

Removal of chlorpyrifos, carbofuran, cyhalothrin, and bifenthrin residues from citrus by using ozonated water

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Abstract

Citrus fruits—oranges, lemons, and grapefruits—are produced and consumed in huge proportions around the globe which contain 91% water and the reason why they are regarded as the most hydrating fruits. Citrus fruits are a vital part of the human diet by providing the essential vitamins, minerals, and antioxidants to the body, but the process of citrus fruits production frequently utilizes the application of pesticides to deal with pests and diseases. Pesticide residues, including chlorpyrifos, carbofuran, cyhalothrin, and bifenthrin, in the fruit can still have an effect on consumers' health. In this study, the process of ozonation was used to remove pesticide residues from citrus fruits such as in oranges, lemons, and grapefruits. Ozonated water helped in the detection and removal of pesticides residues chlorpyrifos, carbofuran, cyhalothrin, and bifenthrin from citrus fruits. The mean residual level of chlorpyrifos was 0.015 ± 0.004 in oranges, 0.014 ± 0.004 in lemons, and 0.022 ± 0.001 in grapefruits; the bifenthrin level was 0.055 ± 0.004 in oranges, 0.055 ± 0.004 in lemons, and 0.054 ± 0.005 in grapefruits; the lambda-cyhalothrin level was 1.104 ± 0.174 in oranges, 1.056 ± 0.210 in lemons, and 1.208 ± 0.172 in grapefruits; and the carbofuran level was 0.625 ± 0.050 in oranges, 0.616 ± 0.046 in lemons, and 0.616 ± 0.050 in grapefruits. Furthermore, residual percentages of chlorpyrifos, carbofuran, cyhalothrin, and bifenthrin were detected in all citrus fruits, with the results showing that maximum levels of pesticide residues were efficiently removed from citrus fruits at 10 ppm (parts per million) ozonation without any poisoning and deterioration of fruits.

Keywords: chlorpyrifos; carbofuran; cyhalothrin; bifenthrin residues; citrus; ozonated water

Introduction

The economic impact of the agriculture sector on profits after the independence of Pakistan was significant, which resulted in almost 21% gross domestic product (GDP) and provided employment to 45% people. Almost 63% of the population lived in rural areas and made their living in a direct or indirect manner from agricultural sources (Raza *et al.*, 2012). Agriculture plays a vital role in the provision of food for existing and rising population, raw material for agribusiness, and for the export revenues. The main subsectors in the agriculture industry are major and minor crops, forestry, live stocks, and fisheries (Jiang and China, 2016). The major pillar of a country's economy is agricultural production. For a balanced diet, the people of Pakistan are more likely to ingest vegetables and fruits. Exporting vegetables and fruits to foreign states, like India, Kingdom of Saudi Arabia, Afghanistan, UAE, and England, are a great source for earning foreign currencies (Syed *et al.*, 2014). A healthy lifestyle can be had by consuming fruits and vegetables due to the high contents of vitamin A, vitamin C, minerals, phytochemicals, electrolytes, antioxidants, and dietary fiber they contain (Yahia *et al.*, 2019). They are also rich in bioactive compounds which offer more health benefits by preventing certain illnesses. (Albuquerque *et al.*, 2020).

Health advantages of fruits and vegetables are lowering cancer risk, boosting brain ability, protecting bones, immune system, teeth, heart, urinary tract, eyesight, aging, and reducing elevated cholesterol levels (Saeid *et al.*, 2021; Islam *et al.*, 2021). Based on the nutritional profile, vitamin A in fruits and vegetables help in reducing the occurrence of night blindness (Kaparapu *et al.*, 2020) plant-based foods containing a high amount of vitamin C help in digestion and boost immunity (Khalid *et al.*, 2022). Over the last two decades, there has been a comparatively sustained increase in the output of citrus all over the world. According to the most recent report by the United States Department of Agriculture (USDA, 2022) on the international citrus markets and trade, favorable weather in Brazil and Turkey is expected to boost orange production globally for 2021/2022, predicted to reach 49.0 million tons over the previous year. Brazil is the top producer of oranges with 16.91 million tons, followed by China with 7.55, European Union with 6.10, Mexico with 4.28, and United States with 3.46 (Ben Hsouna *et al.*, 2023).

Tropical and subtropical regions of various countries are the known areas to produce citrus fruits, with a significant production in Pakistan, Japan, Brazil, the United States, China, Mexico, and the Mediterranean areas. Around 1.9 million tons of citrus fruits are annually produced in Pakistan (Zhong and Nicolosi, 2020); sweet oranges, grapefruits, Mandarins, and lemons are the most cultivated citrus varieties (Hamid *et al.*, 2024). Here, people

mostly consume plant-based foods which account for almost 60% of their daily energy intake. Pakistan is blessed with an adequate supply of quality vegetables and fruits to overcome the shortage of basic food items. Citrus fruits contain high amounts of vitamin A, B, and C, essential minerals such as phosphorus, iron, calcium, and zinc, and an ample amount of carbohydrates, especially necessary for their metabolic activities which are fundamental to human health (Abakpa and Adenaike, 2021).

Different technical packs are used to prevent insect attacks and protect fruit quality during citrus production. As various citrus species contain pesticide residues, several physicochemical, biochemical, and mechanical inputs allow the residues to disperse (Sun *et al.*, 2016). Today, researchers are emphasizing on organic farming to reduce the use of pesticides on citrus production (Kowalska *et al.*, 2022). Various techniques can be used to eliminate the contaminants of pesticides, including cleaning with water and lower the entanglement of chemical solutions like ozone, chlorine, salts, hydrogen peroxide, and detergents; also, other parameters such as pH (potential of hydrogen), oxidation, temperature, metabolism, hydrolysis, depletion, and photolysis can be used to degrade pesticides (Pandiselvam *et al.*, 2020).

