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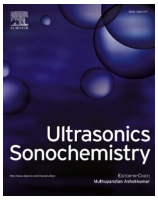

Effect of ultrasound assisted cleaning on pesticide removal and quality characteristics of *Vitis vinifera* leaves

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ABSTRACT

In this study, the pesticide (acetamiprid, deltamethrin, and pyridaben) removal and physicochemical quality improvement of vine (*Vitis vinifera*) leaf were examined using ultrasonic and traditional cleaning for 5, 10, and 15 min. After an ultrasonic cleaning procedure at 37 kHz for 10 min, acetamiprid, deltamethrin, and pyridaben in vine leaf were reduced by 54.76, 58.22, and 54.55 %, respectively. Furthermore, the total phenolic content (TPC) in vine leaf increased to 13.45 mg GAE/g DW compared to that in control samples using traditional cleaning (10.37 mg GAE/g DW), but there were no significant differences in DPPH radical scavenging activity. After 15 min of conventional cleaning, the total chlorophyll and total carotenoid content of leaves were found to be lowest among all samples, at 6.52 mg/kg and 0.48 mg/kg, respectively. In conclusion, when compared to conventional cleaning methods, ultrasonic cleaning with no chemicals or heat treatment has proven to be a successful and environmentally friendly application in reducing commonly used pesticides and improving the physicochemical qualities of leaves.

1. Introduction

Vitis vinifera L. is among the most significant agricultural products grown in the Mediterranean regionin terms of the production of both wine and table grapes [1]. *Vitis vinifera* (grapevine) leaves, like grape skins and seeds, are considered healthy foods with homeostatic, spasmolytic, and astringent propertiesas well as antioxidant, antifungal, antimicrobial, anti-inflammatory, and anti-carcinogenic effectsbecause of their high phenol and flavonoid content[2,3]. In previous studies, infusions of vine leaves were used to cure stomach problems, hepatitis, haemorrhage problems, and diarrhea, while plant extract formulations were utilized to treat abscesses and wounds [4]. *V. vinifera* leaves are produced into dietary components and antioxidant supplements, and are consumed as food, particularly in Mediterranean cuisine, going by the names “yaprak dolma (sarma)” in Turkey, “dolmehbargmo” in Iran, and “warakenab” in Egypt and Lebanon[5,6]. Several organic acids, phenolic acids, flavonols, tannins, procyanidins, anthocyanins, lipids, enzymes, vitamins, carotenoids, terpenes, and reducing or non-reducing sugars have been found in leaves of *V.vinifera*[1,4,7]. Most of the plant’s medicinal qualities are related to phenolic chemicals with antioxidant activity. These discoveries have attracted attention in *V. vinifera* leaves as a source of nutrients and bioactive substances. It also eliminates

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disposal issues from wine and juice residues[5]. Like other crops, it might be harmed by pests or diseases during cultivation or manufacturing, which would result in losses that would reduce its impact and quality[6].

A variety of pesticides are typically sprayed on vineyards at various phases of production and during post-harvest storage to prevent vine crop degradation and control pests and plant infections. Because grapevine is susceptible to a variety of pests and illnesses, a rigorous pesticide program is frequently required to reach production goals[8]. Pesticides, which are used to protect plants from diseases and pests, have been demonstrated in several scientific studies to be harmful to human and environmental health, particularly cancer. More than 300,000 people die from pesticide poisoning every year.Rather than occupational or accidental exposure, predominantly cutaneous or inhalation, self-poisoning by ingestion is the leading cause of death[6,7]. Pesticide residues (PR) in food can be detrimental to people, depending on how and how much they are exposed to them [9]. Direct intake of pesticides from fresh foods is the most common route of pesticide exposure [10,11]. To manage the legitimate use of pesticides, international organizations establish standards for pesticide residual amounts that are permitted by law in food items when they enter the market. These pesticide levels areknown as ''Maximum Residue Levels'' (MRLs)[4]. Items sent out for export may not be accepted and may be sent back at the border because residues are sometimes found in herbal products, which are important in both domestic and international trade, at a rate higher than what is acceptable in international trade[12]. Previous research has revealed that the majority of *Vitis vinifera* leaf samples from nearby farms contain pesticide residues that exceed MRLs, indicating that farmers may not be following good agricultural practices (GAP), which could endanger consumers' health[6].

Since consumers are becoming more sensitive to the presence of pesticide residue in fresh food and agricultural commodities and sincepesticides must be applied to crops within particular dose limits and laws, numerous pesticide removal technologies to eliminate pesticide residue from fresh agricultural products have been investigated in recent decades [13–15]. Pesticides can be eliminated by a variety of methods, but processes to get them out of aqueous solutions emphasis on sorption, coagulation, membrane processes, degradation by sophisticated oxidation techniques, and biodegradation[16]. Surfactants, ozone (O_3), ionic solvents, chlorine treatment and hydrostatic pressure have all been employed to reduce pesticide residue[17,18]. None of these methods, however, has been shown to be effective in eradicating PR from the environment. In addition, chemical washing and high-temperature processing have a number of detrimental effects on agricultural products, including nutritional loss, color retention, and flavonoid loss[11]. As a result, more effective, long-term, and ecologically acceptable pest and chemical removal strategies are urgently required.

