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Effect of ozone, hydrogen peroxide, and chlorine solution in reduction of chlorpyrifos and cypermethrin residues from cauliflower

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ORIGINAL ARTICLE

Abstract

The extensive utilization of chlorpyrifos and cypermethrin in agriculture raises concerns regarding the accumulation of pesticide residues in human food, posing a threat to human health. This study aimed to investigate the effect of ozonation on cauliflower to decrease the level of pesticides. Cypermethrin and organophosphate chlorpyrifos were applied to cauliflower at specific doses. High-performance liquid chromatographic techniques were employed to detect pesticide residues in cauliflower. The effects of common oxidizers (hydrogen peroxide, chlorine, and ozone) on the reduction of pesticide residues were investigated. Solutions of hydrogen peroxide (10 and 100 ppm), chlorine (10 and 100 ppm), and ozone (10 and 100 ppm) were prepared, and the cauliflower sample was immersed for 20 min. The immersion in 10 ppm hydrogen peroxide solution reduced chlorpyrifos and cypermethrin by 68 and 65%, respectively. Meanwhile, at 100 ppm hydrogen peroxide solution, the reduction rates of chlorpyrifos and cypermethrin residues were 72 and 75%, respectively. Immersion in ozone solution at 10 ppm concentration reduced chlorpyrifos and cypermethrin by 58 and 57%, respectively. At 100 ppm ozone, chlorpyrifos was reduced up to 64%, and cypermethrin reduction was 74%. Chlorine immersion at 10 ppm reduced chlorpyrifos residues by 68% and cypermethrin residues by 81%, while a 100 ppm chlorine solution reduced chlorpyrifos residues by 100% and cypermethrin residues by 84%. From these results, it was concluded that chlorine was the most effective of all the cleaning solutions to remove pesticides from vegetables.

Keywords: extraction; health; pesticides; residues; vegetables

Introduction

Pakistan is an agrarian region blessed with plenteous natural occupancy like water irrigation, productive soil and a variety of climates varying from temperate to tropical. In Pakistan, food products encompassing legumes, cereals, fruits, oils seeds, and vegetables are cultivated to feed its rapidly growing population (Gul *et al.*, 2022).

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Moreover, the agriculture sector contributes 18.9% to Pakistan's GDP. Almost 40 different varieties of fruits and vegetables are encouraged by the agrochemical circumstances in Pakistan, and vegetables are a significant portion of their food. The main vegetables cultivated in Pakistan include onion, melon, potato, chili, cucumber, tomato, okra, brinjal, turnip, spinach, cauliflower, and peas. The poisonous chemicals utilized in demolishing, preventing, repulsing, or mitigating pests are termed pesticides (Wang *et al.,* 2018; Keeney and Cruse, 1998). In Pakistan, pesticides are widely used on vegetables, and farmers who employ pesticides on vegetables during the development time even after harvesting are unfamiliar with the technical information and agricultural techniques. Pesticide usage without following proposed doses and time results in its aggregation and assembly as residues in the internal portions of vegetables (Mazhar *et al.*, 2019). Consumers with high pesticide exposure are associated with acute diseases such as headaches, nausea, convulsions, diarrhea, eye irritation, breathing discomfort, and even death. Long-term contact with low doses of pesticides can cause chronic diseases such as cancer, Parkinson's disease, diabetes, congenital disorders and disorders of the reproductive, respiratory, and cardiovascular systems. Moreover, pesticide concentrations increase in the human body due to the biomagnification process, which severely impacts both the environment and human health (Zhou *et al.,* 202; Naveed *et al.,* 2024; Kaur *et al.,* 2024; Sharma *et al.*, 2020).

The name cauliflower is derived from two Latin words "caulis," which means cabbage, and "florist," which means flower. The edible portion of cauliflower known as curd is made up of abortive flowers with juicy, small, closely crowded stems. Cauliflower is probably native to the northern coastline of the Mediterranean region and Western Europe. The cultivation and development of cauliflower is believed to have started in the eastern Mediterranean, (Maggioni *et al*., 2018; Żyła *et al*., 2021) and it has been documented as being under gardening in Europe since the 15th century. It is now cultivated all over the world but only in relatively mild climatic conditions because the inflorescence production is inadequate above 25°C.