Organophosphorus pesticide (OP) and chlorpyrifos (CPS) are the chemicals used for protecting citrus fruits by killing insects and worms (Foong *et al.*, 2020). Ozonation is a proven method for degrading various types of pesticide residues from citrus fruits, due to the fact that ozone oxidizes pesticides by breaking the chemical links in their molecules, confirming that they can no longer be harmful for human health. Ozone treatment with zero residues outcomes are utilized in food product sanitization, air quality improvement, and general disinfectant due to its ability to breakdown pesticides (Díaz-López *et al.*, 2022). In the agricultural production, and since the physicochemical properties of fruits are not altered, ozone is used to degrade pesticide residues during postharvest storage (de Souza *et al.*, 2018). The most appealing features of ozone are: its quick decomposition in molecular oxygen without leaving any trace and low concentrations of ozone can be used for the protection of surfaces from any fungal growth and infection (Liang *et al.*, 2021). The aim of this study was to extract pesticide residues from citrus fruits with the quantification of these residues and using ozonation for the removal of carbofuran, cyhalothrin, chlorpyrifos, and bifenthrin residues from citrus fruits.

Materials and Methods

Insecticides selected for ozonation were carbofuran (Furadan, 3% G, FMC), chlorpyrifos (Lorsban®, 40 EC,

Dow Agro-Sciences), bifenthrin (Talstar® 100 g a.i./l EC; FMC), and cyhalothrin (Karate® 2.5 EC Syngenta, Switzerland) and acetonitrile chemical was procured from JK enterprises (Karachi, Pakistan). Sweet oranges, lemons, and grapefruits were obtained from the market and the nearby fields in Multan, Pakistan and transported to the processing laboratory of the Institute of food Science and Nutrition Bahauddin Zakariya University, Multan. Fruits were kept at the required temperature and humidity. Ozone generator was used to produce ozone and ozone concentrations were evaluated by titration (Wang *et al.*, 2022).

Matrices production of citrus fruits

Tap water was used to wash samples of lemons, grapefruits, and oranges and then cut into slices. All prepared matrices of citrus fruits were subjected to the analysis for pesticide residues.

Solutions preparation

The analytical standard of pesticide was used to prepare pesticide solutions (chlorpyrifos, cyhalothrin, carbofuran, and bifenthrin) as solvent. Pesticide solution of 10 L was prepared by using tap water where each pesticide had a concentration of 200mg/L (Swami *et al.*, 2021).

Loading of pesticides onto matrices

Matrices of 2 kg citrus fruits—lemons, oranges, and grapefruits—were dipped into a prepared solution of pesticides for a duration of 5 minutes. Later, the sample was sundried for 10 minutes and blended in the food processor of GABA National (GN-920, Japan) for 5 minutes. QuEChERS analytical method was used for easy, cost-effective, and safe extraction of loaded pesticides on matrices. Concentrations of pesticides from fruit samples were measured using high-performance liquid chromatography (HPLC) (Lehotay, 2007).

Table 1. Detection of pesticide residues in fruit samples.

Fruit Sample	Pesticide	(Mean ± SD)
Oranges	Chlorpyrifos	0.015 ± 0.004
	Bifenthrin	0.055 ± 0.004
	Lambda-Cyhalothrin	1.104 ± 0.174
	Carbofuran	0.625 ± 0.050
Lemons	Chlorpyrifos	0.014 ± 0.004
	Bifenthrin	0.055 ± 0.004
	Lambda-Cyhalothrin	1.056 ± 0.210
	Carbofuran	0.616 ± 0.046
Grapefruits	Chlorpyrifos	0.022 ± 0.001
	Bifenthrin	0.054 ± 0.005
	Lambda-Cyhalothrin	1.208 ± 0.172
	Carbofuran	0.616 ± 0.050

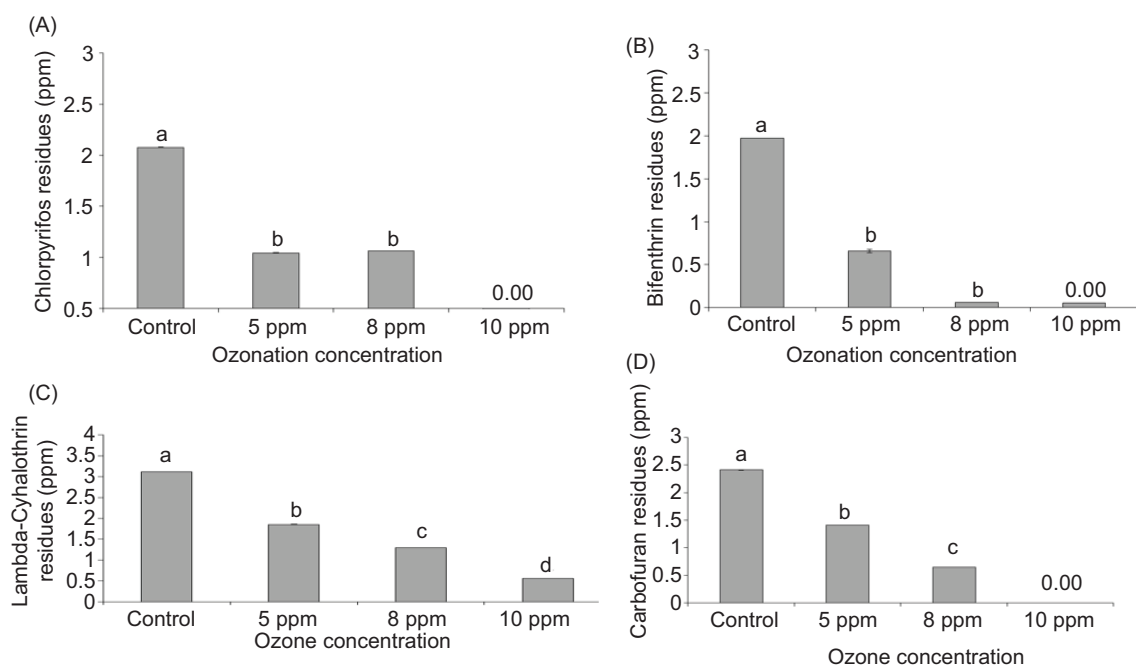


Figure 1. Chlorpyrifos, bifenthrin, lambda-cyhalothrin, and carbofuran residues treated with different ozone concentrations on oranges.