Ultrasonication (US) is one of the most popular non-thermal methods used in "green" chemistry, where sound waves of varying frequencies are used to accomplish specific goals. In this context, "ultrasound" refers to the kinetic energy produced by sound waves with frequencies beyond the range of human hearing (20 kHz to 20 MHz range)[19,20]. The innovative use of ultrasound in food processing has been demonstrated to be an effective method to extend shelf-life, reduce the microbiological load, increase extraction yield, clean pesticide, and raise drying rate with improved quality and nutrition retention, improved emulsification, crystallization, filtration, faster thawing, and many other benefits [19,21].Ultrasound-assisted cleaning (UAC) is a pesticide removal technology that is safer for the environment and more effective than previous approaches. It's also a cleaning method that's both time and energy-efficient[13,22,23]. Pesticide removal with ultrasound has been tested in a number of fruits and vegetables, including grapes, cabbage, carrots, tomatoes, and cucumbers [11,24–26], however no study has been conducted to evaluate ultrasonic cleaning of vine leaves. Therefore, the goal of this research was to determine the effect of ultrasound assisted cleaning (UC) on pesticide residues and quality parameters of *V. vinifera* leaves and compare the results to those ofconventional cleaning (CC).

2. Materials and methods

2.1. Chemical and samples

Samples of vine leaves were collected at a vineyard in Izmir, Turkey. Until they were subjected to analysis, samples were kept at ambient temperature (23 ± 2 °C). The Folin-Ciocalteu reagent and gallic acid standard were obtained from Merck (Darmstadt, Germany). The pesticide standards, DPPH, and solvents such as acetonitrile, acetone, and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). In this work, all reagents and solvents used were ofanalytical grade.

Acetamiprid, deltamethrin, and pyridaben were purchased in powder form. In a properly ventilated fume hood, 100 mg/L pesticide stock solutions were madeusing ultrapure water. Commercial insecticides and fungicides for dipping solutions were prepared according to their prospectuses and allowable legal limits, as well as the doses recommended by agronomists and pharmaceutical representatives. The pesticide solution immersion method was adopted because the amount of pesticide residue in the product varies with spray application. The concentrations of pesticides in the dipping stock solution were 24 %, 56 %, and 20 %, respectively, for acetamiprin, deltamethrin, and pyridaben. The leaf samples were bought from a local farmer and kept at 4 °C in a refrigerator prior to treatment. The size and kind of all the samples were theapproximately the same. Analysis of residues revealed the existence of pesticide residues in untreated leaf samples.

2.2. Pesticide-contaminated sample preparation

In a 2 L container, a 135 mL dipping solution containing pesticide active ingredients was added. To absorb the pesticide components, 100 g of leaf samples were dipped in the pesticide dipping solution for 30 min. Pesticide-contaminated leaf samples were taken out of the container after 30 min of soaking and allowed to air dry at room temperature on filter paper.

2.3. Conventional and ultrasound assisted cleaning conditions

According to previous studies, foods like lettuce, spinach, tomatoes, and strawberries were better decontaminated from pesticides by low frequencies between 20 kHz and 45 kHz, probably because lower frequencies resulted in larger bubbles and greater energy release in those sonicated for up to 20 min[14,22,23]. Therefore, in this study, an ultrasonic bath with a 37 kHz frequency was chosen for ultrasound-assisted cleaning. First, leaves (15 g/replicate) were put in a250 mL beaker, and 135 mL of distilled water wasadded (1:9, leaves: water), and then the beaker was submerged in an Elmasonic S50 R model ultrasonic bath (Germany, capacity: 5L, frequency:37 kHz, power:150 W). In all treatments, the acoustic intensities of the ultrasonic bathwere constant at 100 %.The initial temperature of ultrasonic bath was 24 ± 1 °C. During ultrasound treatment, the process temperature did not rise above 40 °C.Similar to ultrasound assisted cleaning, in conventional cleaning, leaves (15 g/replicate) were put in a 250 mL beaker and 135 mL of distilled water were added (1:9, leaves:water). They were left in distilled water for 5,10 and 15 minat ambient temperature (24 ± 1 °C). After the cleaning was completed, the samples were dried with paper towels to remove excess waterfrom surface. All treatments were conducted in triplicate. The cleaning procedures are presented in Fig. 1.