Pesticide residues in food have been reduced using a variety of ways (Rodrigues *et al*., 2020; Yigit and Velioglu, 2020; Zou *et al*., 2019). Washing processes such as water dipping and rubbing have been demonstrated to reduce pesticide contaminants when used to remove debris and filth. However, this method may be less successful in eliminating more persistent pesticide residues (Gavahian and Khaneghah, 2020; Qi *et al*., 2018). Consumers are concerned about pesticide residues because they are known to cause potentially detrimental impacts on human health, such as interference with the reproductive system and embryonic development as well as their ability to cause cancer and asthma. The pesticidal residues in or on the plants are inevitable even if they are employed by good agricultural practices (Hakme *et al*., 2020; Lehel *et al*., 2022). Processing or in-home practice stages such as rinsing, trimming, and cooking can lessen pesticide residues (Egemen and Ferda, 2021; Hassanzadeh *et al*., 2018).

Certain chemical compounds should be added to the water used for rinsing and cleaning to boost treatment. Acetic acid, chlorine, ozone, potassium permanganate, hydrogen peroxide, sodium chloride, and chlorine dioxide are the generally proposed compounds for residue elimination. Effective ways to lower the amounts of chlorpyrifos in water sources include physical techniques such as filtration and adsorption onto materials like activated carbon. Chemical degradation methods help break down chlorpyrifos molecules into less dangerous chemicals. Some of the examples of these methods are advanced oxidation processes (AOPs) that employ UV irradiation. Bioremediation, which uses specialized microorganisms that can metabolize chlorpyrifos, is one of the biological methods that offer environmentally benign pesticide removal solutions for soil and water settings (Ayilara *et al*., 2023). Pesticide residues in food samples are reduced by using chemical agents (Campayo *et al*., 2019; Tomer *et al*., 2019; Tongjai *et al*., 2021; Yigit and Velioglu, 2020; Zheng *et al*., 2019) such as hydrogen peroxide, chlorine, and ozone as these were considered as less harmful and viable options for more complex pesticide reduction methods. However, because of the interaction between pesticides and ozone in raw materials, ozonation may yield by-products.

It has been suggested that the by-products of some organophosphate pesticides may be more harmful than the insecticides themselves. Postharvest chlorine treatments have also been shown to be effective in reducing pesticide residues in apple fruits (Weber *et al*., 2016). Hydrogen peroxide treatment is considered to be the most effective in reducing pesticide residues and potent for the removal of microbes. Some findings report that hydrogen peroxide is a good alternative to chlorine. As an alternate postharvest treatment strategy, ozonated water dips have similar potential. Several reports indicated that pesticide actions are influenced by a variety of circumstances during the procedure of ozonation (Baghirzade *et al*., 2021). Some pesticides such as triazines (Aldeguer Esquerdo *et al*., 2020), organophosphorus pesticides (Tamaki and Ikeura, 2012), trifluralin, and aldrin can be effectively removed from water by ozonation. The effect of ozone mist on the inactivation of *Escherichia coli* from the surfaces has also been reported by Cabral *et al.* (2023). Two pesticides selected for this study were chlorpyrifos and cypermethrin.

Material and Method

Chemicals and equipment

High-performance liquid chromatography (HPLC) is performed by using an isocratic high-pressure liquid chromatography system and variable wavelength programmable UV/Visible detector. Data was analyzed by using PEAK software (Hogan *et al.*, 2018). The sample was weighed by using an electronic weighing balance. Acetonitrile, HPLC-grade ethyl acetate (200 mL), technical grade pesticide standards (chlorpyrifos, cypermethrin), anhydrous sodium sulphate for the analysis of residues, a pure nitrogen source in the extraction step for evaporation to dryness, chlorine, and hydrogen peroxide were used, and ozone was generated by an ozone generator.