Extraction of pesticide residues

For the extraction of pesticide, 500 g of clamshell was mixed in a blender for 2 minutes; 3 g of parent mixture was poured into a tube, when 12 mL distilled water was added; later, all the chemicals were mixed in a vortex tube for 2 minutes. After a gentle shaking of the tube, it was placed in the centrifuge at 4000 rpm for 2 minutes. In another tube, 8 mL of supernatant was added, after which 1.2 mg magnesium sulfate and 0.4 g Primary and Secondary Amines (PSA) were added gradually. After shaking the sample and performing centrifugation, 1 mL supernatant was added into the vial with 4 mL water and methanol. The prepared solution was then added to HPLC in which 20 µl sample was used.

Generation and verification of ozone

A corona discharge (Xetin ozone air and water purifier, Model-XT301, Taiwan) was used to generate ozone gas (100 ppm at air flowrate of 2.5 L/minute and 32.10 mg/hour ozone output). To guarantee unswerving ozone production output, the ozone generator was warmed up for 15 minutes before the experiment. The levels of dissolved gas were measured using a portable ozone detector (DO3, Echo Sensors Inc., USA) with an accuracy of 0.01 mg/L in the range of 0–10 mg/L; the dissolved ozone was 0.054 mg/L.

Pesticides removal through ozonation

A total of 30 L tap water was used for ozonation in a reactor at 10°C. Then 1 kg matrices consisting of pesticide were added in the ozonated water for 5 minutes in the required concentrations of 5 ppm, 8 ppm, and 10 ppm. Citrus slices were then sun-dried after the completion of ozonation procedure.

Pesticides extraction after ozonation

For the evaluation of pesticides persistent concentrations in samples, 1 kg of ozonated matrix was separated out regimented in the food processor. After homogenization, the organic layer of pesticide was extracted from the 15 g of sample taken, according to the QuEChERS method for HPLC study (Gonzalez-Curbelo *et al.*, 2022).

HPLC analysis of pesticides

Pesticide levels were identified and quantified by ultraviolet (UV) visible detector and HPLC was used to analyze aliquots. Separation was performed by C₁₈ (a straight-chain

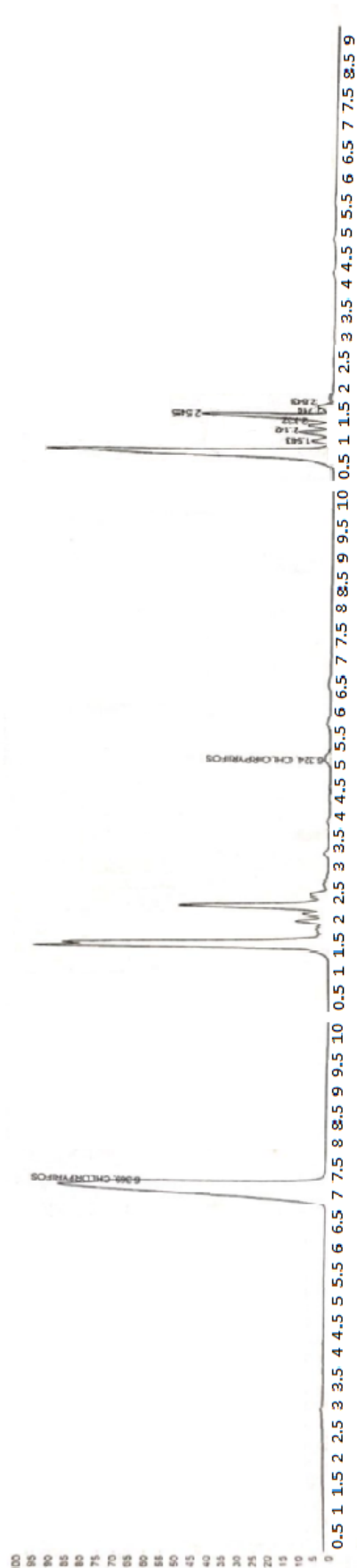


Figure 2. HPLC chromatograms of chlorpyrifos for oranges. A: Grade standard of chlorpyrifos; B: Oranges contaminated with chlorpyrifos before ozonation; C: Oranges contaminated with chlorpyrifos after ozonation.

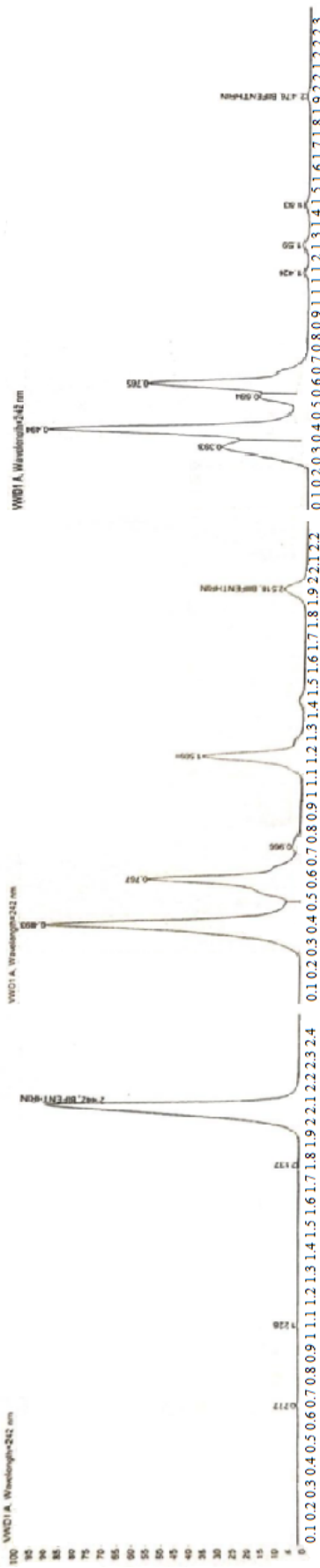


Figure 3. HPLC chromatograms of bifenthrin for oranges. A: Grade standard of bifenthrin; B: Oranges contaminated with bifenthrin before ozonation; C: Oranges contaminated with bifenthrin after ozonation.