2.4. pH and moisture content

The emulsion of vine leaves was produced by blending 10 g of sample with 50 mL of distilled water using a blender (Fakir Arms Trex Dual 500 W, Staufen, Germany) [28]. The pH of the leaf emulsions was

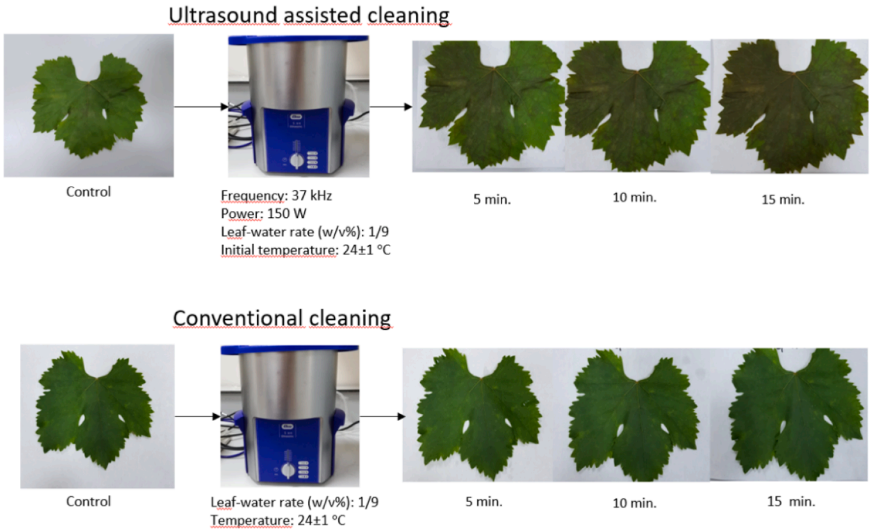

Fig. 1. Schematic view of ultrasonic and conventional cleaning.

measuredusing a table type digital pH meter (Ohaus, Model ST2100-F, Parsippany, NJ). The method of oven drying was employed to calculate the moisture content. This technique involved homogenizing and drying pesticide-contaminated leaves at 120 °C to a consistent weight [29].

2.5. *Color parameters*

Using a colorimeter (Konica Minolta CR 300, VA), color parameters of leaves including L*, a*, and b* were assessed in the CIE-LAB system. A black and whitesurface was used to calibrate the colorimeter. Three replicate readings were performed for each sample. The data was presented in the form of a meanand standard deviation[30].Total color variance (E) values were obtained by equation 1 to show the color variations between the control sample and the samples after cleaning procedures[31].

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^{*2})]^{1/2} \quad (1).$$

where L*, a*, and b* represent the differences in these values between the control sample and the samples after cleaning.

2.6. *Total phenolic content (TPC)*

TPC was calculated using the Folin-Ciocalteu assay [3]. To make a plant extract solution containing 1 mg/mL, 10 mg of dried extract were dissolved in 10 mL of 50 % ethanol.The directions were followed in the preparation of the reagents. The standard curve was createdusing gallic acid, and 0.02 mL of plant extract solution was employed in a microplate reader (Thermo Scientific Multiskan Go Microplate Spectrophotometer, Model 1510, Vantaa, Finland). Then, 0.08 mL of 7.5 percent Na_2CO_3 and 0.1 mL of a 1:10 solution of Folin-Ciocalteu reagent were added. The samples were held in the dark for 30 min at room temperature (23 ± 2 °C). After incubation, the absorbances were read at 765 nm.

2.7. *Total antioxidant (DPPH) capacity*

According to a documented method, the DPPH (2,2-diphenyl-1-picrylhydrazyl) analysis was employed to assess the antioxidant activity of samples [32]. In 50 % ethanol, the extract concentration was 1 mg/mL. The analysis was carried out in accordance with the protocol in the microplate reader. 10 μL of extract were put into each well, followed by 90 μL of distilled water. 100 μL of DPPH solution were added to the plates, which were incubated at room temperature (23 ± 2 °C) in the dark for 30 min. To create a blank solution, the same procedure was employed, but 50 % ethanol was substituted for extract. The absorbance samples were measured at 517 nm, and DPPH activity was calculated as a percentage of inhibition.

2.8. *Total carotenoid and chlorophylls*

The measurements of carotenoid and chlorophyll pigments were performed according to [33]. Briefly, 0.1 g of homogenized plant material was put in a 50 mL flask and mixed with 15 mL of 80 % acetone. The flask was cleaned using an ultrasonic cleaner for five minutes. The extract was placed in a tube and centrifuged for ten minutes at a speed of 10,000 rpm. The absorbance of the samples was calculated in comparison to a blank sample (80 % acetone) at different nanometers, such as 441, 646, 652, and 663, respectively, for total carotenoids, chlorophyll *b*, total chlorophyll, and chlorophyll *a*. The following equations were used to determine the amounts of chlorophyll and carotenoid pigments in samples:

$$\text{Chlorophyll } a\ (\mu g/g\ FW) = (12.21 \times E663 - 2.81 \times E646) \times (V/1000 \times m) \quad (2).$$

$$\text{Chlorophyll } b\ (\mu g/g\ FW) = (20.13 \times E646 - 5.03 \times E663) \times (V/1000 \times m) \quad (3).$$

$$\text{Total chlorophyll } (\mu g/g\ FW) = (27.8 \times E652) \times (V/1000 \times m) \quad (4).$$

$$\text{Total carotenoids } (\mu g/g\ FW) = [(1000 \times E441) - 3.27 \times (12.21 \times E663 - 2.81 \times E646) - 104 \times (20.13 \times E646 - 5.03 \times E663)\ ] \times [V / 1000 \times (m \times 229)] \quad (5).$$

where, E is the absorbance at a specific wavelength and V is the volume of a measuring flask.

2.9. *Determination of pesticide residues*

The LC-MS/MS method was used to measure pesticide residues[4]. The liquid chromatography equipment with tandem mass spectrometer employed was an Agilent Technologies 1200 Infinity class liquid chromatography system. For chromatographic separation, a Synergia (Phenomenex, Torrance, CA, USA) reverse-phase analytical C18 column with a guard-column of 150 × 2 mm and 2.5 m particle size was utilized. In each sample, the mobile phase was a water-methanol solution containing 5 mM ammonium acetate. The gradient program ran like this: Over the course of 12 min, 2 % B climbed to 100 % B, stayed at 100 % B until 20 min, and then decreased to 2 % B at 25.01 min. At a flow rate of 0.4 mL min^{-1}, the overall run time was 30 min.The injection volume was 5 μL and the column was held at 25 °C.

The LC was connected to an AB SCIEX 3200 QTRAP Triple Quadrupole Mass Spectrometer in positive ion mode, with an electrospray chemical ionization (ESI) source. The data was gathered using the multiple reaction monitoring (MRM) method. The ion spray voltage was set at 5 kV, while the source temperature was set to 500 °C.

The degradation of pesticides was determined bythefollowing equation:

$$\text{Degradation (\%)} = (C_0 - C_t) / C_0 \text{ X } 100 \quad (6)$$

Where: $C_0$is the concentration of the pesticide residues at the beginning of a test and C_t is the concentration of the pesticide residues at time t.

2.10. *Statistical analysis*

One-way analysis of variance (SAS 9.2, SAS Institute Inc., Cary, NC, USA) was used to figure out the difference between the means of several parameters.Effects were significant when $p \leq 0.05$. Tukey's HSD test was used for the post-hoc process[34].

3. Results and discussions

3.1. *Effect of ultrasonic cleaning on pH and moisture content of vine leaves*

The pH of fruits, vegetables, and green leaves is an important quality parameter that is related to microbial spoilage. The majority of spoilage microorganisms, particularly bacteria, prefer a pH near 4.5. Fungi are much less sensitive to pH and can grow in a wide range, most commonly 3–8, demonstrating much better acid tolerance [35]. In general, the pH of fruits and green leaves is low, and fungi are more commonly responsible for spoilage than bacterial strains. As the pH increased, the leaf became more susceptible to spoilage [36]. It was determined that different cleaning methods and times did not cause a significant change in the pH value of the vine leaves, and the pH value of the pesticide control samples was 4.23, resulting in an extremely low decrease as a result of both conventional cleaning and ultrasonic cleaning.

Moisture content and dry matter varied from 74.82 % to 82.22 % and 17.78 % to 25.18 % for the vine leaves, respectively (Table 1). Considering the changes on the quality, the dry matter amount in the raw vine leaf obtained by using the oven was 74.82 %. After 5 min of conventionalcleaning, the moisture content increased by 0.93 % and reached 75.52 %. The highest increase in moisture content was recorded in samples exposed to ultrasound for 5 min, which increased by 8.87 % and reached the highest value of 82.22 %.

Table 1
Effect of ultrasound assisted cleaning on pH, moisture content, dry matter, phenolic content and antioxidant activity of vine leaves.

Samples	pH	Moisture Content (%)	Dry Matter (%)	TPC (mg/ g DW)	DPPH (%)
Control	4.23 ± 0.05^{a}	74.82 ± 0.11^{d}	25.18 ± 0.11^{a}	10.37 ± 0.75^{c}	73.16 ± 2.13^{a}
CC 5 min	4.16 ± 0.04^{a}	75.52 ± 0.91^{cd}	24.48 ± 0.91^{ab}	10.62 ± 0.53^{c}	64.21 ± 1.54^{b}
CC 10 min	4.10 ± 0.06^{a}	77.70 ± 0.09^{bcd}	22.30 ± 0.09^{abc}	8.72 ± 1.46^{cd}	53.28 ± 2.75^{c}
CC 15 min	4.11 ± 0.03^{a}	81.43 ± 0.58^{ab}	18.57 ± 0.58^{cd}	9.89 ± 0.31^{b}	47.24 ± 3.01^{d}
UC 5 min	4.01 ± 0.08^{a}	82.22 ± 1.76^{a}	17.78 ± 1.76^{d}	12.84 ± 2.61^{ab}	72.10 ± 1.98^{a}
UC 10 min	4.12 ± 0.09^{a}	78.95 ± 1.48^{abc}	21.05 ± 1.48^{bcd}	13.45 ± 1.54^{a}	69.63 ± 2.09^{ab}
UC 15 min	4.13 ± 0.01^{a}	78.61 ± 0.22^{abcd}	21.39 ± 0.22^{abcd}	8.22 ± 1.51^{d}	70.66 ± 3.11^{a}