Sample collection

Cauliflowers were collected from the fields of Multan (Bund Bosan, Sher Shah and Qadir Pur Rawan) and Muzaffargarh regions (Shah Jamal, Meher Pur and Khan Garh) to detect the pesticide residues. Three samples were collected randomly from each region. Cauliflowers were harvested using fixed-blade knives and packed in plastic bags. Dry ice was used to prevent the disintegration of pesticide residues. The samples were then brought to the laboratory of the Institute of Food Science and Nutrition, BZU Multan. The cauliflowers were then washed and stored at 4°C until processing.

Field trials

Chlorpyrifos and cypermethrin were applied to the cauliflowers, cultivated in the fields, at recommended doses and were collected when they reached maturity. 200 mL of each pesticide was mixed with 100 L of water and applied to the cauliflowers. During their growth period, pesticides were applied twice. In another field trial, no pesticides were applied, and this was used as the control. After harvesting, the cauliflowers were packed in plastic bags and transported to the laboratory of the Institute of Food Science and Nutrition, where they were stored at 4°C until experimentation. All tests were executed at 20°C room temperature. For agricultural uses, the recommended dosages of the widely used insecticide cypermethrin typically range from 5 to 20 g of active ingredient per hectare; while for domestic pest management, the concentrations in formulated solutions may vary between 0.1 and 0.25%. Chlorpyrifos, another pesticide, is frequently used in agriculture at the rates of 0.5 –2 kg of active ingredient per hectare, with formulations and concentrations designed to target pests and crops. Depending on the kind of food product, the FDA has set the maximum residual levels (MRLs) that are usually expressed in parts per million (ppm). For instance, the FDA has set the MRL for cypermethrin in various food items from 0.01 to 3.0 ppm, and that for chlorpyrifos that may vary from 0.01 to 0.1 ppm.

Dipping solution experiments

Six experiments were performed: 1) chlorine dipping at 10 mg/L; 2) chlorine immersion at 100 mg/L; 3) hydrogen peroxide immersion at 10 ppm; 4) hydrogen peroxide (H_2O_2) immersion at 100 mg/L; 5) ozone immersion at 10 ppm; and 6) ozone dipping at 100 mg/L for 20 min. After each experiment, the residues on the samples were compared with those of the control group. The temperature of the immersion solutions was 20 ± 02°C. After dipping, the surface of the vegetables was air dried by static air for 24 h at ambient temperature.

Preparation of cleaning solutions

Cleaning solutions were prepared in three different ways.

Ozone solution

Ozone is a strong-acting oxidizer that oxidizes pesticides and other chemicals from vegetables effectively. Ozone is a triatomic form of oxygen and unstable gas in deionized water. It has a half-life of 20–30 min after which it degrades to oxygen and leaves no residues in food. Thus, ozone is considered safe for use in human foods. Due to these characteristics, ozone is used to mitigate fungi and insects in products during storage and is utilized however in vegetables and fruits (Anjali *et al.*, 2022; Chuwa *et al.*, 2020). Ozone is generated by a generator. Aqueous solution of ozone is prepared by dissolving ozone in water. Two dosage solutions were prepared by exposing to ozone gas for 40–42 min: 1) 10 mg dissolved in 1 L (10 ppm) and 2) 100 mg dissolved in 1 L of (100 ppm) (Özen *et al.*, 2021).

Hydrogen peroxide solution

Hydrogen peroxide is highly effective in the disintegration of organophosphorus pesticide residues and less effective for organochlorine pesticides. Hydrogen peroxide in liquid form was used. Two solutions were prepared: 1) In the first solution, 0.1 mL of hydrogen peroxide was pipetted out and dissolved in 1 L of water. 2) The second solution was prepared by dissolving 0.01 mL of hydrogen peroxide in 1 L water. The study was conducted at room temperature (25°C) and pH 6 ± 0.2. The samples were dipped in 10 and 100 ppm hydrogen peroxide solutions for 10 and 20 min durations, respectively (Swami *et al.*, 2021; Rodrigues *et al.*, 2019; Cengiz *et al.*, 2014)

Chlorine solution

The most significant use of chlorine is water purification. Tap water with chlorine is used mostly for the postharvest cleaning of vegetables. (1) 10 mg of chlorine in granule form was dissolved in 1 L of water. The solution was shaken until chlorine dissolved completely in water. (2) 100 mg of chlorine was dissolved in 1L of distilled water.