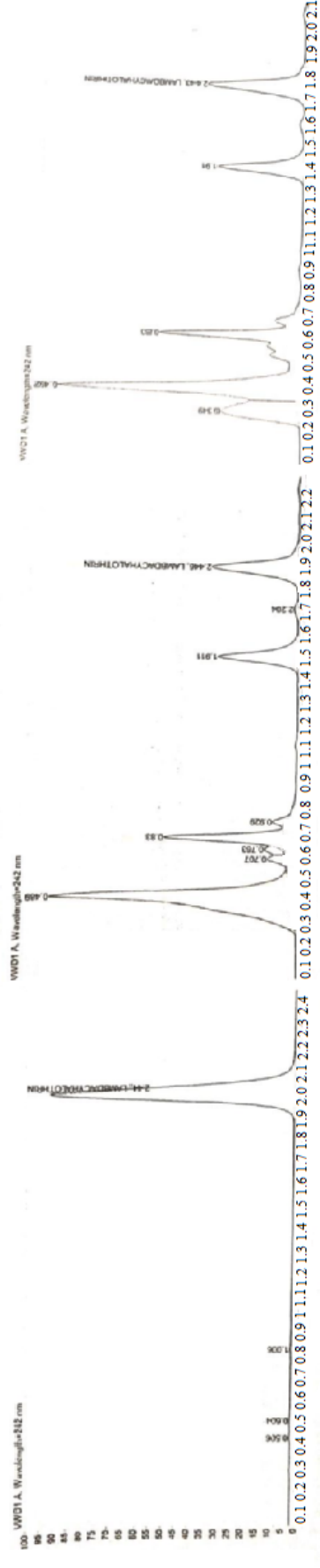


Figure 4. HPLC chromatograms of lambda-cyhalothrin for oranges. A: Grade standard of lambda-cyhalothrin; B: Oranges contaminated with lambda-cyhalothrin before ozonation; C: Oranges contaminated with lambda-cyhalothrin after ozonation.

alkane with 18 carbon atoms) (250×4.6 mm i.d) and UV 254 nm reversed phase column using the direct gradient of solvent at the adjustment of 0.3 mL/minute. The limit of detection for target compounds was in the range of 1.5 $\mu\text{g}/\text{kg}$ to 10 $\mu\text{g}/\text{kg}$. Deionized water was denoted as A: acetonitrile as solvent B (70:30, v/v [volume per volume]) with 20 μl volume of injection. To the UV visible wavelengths, calibration of pesticide detectors was initiated at 242 nm. For the identification of the residue, retaining time of pesticide residues was compared with reference standards. Determination of the reduction percentage was done by comparing the concentrations of pesticides residue in ozonated water for washed fruits to those for the unwashed (Vuthijumnonk and Shimbhano, 2019).

Statistical analysis

The subjected data or treatments were performed in triplicate for each commodity and the result was investigated using ANOVA (Analysis of Variance), which was depicted as mean \pm standard deviation (SD) at a significance of 0.05 or less. All the chemometric calculations were done by applying the statistics package of the program (Engle and Granger, 1987).

Results and Discussion

Lemons, oranges, and grapefruits were selected for the determination of pesticide residues after spraying and

before ozonation. The supreme residue limit of bifenthrin, chlorpyrifos, carbofuran, and lambda-cyhalothrin were 0.05 ppm, 0.01 ppm, 0.5 ppm, and 1 ppm, respectively (Table 1). Different pesticides residues were detected in different concentrations in these citrus fruits when the concentration of ozonated water changed. The mean residual level of chlorpyrifos in oranges, lemons, and grapefruits was 0.015, 0.014, and 0.022, respectively (Figure 1). The bifenthrin pesticide's concentration in oranges and lemons was similar (0.055) and 0.054 was observed in grapefruits. The mean residual level of lambda-cyhalothrin was 1.104 in oranges, 1.056 in lemons, and 1.208 in grapefruits; the mean residual level of carbofuran was 0.625 in oranges and 0.616 in lemons as well as in grapefruits. Pesticide residues that are highly soluble, wobbly, and weakly bound to the fruit surface are removed more easily, whereas fat-soluble, stable, and strongly adsorbed entail intensive treatments (Dhankhar *et al.*, 2023). Likewise, fruits with smooth, non-waxy, and thick peels consent higher removal percentages of pesticides residues as compared to porous, waxy, or thin-skinned fruits or vegetables (Lemic *et al.*, 2025; Osman *et al.*, 2024). Combining multiple techniques, such as ozonation followed by rinsing or surfactant washes, often yield the highest pesticide removal efficiency (Pal and Kioka, 2025).

Effect of different ozone concentrations on sanitization of oranges treated with different pesticides

Nontreated fruits were found to have maximum residues of chlorpyrifos, whereas the minimum levels of residues