Meanvalues ± SD of three independent measurement. The values in each column bearing the same superscriptare not significantly different ($p < 0.05$).

3.2. *Effect of ultrasonic cleaning on color parameters of vine leaves*

Color is another significant quality characteristic of both raw and processed fruits and vegetables. In addition to antioxidant activity, pigments are responsible for color, which is one of the factors that affect consumer preferences and is regarded as a quality parameter [37]. Natural pigments' structural properties are frequently altered during food processing, changing the color of the final product. Although chlorophyll degradation is undesirable in many fruits and vegetables, it is critical in pickled cucumbers, olives, okra, and vine leaves that chlorophylls be converted into pheophytins and pheophorbides. These products are preferred by customers to be olive yellow in color [38].The change in color parameters was illustrated in Table 2. Although nostatistically significant difference was observed in theL* values of thesamples, the biggest total color change wasfound in the samples that were washed ultrasonically for 10 min (ΔE = 6.39). After interacting with plant material, the cavitational impact of ultrasonic waves enhances the release of extractable chemicals and increases mass transfer by destroying plant cell walls. Ultrasound waves also change the physical and chemical properties of the material[39]. The bigger ΔE observations could be explained by the fact that the cavitation phenomena might facilitate the mass transfer of pigments and other impurities from the leaves to the water. The a* value represents a color's red-green component, with a* (positive) and a* (negative) representing red and green values, respectively [40]. Thehighesta* value (-3.94) was determined at samples exposed to 15 min of ultrasound, and it was significantly different from all other a* values of leaves.

3.3. *Effect of ultrasonic cleaning on total phenolic content of vine leaves*

According to the total phenolic content determination, the TPC value in the raw vine leaves was determined as 10.37 mg GAE/gDW. Güler and Candemir (2014), who conducted research on five leaf samples from Turkey's Manisa region, discovered similar levels to what we obtained in our study, ranging from 9.72 to 14.25 mg GAE/g DW [41]. The greatest loss occurred after 15 min of ultrasonic cleaning, with the TPC value dropping to 8.22 mg GAE/g DW. The most significant rise was seen after 10 min of ultrasonic cleaning, with the TPC value reaching 13.45 mg GAE/g DW in these samples. It was discovered that using ultrasound for up to 10 min increased the TPC value in the ultrasound-treated samples, while using it for 15 min caused the phenolic compound to be identified below that of the control sample. As the ultrasound treatment time in vine leaves was increased from 10 to 15 min, the total phenol content declined. The oxygen, whichacts as acofactor in numerous enzymatic reactions and as acatalyst for non-enzymatic oxidations, can be used to explain this result. Phenols, which are potential

Table 2
Effect of ultrasound assisted cleaning on color properties of vine leaves.

Samples	L*	a*	b*	ΔL
Control	36.88 ± 2.35^{a}	-7.55 ± 0.31^{c}	17.19 ± 1.05^{ab}	–
CC 5 min	38.03 ± 2.96^{a}	-7.94 ± 0.28^{c}	17.86 ± 0.91^{a}	1.39
CC 10 min	36.16 ± 1.66^{a}	-7.58 ± 0.44^{c}	16.69 ± 0.95^{ab}	0.86
CC 15 min	38.22 ± 2.27^{a}	-7.83 ± 0.26^{c}	17.39 ± 0.96^{ab}	1.42
UC 5 min	35.65 ± 2.75^{a}	-5.53 ± 0.28^{b}	16.02 ± 0.88^{ab}	2.64
UC 10 min	31.69 ± 1.58^{a}	-5.17 ± 0.27^{b}	14.32 ± 1.23^{b}	6.39
UC 15 min	33.38 ± 2.50^{a}	-3.94 ± 0.35^{a}	14.65 ± 2.24^{ab}	5.63

Mean values ± SD of three independent measurement. Thevalues in each column bearing the same superscript are not significantly different ($p < 0.05$).

substrates for the oxidizing enzymes polyphenol oxidase and peroxidase, were released when the leaves were immersed in ultrasound.And they were reduced by the extension of ultrasonic treatment[29]. In addition, it has been noted in earlier studies that prolonged exposure to ultrasonic waves can cause excessive cavitation and cell disruption in foods, which can lead to the degradation of polyphenols[42]. It is thought that the decrease in TPC in the samples that were exposed to ultrasound for 15 min was caused by the increased cavitation and warming caused by the ultrasound. This caused a greater amount of phenolic substances to be transported to the water through the microchannels formed in the leaves.