Pesticide residue analysis

Extraction

Cauliflowers were collected randomly from the fields. Each vegetable sample (80 g) was chopped into pieces and homogenized by a kitchen blender, packed into polythene bags, and stored at a temperature below −15°C. The blended sample of cauliflower (80 g) was mixed with anhydrous sodium sulphate (50 g) in a conical flask and extracted with ethyl acetate (200 mL) for 4–5 min using an Ultra-Turrax (IKA-WERK). The ethyl acetate extract was then filtered through a Buchner funnel fitted with a filter paper covered by 20 g of anhydrous sodium sulphate after the content had settled for about half an hour. Following filtration, the extract was dried by evaporating and redissolving in 5 mL acetonitrile, by a rotary vacuum evaporator. The final volume was adjusted to 2 mL by adding acetonitrile drop by drop. The solution was then centrifuged and filtered. The clean organic layers were taken and analyzed in the NUCHEM (PVT) LTD laboratory using an HPLC with UV/Visible detector.

Results

The effectiveness of three cleaning solutions, ozone, hydrogen peroxide, and chlorine, was determined using HPLC. HPLC is used for quantitative and qualitative analyses of dissolved compounds in solvents. The most utilized pesticides were determined using HPLC with UV detection. 2.703 ppm of chlorpyrifos residues and 0.991 ppm of cypermethrin residues were detected. The samples were dipped in these cleaning solutions for 20 min. The samples were taken for analysis of pesticide residues after 20 min.

Table 2 demonstrates the efficacy of ozone based on their elimination rates for cypermethrin and chlorpyrifos in cauliflower. In the pesticides-loaded cauliflower sample, 2.703 ppm of chlorpyrifos was present. Following ozone treatment with 10 ppm ozone solution, 1.128 ppm chlorpyrifos was detected. Chlorpyrifos levels reduced from 2.703 to 0.967 ppm in a 100 ppm ozone solution. In the pesticide-loaded sample, cypermethrin residues were present at a concentration of 0.991 ppm. Cypermethrin residues were reduced to 0.256 ppm after ozone treatment in 10 ppm solution and to 0.423 ppm after ozone treatment in at 100 ppm solution. The removal rate of ozone for chlorpyrifos and cypermethrin was the same at the concentration of 10 mgL^{-1}. At 100 mgL^{-1}, the reduction rates for chlorpyrifos and cypermethrin were 64 and 74%, respectively. These results indicated that ozone was more effective at the rate of 100 ppm than 10 ppm.

Table 1. Chlorpyrifos and cypermethrin residues in cauliflower samples from different regions.

Vegetable	Sample collected (triplicate)	Collection area	Chlorpyrifos residues (ppm)	Cypermethrin residues (ppm)
Cauliflower	Sample 1	Bund Bosan	1.319	0.056
	Sample 2	Sher Shah	1.213	0.063
	Sample 3	Qadir Pur Rawan	1.323	0.196
	Sample 4	Shah Jamal	0.069	0.179
	Sample 5	Meher Pur	1.206	0.117
	Sample 6	Khan Garh	0.071	0.057

Table 2. Leftover and removed pesticide percentages after treatment by ozone, hydrogen peroxide, and chlorine.

Vegetable sample	Treatment chemical and dosage (ppm)	Chlorpyrifos (2.703 ppm)		Cypermethrin (0.991 ppm)	
		Leftover pesticide (ppm)	Removed percentage (%)	Leftover pesticide (ppm)	Removed percentage (%)
Cauliflower	Ozone (10)	1.128	58	0.423	57
	Ozone (100)	0.967	64	0.256	74
	Hydrogen peroxide (10)	0.870	68	0.349	65
	Hydrogen peroxide (100)	0.752	72	0.249	75
	Chlorine (10)	0.860	68	0.182	81
	Chlorine (100)	0.00	100	0.160	84