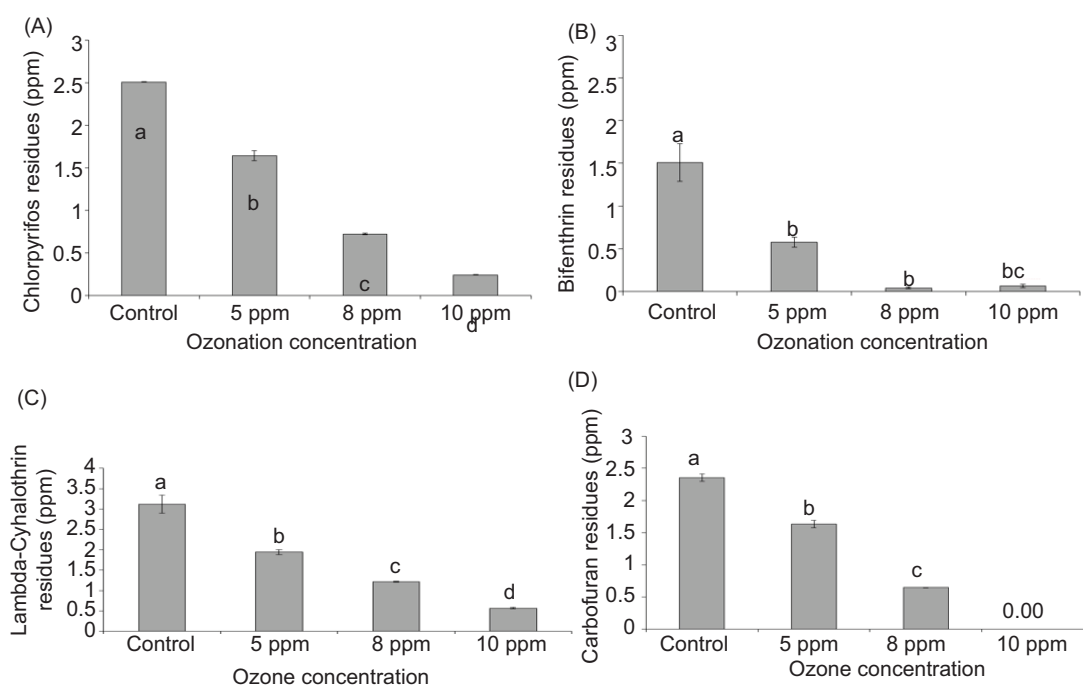


Figure 5. Chlorpyrifos, bifenthrin, carbofuran, and lambda-cyhalothrin residues on lemons treated with different ozone concentrations.

were obtained when fruits were treated with a higher dose of ozone. The results of the ozonated oranges were found to be significant ($p < 0.05$) for the reduction of pesticides; chlorpyrifos residue decreased noticeably, that is, 49.8%, 48.8%, and 100% at 5 ppm, 8 ppm, and 10 ppm of ozone from oranges, respectively. Likewise, bifenthrin residues were significantly reduced after ozonation as compared to nontreated oranges; the maximum decline (97.6%) in residue was notified at a higher dose of 10 ppm ozone while minimum levels (68.4%) of residue were investigated at 5 ppm. Moreover, bifenthrin removal at 8 ppm was slightly lower (97.2%) than the 10 ppm ozone solution (Figure 2). Similarly, the results for lambda-cyhalothrin indicated significant decline with an increased range of ozone, that is, 10.7%, 36.5%, and 75.8% at 5 ppm, 8 ppm, and 10 ppm of ozone in oranges.

These results were in accordance with the study proposed by Wu *et al.* (2019): by using the method of ozonation, the removal percentages for all these pesticide residues from spinach were similar. For the removal of carbofuran residues from oranges, different concentrations of ozone were used. Carbofuran residues were significantly reduced after ozonation while using different concentrations of ozone. Nontreated fruits were found to have maximum residues; however, elevated ozone concentration in the experiment resulted in the reduction of pesticide residues of carbofuran. Removal percentages for carbofuran were 31.3%, 69.2%, and 100% at 5 ppm, 8 ppm, and 10 ppm, respectively, of ozone from oranges. In the results supported by Pandiselvam *et al.* (2020), 100% chlorothalonil residues were removed by using 10 ppm ozone, followed by 98.5% tetradifon and 94.4% chlorpyrifos ethyl from citrus fruits.

Effect of different ozone concentrations on sanitization of lemons treated with different pesticides

The results for chlorpyrifos residues were found to be significant for the reduction of pesticides in lemons at different concentrations of ozone as compared to nontreated lemons. The maximum decline of pesticides was indicated at 10 ppm ozone level while the lowest removal was notified at 5 ppm (Figure 5).

Bifenthrin residues were found to have removal percentages of 61.7, 95.8, and 98.1 at 5 ppm, 8 ppm, and 10 ppm of ozone, respectively. Cyhalothrin residues were significantly reduced after ozonation and percentages for the removal of lambda-cyhalothrin were obtained as 38.4%, 61.7%, and 82.1% at 5 ppm, 8 ppm and 10 ppm of ozone, respectively. Similarly, carbofuran residues were significantly reduced after ozonation while using different concentrations of ozone and removal percentages were determined as 31.1%, 71.9%, and 100% at 5 ppm, 8 ppm,