3.4. Effect of ultrasonic cleaning on antioxidant activity of vine leaves

The total amount of antioxidant substance was determined according to theDPPH free radicals shown in Table 1. The DDPH value in raw grape leaves was found to be 73.16 %. It was observed that the ultrasonic cleaning method and duration did not have a significant effect on the antioxidant activities of the samples; however, theDPPH values of conventionally washed leaves were substantially lower than those in the control sample. The interval with the highest DPPH value wasmeasured 72.10 % in 5 min of ultrasoniccleaning.Significant differences were observed in antioxidant activity among all conventionally cleaned samples (p greater than 0.05). The15-minute conventional cleaning was found to have the greatest reduction in DPPH value, with 47.24 %.In this study, it was found that a high amount of total polyphenols is not always linked to a lot of antioxidant activity.

3.5. Effect of ultrasonic cleaning on thecarotenoid and chlorophyll content of vine leaves

Total carotenoids, total chlorophyll, chlorophyll *a*, chlorophyll *b*, and the ratio between chlorophyll *a* and b were illustrated in Table 4. When the chlorophyll and carotenoid analyses were examined, one of the results obtained was that the chlorophyll *a*/b ratio in vine leaves was approximately 4/1. The total amounts of chlorophyll in the vine leaves, which were subjected to conventional cleaning for 5, 10, and 15 min, were determined to be 10.66, 10.24, and 6.52 mg/kg, respectively (Table3). In ultrasonic cleaning, the highest loss was obtained with 5.69 mg/kg in 15 min of application. When high-carotenoid vegetables, including carrots, are subjected to air and high temperatures, the high degree of unsaturation in their structure causes color degradation, making them vulnerable to oxidation[43]. The initial total amount of carotenoids was 2.18 mg/kg. The leaves that were subjected to ultrasound for 15 min had the lowest carotenoid concentration among theultrasound treated samples due to the water temperature reaching 35 °C. The carotenoid value decreased to 0.73 mg/kg in the vine leaves, which were subjected to cleaning for 5 min, and this value decreased to 0.48 mg/kg, showing the maximum decrease at the end of conventional 15 min cleaning.The contents of chlorophyll *a* and chlorophyll *b* in the vine leaves exposed to 15 min of conventional and ultrasonic cleaning were significantly lower than control samples ($p < 0.05$) pigment levelsdecreased most likely due to degradation of the tissues.

Table 3
Effect of ultrasound assisted cleaning on pesticide residues of vine leaves.

Samples	Acetamiprid (mg/kg)	Deltamethrin (mg/kg)	Pyridaben (mg/kg)
Control	0.042 ± 0.030^{a}	187.71 ± 86.804^{a}	0.033 ± 0.023^{a}
CC 5 min	0.038 ± 0.011^{a}	123.05 ± 59.602^{a}	0.021 ± 0.022^{a}
CC 10 min	0.020 ± 0.011^{a}	79.90 ± 39.598^{a}	0.015 ± 0.012^{a}
CC 15 min	0.026 ± 0.011^{a}	80.91 ± 41.033^{a}	0.018 ± 0.012^{a}
UC 5 min	0.029 ± 0.013^{a}	92.82 ± 46.775^{a}	0.029 ± 0.013^{a}
UC 10 min	0.019 ± 0.010^{a}	96.34 ± 46.089^{a}	0.024 ± 0.007^{a}
UC 15 min	0.023 ± 0.014^{a}	78.43 ± 39.144^{a}	0.024 ± 0.015^{a}

Meanvalues ± SD of three independent measurement. The values in each column bearing the same superscript are not significantly different ($p < 0.05$).

3.6. Effect of ultrasonic cleaning on pesticide residues of vine leaves

A typical method for eliminating pesticide residue from food surfaces is washing the food in distilled water.It has been discovered that when sonication is employed during the washing process, pesticides are effectively removed by disrupting cell membranes, and ultrasound can improve the surface area of solvent and plant material interaction[44]. Ultrasound-assisted cleaning (UAC) is also thought to be an ecofriendly and effective pesticide removal technology that is superior to conventional approaches in its ability to remove pollutants. It is a time and energy-saving cleaning approach[11]. UAC in combination with electrical current was found to reduce pesticide levels in lettuce by 92.57, 81.9, and 93.1 %, respectively, for captan, thiamethoxam, and metalaxyl, respectively[27].