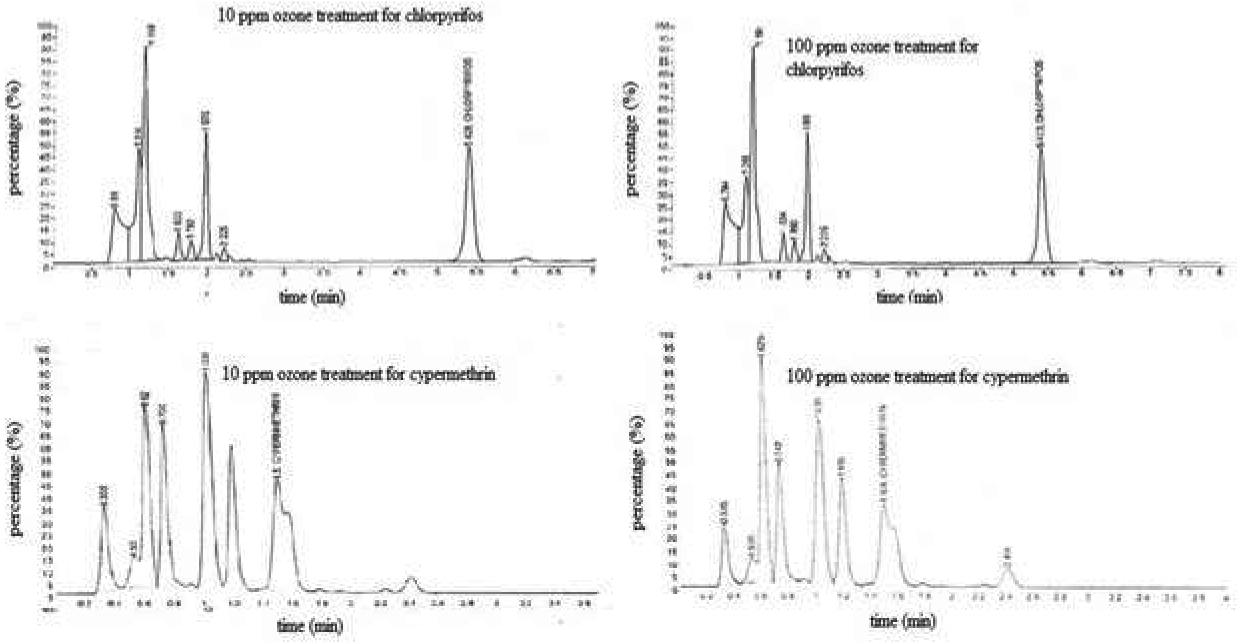

Figure 1. Ozone treatment for chlorpyrifos and cypermethrin residues.

The effectiveness of hydrogen peroxide is shown in Table 2. The hydrogen peroxide solution is a weak acid and can effectively remove the residues of chlorpyrifos and cypermethrin. When the acidity of a solution increases, its efficiency in removing pesticide residues reduces. In the pesticide-treated cauliflower sample, 2.703 ppm chlorpyrifos residues were present when analyzed without applying any treatment. When treated with hydrogen peroxide at 10 ppm solution, 0.870 ppm chlorpyrifos residues were left; but at a concentration of 100 ppm, chlorpyrifos residues reduced to 0.752 ppm. There was a significant increase in the reduction rate for pesticide remnants. The cypermethrin residues (0.991 ppm) were present in untreated cauliflower samples. When treated with 10 and 100 ppm hydrogen peroxide solution, 0.349 and 0.249 ppm cypermethrin residues were detected, respectively. At concentrations 10 and 100 mgL^{-1}, it removed 66 ± 02 and 73 ± 02% of chlorpyrifos and cypermethrin, respectively, from cauliflower samples. 100 mgL^{-1} hydrogen peroxide solution was found to be more effective than l0 mgL^{-1}.

