–1089. https://doi.org/10.1016/j.talanta.2010.06.043

Sheikh, A. S., Panhwar, A. A., Khan, S., Soomro, H. A., & Khaskheli, G. Sh. (2015). Removal of pesticide residues in bitter gourd peel by traditional processing methods. *International Journal of Biology, Pharmacy and Allied Sciences*, 4(12), 6639–

6648. Retrieved September 23 2021 from https://ijbpas.com/pdf/2015/December/1448533291MS%20IJBPAS%202015%203438.pdf

Simon, S., Brun, L., Guinaudeau, J., & Sauphanor, B. (2011). Pesticide use in current and innovative apple orchard systems. *Agronomy for Sustainable Development*, 31, 541–555. https://doi.org/10.1007/s13593-011-0003-7

Singh, N., Wang, C., & Cooper, R. (2014). Potential of Essential Oil-Based Pesticides and Detergents for Bed Bug Control. *Economic Entomology*, 107(6), 2163–2170. http://dx.doi.org/10.1603/EC14328

Stachniuk, A., Szmagara, A., Czeczko, R., & Fornal, E. (2017). LC-MS/MS determination of pesticide residues in fruits and vegetables. *Journal of Environmental science and Health, Part B*, 52(7), 446–457. https://doi.org/10.1080/03601234.2017.1301755

Standardization Institute of the Republic of Macedonia. (2011). Macedonia MKS EN 15662: 2011 – Foods of plant origin – Determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE – QuEChERS-method.

Štěpán, R., Tichá, J. Hajšlová, Kovalczuk, T., & Kocourek, V. (2005). Baby food production chain: Pesticide residues in fresh apples and products. *Food Additives & Contaminants*, 22(12), 1231–1242. https://doi.org/10.1080/02652030500239623

Suárez-Jacoboa, A., Alcantar-Rosales, M.V., Alonso-Segura, D., Heras-Ramírez, M., Elizarragaz-De La Rosa, D., Lugo-Melchor, O., & Gaspar-Ramirez, O. (2017). Pesticide residues in orange fruit from citrus orchards in Nuevo Leon State, Mexico. *Food additives & Contaminants: Part B*, 10(3), 192–199. https://doi.org/10.1080/19393210.2017.1315743

Yang, T., Doherty, J., Zhao, B., Kinchla, A., Clark, J., & He, L. (2017). Effectiveness of commercial and homemade washing agents in removing pesticide residues on and in apples. *Journal of Agricultural and Food Chemistry*, 65(44), 9744–9752. https://doi.org/10.1021/acs.jafc.7b03118