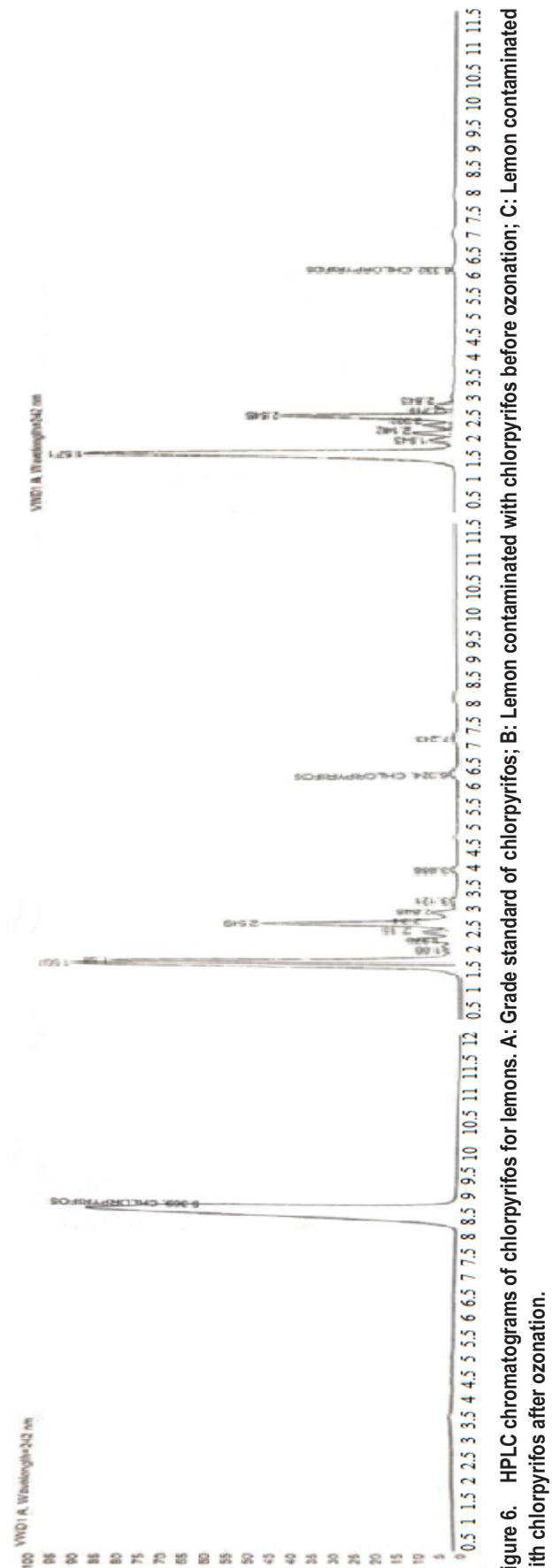


Figure 6. HPLC chromatograms of chlorpyrifos for lemons. A: Grade standard of chlorpyrifos; B: Lemon contaminated with chlorpyrifos before ozonation; C: Lemon contaminated with chlorpyrifos after ozonation.

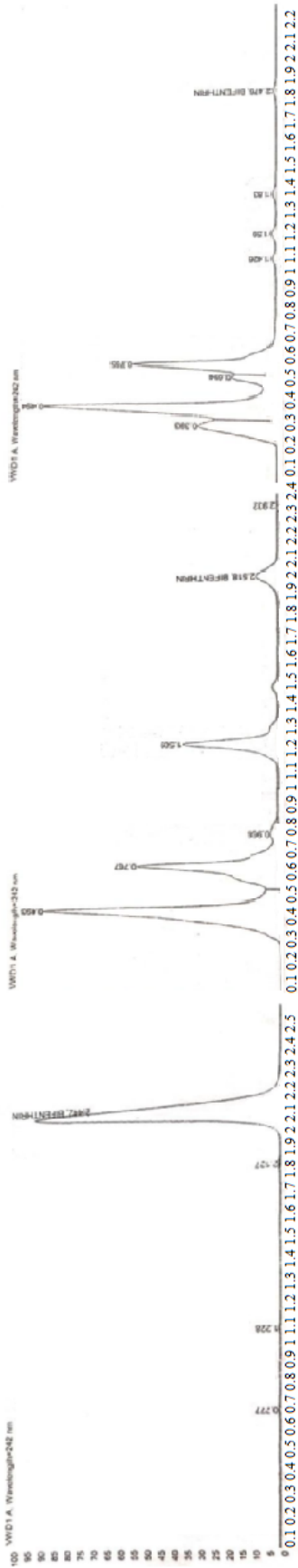


Figure 7. HPLC chromatograms of bifenthrin for lemons. A: Grade standard of bifenthrin; B: Lemons contaminated with bifenthrin before ozonation; C: Lemons contaminated with bifenthrin after ozonation.

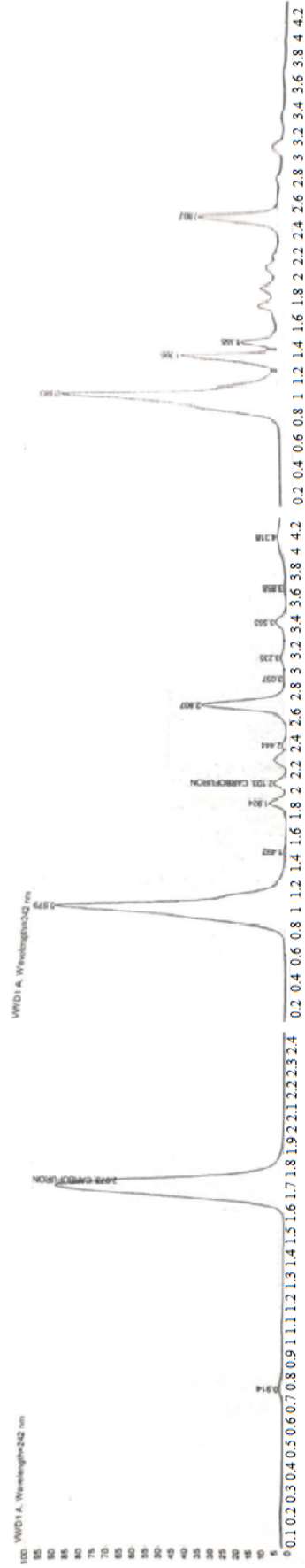


Figure 8. HPLC chromatograms of carbofuran for lemons. A: Grade standard of carbofuran; B: Lemons contaminated with carbofuran before ozonation; C: Lemons contaminated with carbofuran after ozonation.

and 10 ppm of ozone, respectively. A study by Swami *et al.* (2021) supported these outcomes as it revealed that pesticide residues were removed from a variety of citrus fruits when treated with ozone water. Likewise, an earlier study by Siddique *et al.* (2021) investigated the impact of sonolytic ozonation to degrade pesticides residues in fruits and vegetables; the results showed significant decline in the mean residues of thiamethoxam, imidachloprid, acetamiprid, thiachloprid, and carbendazim, that is, 99.3%, 52.6%, 65.2%, 87.3%, and 72%, respectively, in time optimization range from 5 minutes to 15 minutes.

Effect of different ozone concentrations on sanitization of grapefruit treated with different pesticides

Residues of chlorpyrifos were found to have removal percentages of 61.2, 44.1, and 100 at 5 ppm, 8 ppm, and 10 ppm of ozone, respectively (Figure 9). Similarly, bifenthrin residues were reduced significantly as compared to nontreated fruits and the maximum residues of bifenthrin found to have removal percentages of 68.9, 98.1, and 96.8 at 5 ppm, 8 ppm, and 10 ppm of ozone, respectively. Furthermore, removal percentages of cyhalothrin residues were found as 39.9% for 5 ppm, 64.2% for 8 ppm, and 80.2% for 10 ppm of ozone in grapefruits, respectively. Carbofuran residues were also removed from grapefruits at various ozone concentrations and significant reduction

in residues indicated 41.1%, 99%, and 100% at 5 ppm, 8 ppm, and 10 ppm of ozone, respectively. A study by Shakouri *et al.* (2014) revealed that thalonil pesticide residues were removed by ozonation without effecting the quality of food commodities. Similarly, Özen *et al.* (2021) observed the ozone treatment effect to remove pesticides residues in green peppers where the outcomes demonstrated significant decline in residue: 70.08%, 84.80%, and 100% for acetamiprid, malathion, and emamectin benzoate, respectively. In another study by Li *et al.* (2024) demonstrated the pesticide residues removal with ozone microbubbles and results showed significant decline or removal of azoxystrobin, emamectin benzoate, difenconazole, and boscalid under long washing (18 minutes).