As indicated in Table 2, chlorine was more effective than other cleaning solutions (ozone and hydrogen peroxide) when dipped in pesticide solution. When treated with chlorine at a concentration of 10 ppm, 2.703 ppm chlorpyrifos was detected in the cauliflower samples, and 0.860 ppm residues were present when analyzed after 20 min of dipping in chlorine solution. When dipped in chlorine solution at the concentration of 100 ppm, no chlorpyrifos residues were observed. This indicated that chlorine is the most effective cleaning solution (ozone, hydrogen peroxide). The cypermethrin residue levels before and after treatment with 10 ppm chlorine were 0.991 and 0.182 ppm, respectively. Treatment with a 100 ppm concentration solution detected 0.160 ppm cypermethrin residues. As shown in the table, chlorine solution removed 68% of chlorpyrifos and 81% of cypermethrin at the concentration of 10 mgL^{-1}, and 84% of cypermethrin and 100% of chlorpyrifos at 100 ppm.

The effectiveness of three cleaning methods in removing pesticides from cauliflower

The efficacy of three cleaning solutions was compared after the cleaning of cauliflower for the chlorpyrifos and cypermethrin residues. Following treatment with these cleaning methods, cauliflower was tested for pesticide residues. Chlorine eliminated up to 100% of chlorpyrifos residues. The results showed that among the three cleaning solutions, chlorine at 100 mgL^{-1} was the most effective, and ozone in an aqueous medium was the least effective.

Discussion

The results revealed that hydrogen peroxide, chlorine, and ozone are effective against the reduction of chlorpyrifos and cypermethrin residues in cauliflower. Cypermethrin is a pyrethroid insecticide that has been extensively used worldwide after the ban on organophosphate pesticides by EPA. Pesticides jeopardize the environment and are

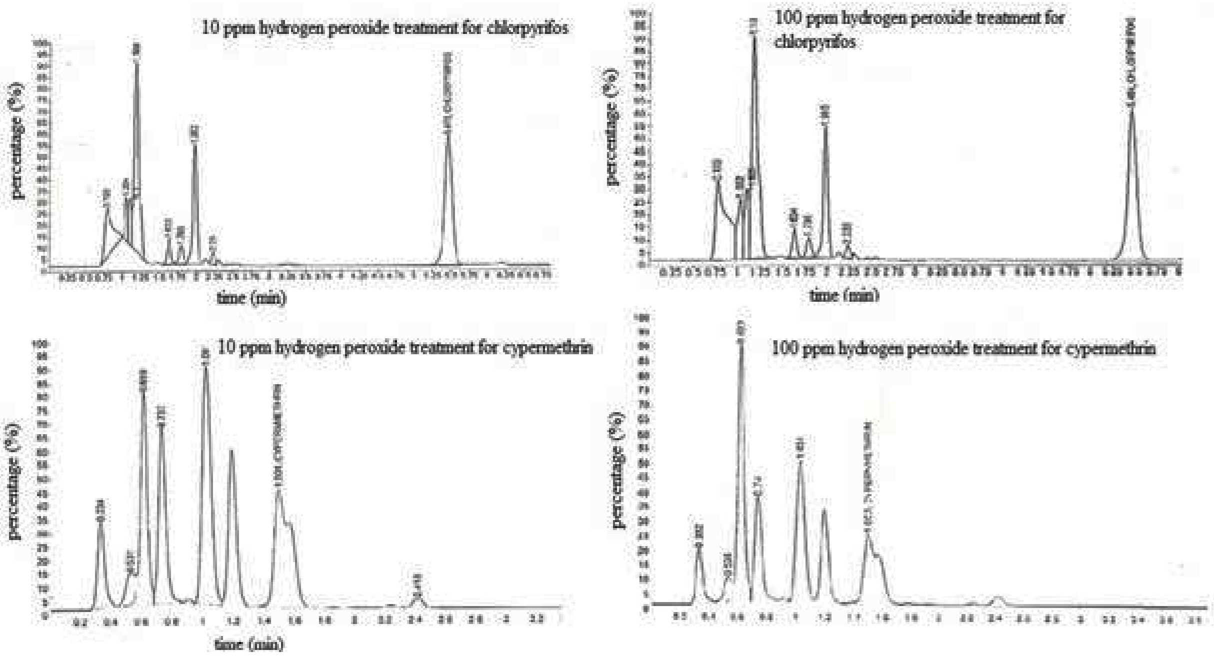

Figure 2. Hydrogen peroxide treatment for chlorpyrifos and cypermethrin residues.