In this study, acetamiprid residues in leaf samples were decreasedin ultrasonic cleaning by 30.95, 54.76, and 45.24 %,respectively, for 5, 10, and 15 min of treatment. Whereas acetamiprid residues in leaf samples were reducedin conventional cleaning by 9.52, 52.38, and 38.10 %, respectively, for 5, 10, and 15 min of treatment. Ultrasonic cleaning reduced deltamethrin residues in leaf samples by 50.55, 48.68, and 58.22 % for 5, 10, and 15 min of treatment, respectively. Deltamethrin residues in leaf samples were reduced by 34.45 %, 57.43 %, and 56.90 %, respectively, after 5, 10, and 15 min of treatment with conventional cleaning. Pyridaben residues in leaf samples were decreased 36.36 %, 54.55 %, and 45.45 %, respectively for 5, 10 and 15 min of ultrasonic cleaning, while the decrease in pyridaben residues was 12.12 %, 27.27 %, and 27.27 %, respectively for 5, 10, and 15 min of conventional cleaning (Fig. 2). The ultrasound application facilitates the release of water from the sample by forming microchannels in plant cells; additionally, the length of the sonication treatment increases the size and amount of microchannels, and acoustic cavitation increases mass transfer between media and plant[45]. The rise in pesticide residues between 10 and 15 min of ultrasonic treatment is attributable to reverse mass transfer between plant and water resulting from extended sonication.Among all treatments, the lowest degradation was observed in pyridaben residue in those exposed to 5 min of conventional cleaning. In a recent study published by [14], pesticide residue reductions of 76.52 %, 37.74 %, and 75.37 % were discovered in tomato samples utilizing an ultrasonic bath. The elimination of captan and metalaxyl residues was higher in tomato samples compared to lettuce samples, that could be attributable to the textural properties of the lettuce. Because lettuce has a bigger surface area than tomatoes, pesticides may permeate more regions, making cleanup more difficult. Similarly, in our study, because vine leaves have a larger surface area, pesticide removal results were lower than tomato results, which Cengiz et al. (2018) observed. Because of the large surface area, ultrasonic treatments may have difficulty reaching insecticides on inner surfaces. Because ultrasonic energy degrades as it passes through different materials, less energy reaches the internal parts of the leaves. As a result, researchers discovered that using ultrasound to remove pesticides absorbed into the leaves may be more difficult than removing pesticides from the tomato surface. In another study, removal of five pesticides frequently used on rape and grapes using an ultrasonic washing was compared to normal water washing. It was found that ultrasonic washing was more efficient for pesticide elimination, with removal rates ranging from 14.7 % to 59.8 % on rape and between 72.2 % and 100 % on grapes[24].

In 2019, the Institute of Food Physical Processing at the School of Food and Biological Engineering at Jiangsu University in Zhenjiang, Jiangsu, China, built industrial-scale multi-frequency power ultrasonic equipment for cleaning fruits and vegetables that could clean more than 2000 kg of food per hour. On the conveyor belt cleaning tank, six

Table 4
Effect of ultrasound assisted cleaning on carotenoid and chlorophyll contents.

Samples	Total chlorophyll (mg/kg)	Total carotenoid (mg/kg)	Chlorophyll *a* (mg/kg)	Chlorophyll *b* (mg/kg)	Chlorophyll *a*/b
Control	10.46 ± 0.18^{a}	2.18 ± 0.08^{a}	6.98 ± 0.30^{ab}	2.26 ± 0.18^{a}	3.09 ± 0.12^{c}
CC 5 min	10.67 ± 0.16^{a}	0.73 ± 0.07 ^{cd}	7.58 ± 0.0.81^{a}	1.52 ± 0.04^{b}	4.98 ± 0.39^{a}
CC 10 min	10.25 ± 0.18^{a}	0.70 ± 0.27 ^{cd}	6.97 ± 0.16^{ab}	1.85 ± 0.13^{ab}	3.79 ± 0.34^{bc}
CC 15 min	6.52 ± 0.05^{c}	0.48 ± 0.18^{d}	4.25 ± 0.06^{d}	1.43 ± 0.07^{b}	2.98 ± 0.18^{c}
UC 5 min	8.10 ± 0.49^{b}	1.18 ± 0.04^{bc}	5.28 ± 0.09 ^{cd}	1.83 ± 0.14^{ab}	2.89 ± 0.17^{c}
UC 10 min	8.23 ± 0.15^{b}	1.31 ± 0.12^{b}	5.67 ± 0.13^{bc}	1.51 ± 0.08^{b}	3.77 ± 0.28^{bc}
UC 15 min	5.68 ± 0.12^{c}	0.69 ± 0.04 ^{cd}	4.09 ± 0.04^{d}	0.94 ± 0.06^{c}	4.36 ± 0.31^{ab}

Mean values ± SD of three independent measurement. The values in each column bearing the same superscript are not significantly different ($p < 0.05$).