Drawbacks of ozonation

In this advanced era of food industries, several concerns create barriers to saturate consumers' demand for food—healthy and high-quality food with zero hazards. To overcome this issue, consistent monitoring at every stage should be ensured, which is only possible when advanced technologies support the concept of food safety; ozonation is one that is cheap as well as assist in removing pesticides from food products, including meat, fruits, and vegetables (up to 100%) without effecting the sensory, nutritional,

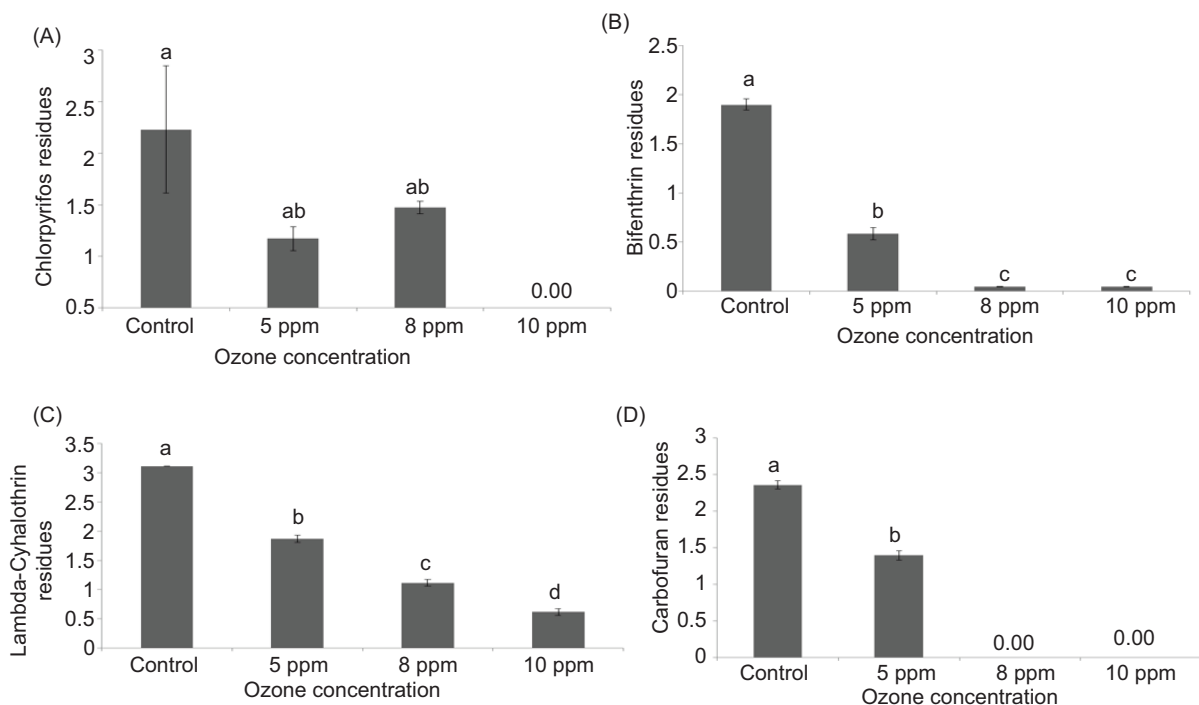


Figure 9. Chlorpyrifos, bifenthrin, lambda-cyhalothrin, and carbofuran residues on grapefruits treated with different ozone concentrations.

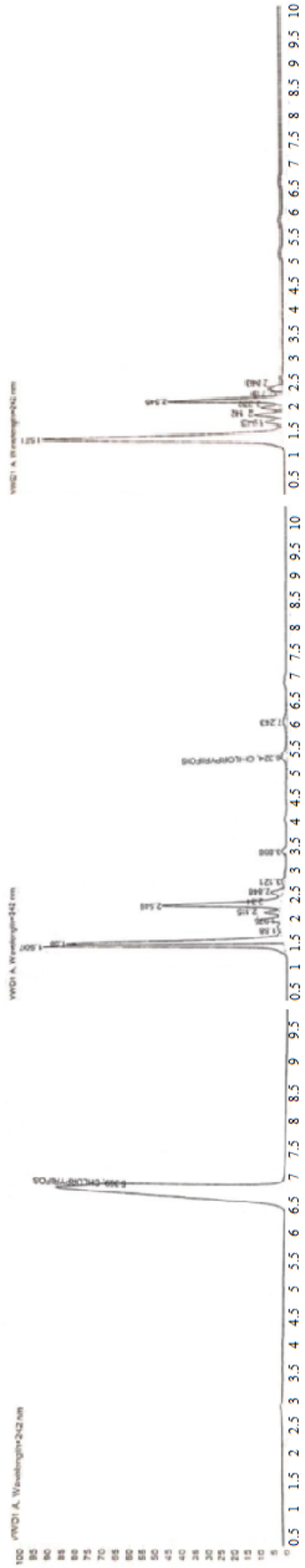


Figure 10. HPLC chromatograms of chlorpyrifos for grapefruits. A: Grade standard of chlorpyrifos; B: Grapefruits contaminated with chlorpyrifos before ozonation; C: Grapefruits contaminated with chlorpyrifos after ozonation.