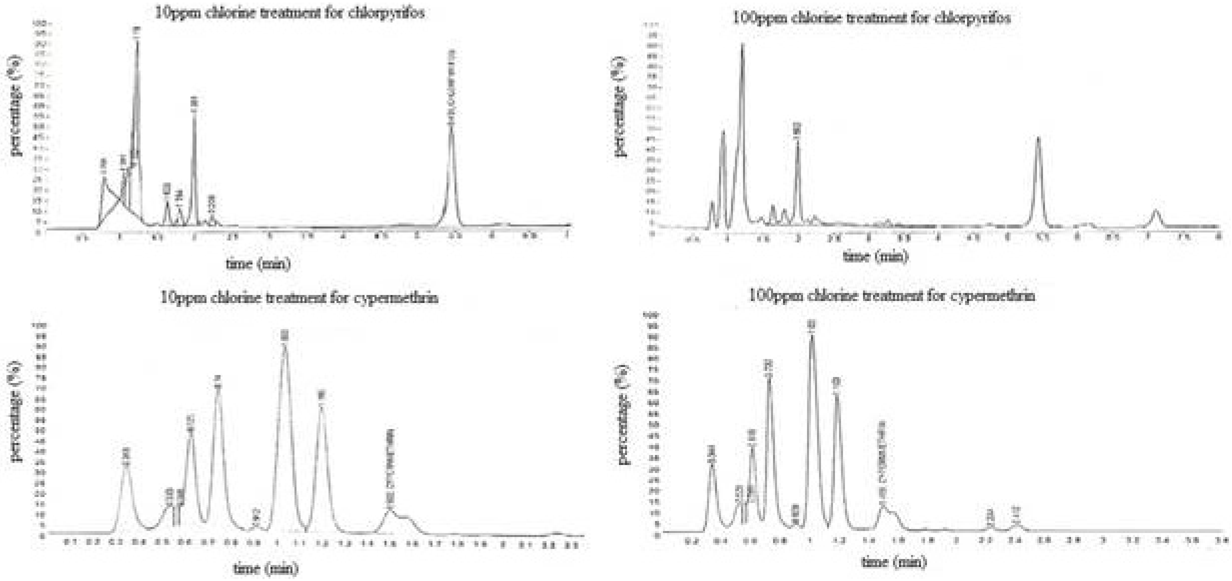

Figure 3. Chlorine treatment for chlorpyrifos and cypermethrin residues.

dangerous to human health. When pesticides are applied to agricultural commodities, some of their residues persist on the commodities after disintegration, which are considered toxic to humans; the elderly are more susceptible to these chemicals (Iqbal *et al.,* 2022). Because Pakistani farmers are no longer trained, they apply pesticides more than the recommended limits, resulting in a growing residual problem. These insecticides are minimized to reasonable ranges using washing processes. However, washing is not just enough for the effective removal of pesticide residues (Hussain *et al.,* 2022). The current study results revealed that 100 ppm of ozone, hydrogen peroxide, and chlorine gas showed a higher reduction of pesticide residues as compared to 10 ppm. Another research depicted those soaking cucumbers in tap water for 5 min reduced the residue levels of dichlorvos, trichlorfon, chlorpyrifos, fenitrothion, and dimethoate in or on cucumber samples by 14, 22, 53, 13, and 15%, respectively.

Satpathy *et al.* (2012) found that submerging tomato samples in water for 15 min reduced residues of methyl parathion, formathion, fenitrothion, malathion, chlorpyrifos, and parathion by 32, 27, 34, 41, 39, and 37%, respectively. This difference in pesticide reduction is due to the varied surface characteristics of food commodities. Such a difference between the reduction of pesticide residues depends upon five important factors: (1) pesticide particularization such as water solubility; (2) food particularization such as attributes of surface; (3) during the spraying of insecticides, the surrounding environment, that is, temperature and humidity; (4) environmental conditions during the chemical treatment, such as pH and temperature; (5) specifications of the reducing agent.