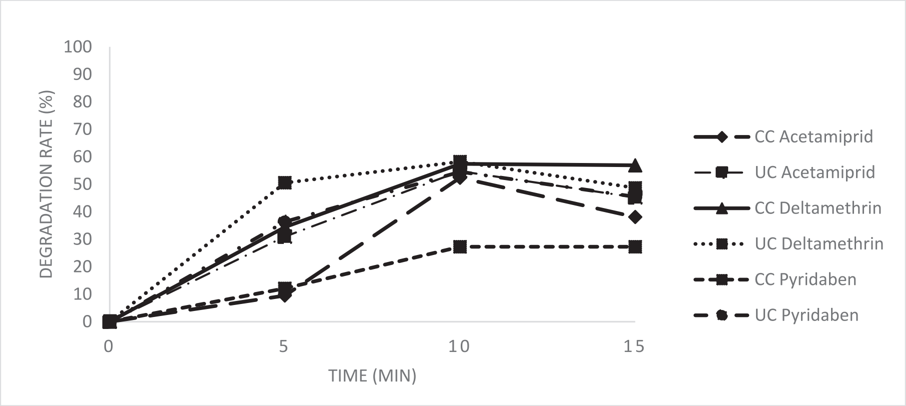

Fig. 2. Effect of ultrasound assisted cleaning on degradation rate of pesticides.

ultrasonic vibration boxes, which can work with different frequencies (20, 28, 33, 40, 68, and 80 kHz), were set up in a row. The transducers were spread out in the box, and each box has a power of 2 kW. This tool could be used to remove sediment, microorganisms, and pesticides from the root, stem, leafy vegetables, and fruits. A different industrial use of ultrasonic centrifugal cleaning technology has also been created, and it operates at various frequencies (20, 28, 35, 40, 50, and 66 kHz). While the machine is being used to wash the product, the basket of fruits and vegetables could be placed inside. Users can remove the sediment, bacteria, and pesticides using centrifugal forces and ultrasonic treatment. These washers were great for washing sensitive leafy vegetables like lettuce, chives, and other greens[11,21]. In recent studies, ultrasound was also combined with low intensity electric current [27], matrix solid-phase dispersion[46], and aqueous ozone[47] to increase the efficiency of removal pesticide from fruits and vegetables. In our study, the selected pesticide cleaning parameters were 37 kHz, 5 to 15 min, and 1:9, respectively, for frequency, time, and concentration of leaves in water. Future research should emphasize on identifying the optimum pesticide removal conditions for various ultrasonic parameters, such as time, temperature, type (probe or bath), frequency, density, etc. Prior to industrial applications of pesticide removal from Vine leaves, it is also critical to thoroughly assess the combined impact of other techniques with ultrasound.

4. Conclusion

The impact of ultrasound on the qualitative characteristics and degradation of extensively used pesticides (acetamiprid, deltamethrin, and pyridaben) for vine leaves was studied for time periods ranging from 5 to 15 min, and the results were compared to those obtained using traditional cleaning methods. According to the findings, ultrasonic cleaning (37 kHz) for 10 min was found to be the most efficient method for reducing pesticide residues. When leaves were exposed to 10 min of ultrasonic cleaning, the greatest reduction of acetamiprid (54.76 %), deltamethrin (58.22 %), and pyridaben (54.55 %) was recorded. In comparison to the conventional cleaning method, the UC process was proven to be a more successful application for reducing selected pesticides that were widely utilized for leaves. During the cleaning process, using the ultrasonic for 10 min increased the release of TPC (found in tissues) in the leaves; however, as the sonication proceeded, the release of phenolics reduced, most likely due to tissue breakdown. The findings of the study revealed that sonication up to 10 min might be used as a pesticide removing method without decreasing the qualitative properties of *Vitis vinifera* leaves. Even though it is likely that in the near future, ultrasonic cleaners will be used in factories to get rid of pesticides, consumers will not be able to consume foods that are safer and free of impurities until scientists determine the most effective ultrasonic working parameters for various types of foods. As a result, more optimization studies are needed to determine the optimal ultrasonic pesticide removal conditions for each product before developing innovative ultrasonic cleaners.

CRediT authorship contribution statement

Alev Yüksel Aydar: Writing – original draft, Methodology, Formal analysis, Investigation. **Tuba Aydın:** Methodology, Formal analysis, Formal analysis, Investigation. **Alican Karaiz:** Methodology, Formal analysis, Investigation. **Furkan Alabey:** Methodology, Formal analysis, Investigation. **Anjineyulu Kothakota:** Resources, Formal analysis, Writing – original draft. **António Raposo:** Validation, Funding acquisition, Resources. **Najla Abdullah Albaridi:** Validation, Funding acquisition, Resources. **R. Pandiselvam:** Supervision, Formal analysis, Validation, Software, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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