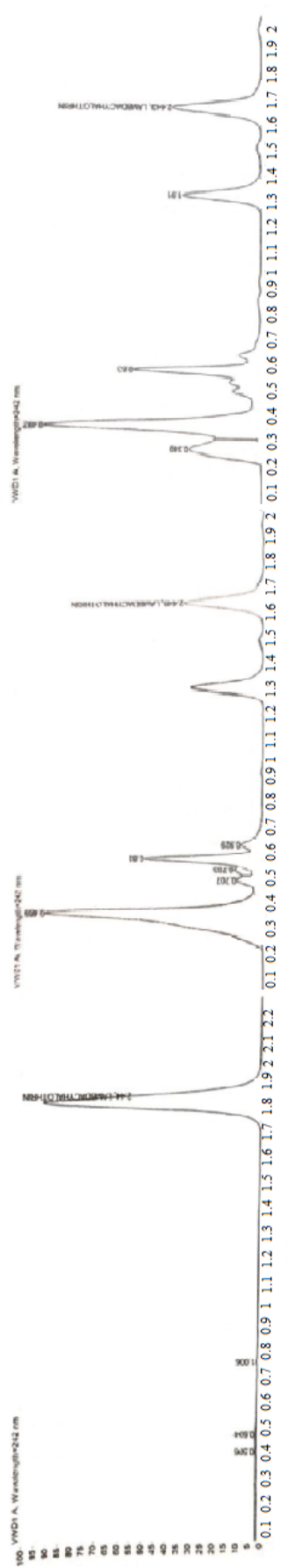


Figure 11. HPLC chromatograms of lambda-cyhalothrin for grapefruits. A: Grade standard of lambda-cyhalothrin; B: Grapefruits contaminated with lambda-cyhalothrin before ozonation; C: Grapefruits contaminated with lambda-cyhalothrin after ozonation.

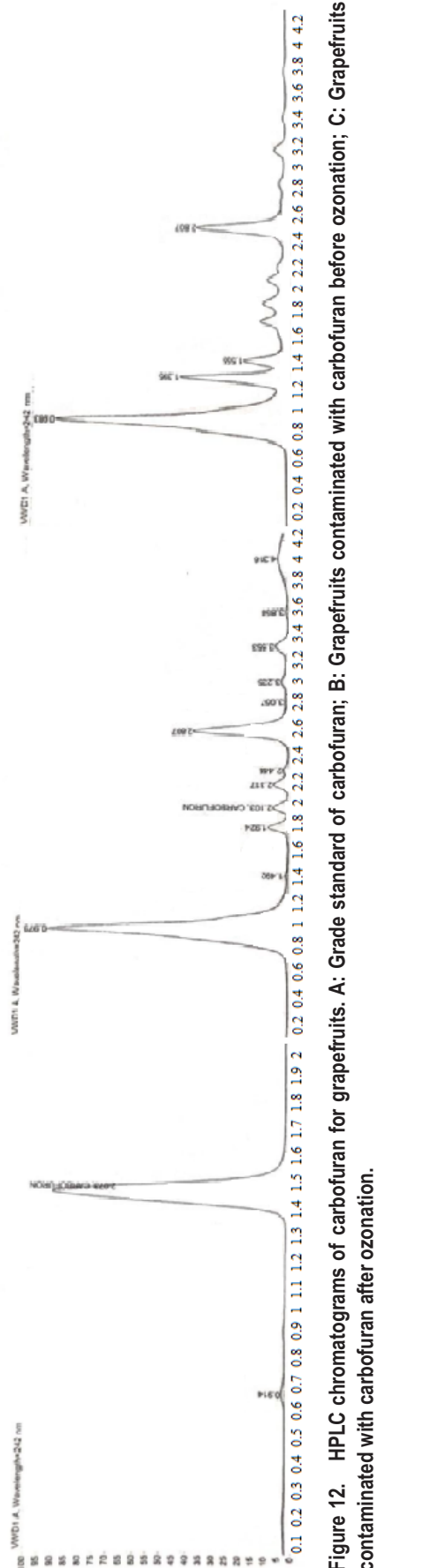


Figure 12. HPLC chromatograms of carbofuran for grapefruits. A: Grade standard of carbofuran; B: Grapefruits contaminated with carbofuran before ozonation; C: Grapefruits contaminated with carbofuran after ozonation.

and physicochemical properties (Dubey *et al.*, 2022). However, it has certain drawbacks in contrast to other techniques—it is less effective against organochlorines (as compared to washing saline solution) and can lead to fruit damage if optimal conditions are not ensured like exposure time, pH, and various types of fruits (Botondi *et al.*, 2021; Gonçalves-Magalhães *et al.*, 2025). For instant, it can decrease concentration of vitamin C and polyphenols and brings down the nutritional value of fruits and can be harmful on reacting with pesticides (Kaur *et al.*, 2022). Furthermore, active carbon filtration, thermal processing, and hydrogen peroxide or chlorine can degrade potential food pesticides residues, but at the same time can be impractical in encountering the concerns for food texture and chemical composition (Pandiselvam *et al.*, 2022; Zhang *et al.*, 2022).

Food safety regulations

Ozone treatment is usually considered safe by the US Food and Drug Administration (FDA) and approved as a food additive by the USDA for pesticide degradation. The European Union also permits ozone use for food processing with specification that it does not leave harmful residues.

Conclusion

Fruits and vegetables play an important part of daily diet for humans. Unfortunately, as these plants are not handled well, pesticide residues remain dominant in causing serious ailments and lifetime health concerns. Usage of pesticide and its detrimental effects largely affect crops and livestock, thus destroying the food production systems. The aim of this study was to remove trouble-causing pesticide residues from the citrus fruits. The results showed that maximum levels of pesticide residues were removed from citrus fruits at 10 ppm ozonation without any poisoning and worsening conditions of fruits. As a developing process, the quality of food products can be improved with the use of different ozonation methods when contaminated with any harmful agent. By protecting fruits from pesticides, it is possible to get rid of pesticide-induced chronic diseases such as cancer, Parkinson's disease, Alzheimer's disease, and other neurological ailments.

Competing Interests

The authors had no relevant financial or nonfinancial interests to disclose.

Author Contributions

All authors contributed equally to this article.

Conflicts of Interest

The authors declare no conflict of Interest.

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