When subjected to oxidizing chemicals such as ozone, hydrogen peroxide, and chlorine, permethrin a synthetic pyrethroid that was frequently employed as an insecticide goes through several different reaction pathways. Cypermethrin goes through an ozone-mediated oxidation process in the presence of ozone, which breaks the carbon–carbon double bonds and produces a smaller, large number of polar molecules. Initiating oxidative processes with hydrogen peroxide may result in the production of hydroxyl radicals that target the cypermethrin molecule and disassembles it into less hazardous, simpler compounds. Comparably, cypermethrin and chlorine may interact through chlorination processes, which changes the chemical structure of the cypermethrin molecule and perhaps lessen its insecticidal efficacy by replacing hydrogen with chlorine atoms (Cengiz *et al.,* 2014)

An organophosphate pesticide called chlorpyrifos is susceptible to several reaction processes from oxidizing chemicals such as hydrogen peroxide, ozone, and chlorine. Chlorpyrifos is susceptible to ozone-mediated oxidation, which breaks down its functional groups that contain phosphorus and forms a variety of oxidation products. Hydrogen peroxide can start oxidative reactions, which may lead to the production of reactive oxygen species that target and break down chlorpyrifos into less hazardous, simpler molecules. Through chlorination processes, chlorine can react with chlorpyrifos to replace hydrogen atoms in the molecule, changing its chemical structure and perhaps decreasing its insecticidal effectiveness (Swami *et al.,* 2018).

Due to the lack of technical guidance on the use of pesticides, most of the farmers use toxic pesticides on vegetables that persist in residues above MRL even after washing and other processing techniques. The finding by Macha (2022) supported these results, who reported that farmers apply these pesticides 2–3 times a week and supply the produce to the market one day after spraying, with adulteration beyond their MRLs. Some farmers apply pesticides just before harvesting. The withholding times are the shortest periods between spraying an agrochemical and harvesting, which provide adequate times to reach an appropriate level (Wang *et al*., 2019). According to Ahmed *et al.* (2016), the removal of residue in and on plants is caused by a combination of environmental elements such as wind, rain, sun, humidity, and temperature, as well as chemical and physical parameters such as volatilization and plant growth (Velioglu *et al*., 2018). The pesticide (captan and formetanate hydrochloride) residues were eliminated from apple sauces and fresh apples by 50–100% by water containing ozone. According to Sujayasree *et al*. (2022), patulin, a food contaminant prevalent in apples and pear juices, was eliminated by ozone. Ozone is a strong oxidant that effectively removes the pesticide residues from food commodities.

Ozone is safer to use in food products and effective for the first 5–15 min after which it decomposes into oxygen molecules. According to this study, ozonated water at 10 ppm for 15 min reduced the chlorpyrifos and cypermethrin residues by 57 ± 1% in cauliflower. Our findings were in line with those that discovered that utilizing a diffused ozone solution of 2.0 ppm for 30 min eliminated 61% of cypermethrin, 47% of methyl parathion, 53% of diazinon, and 55% of parathion from Brassicaceous plants. Hydrogen peroxide at 100 ppm for 20 min reduced 72% of chlorpyrifos and 75% of cypermethrin residues in cauliflower. It has also been mentioned that pesticides are sprayed on cauliflower up to 17 times on average and 15 times during the growth cycle, making it necessary to oversee pesticide usage worldwide to assess the environmental load of pesticide residues that make their way into the human body via food. As a result, pesticide residue assessment in food and their removal through traditional processing has become a requirement (Barbosa *et al*., 2020).

Conclusion

Pakistan is an agriculture-based country, and its economy depends on good agricultural practices. With the increase in the population and advances in technology, the usage of chemicals or pesticides increased for disease prevention in vegetables as well as higher food production to fulfil population needs. These pesticides remained in food and resulted in health challenges. To address this concern, employing the ozonation process could be effective for the removal of pesticides from vegetables.

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Ethical Approval

Not applicable.

Competing Interests

Financial interests: The authors declare no financial interests.

Availability of Data and Materials

The data and material are available.

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