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Research Article

Chlorpyrifos Pesticide Removal from Black Grapes Using Plasma-Activated Water Produced by Plasma Bubbling Technology

B. Rakesh, R. Anbarasan, B. Kamalapreetha, R. Purushothaman, B. Ram Ashutosh, and R. Mahendran

Centre of Excellence in Nonthermal Processing, National Institute of Food Technology, Entrepreneurship, And Management-Thanjavur (NIFTEM-T), Thanjavur, India

Correspondence should be addressed to R. Mahendran; mahendran@iifpt.edu.in

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This study is aimed at evaluating the ability of plasma-activated water (PAW) to reduce the chlorpyrifos pesticide residue in black grapes. Raw black grapes were spiked with a known concentration (10, 15, and 20 ppm) of chlorpyrifos (20% EC) and bubbled with 120 V air plasma at three different time intervals (5, 10, and 15 min). GC-MS analysis of these plasma-bubbled grapes showed up to 65.25% reduction (20 ppm sample) in chlorpyrifos content after 15 min of treatment. However, the treatment also reduced the grape juice's ascorbic acid (19.97 ± 2.69 to 9.15 ± 2.02 mg/ml), antioxidant scavenging activity (77.42 ± 2.97 to 53.30 ± 4.77%), total flavonoids (3.00 ± 0.53 to 2.61 ± 0.57 mg QE/ml), total soluble solids (14.23 ± 1.96 to 11.95 ± 1.86°), total suspended solids (1.95 ± 0.16 to 1.80 ± 0.03 g/l), and turbidity (246.63 ± 11.42 to 224.1 ± 24.85 NTU). Meanwhile, other physicochemical attributes such as pH, titrable acidity, total phenol content, color index, and texture values had slight changes after plasma bubbling. Thus, plasma bubbling proved to be an effective method to remove the chlorpyrifos pesticide present in grapes, and the techniques also preserve the quality of the commodity.

1. Introduction

Grapes (*Vitis vinifera L.*) are among the most popular non-climacteric cluster fruits eaten worldwide. It belongs to the family *Vitaceae* and comes under the species of vining plants [1]. The annual production of grapes is around 75 million tonnes, with the largest production in Europe (about 41%), followed by Asia (29%) and the Americas (21%) [2]. Grapes can be used to prepare wine, grape juice, jam, raisins, etc. Approximately 50% of the global grape production is utilized for breweries and beverages from a total production of 76.75 million tonnes [3]. Several studies have revealed that grapes have antioxidant, anticarcinogenic, anti-inflammatory, antidiabetic, cardioprotective agent, gut-microbiota regulation, and multidrug resistance properties [4].

According to statistics, around an 80% increase in food production is required to cope with the population of 9.7 billion in 2050 [5]. Therefore, overusing pesticides, insecticides, and weedicides on food crops increased over time to reduce food losses and maintain the essential food supply [6]. However, several adverse health (i.e., cancer, Parkinson, and Alzheimer's disease) and environmental effects were also reported due to pesticide residues present in the soil, air, and water [7]. It is insisted that agricultural and processed products follow the prescribed safety level or maximum residue level (MRL) under the regulations of various statutory and regulatory bodies worldwide. Numerous synthetic pesticides including organochlorines, organophosphorus, carbamates, and pyrethrins are used in agricultural practices. Among them, chlorpyrifos is one of the most widely used pesticides in vine crops such as table and wine grapes [8]. Particularly, wine grapes had a 0.05 to 14 mg/kg residue level, but the MRL allowed for the grapes is 0.5 mg/kg [9]. Depending on the level, mode, and duration of exposure, chlorpyrifos can have a variety of consequences on the neurological system, from headaches, impaired vision, lacrimation, excessive salivations, tremors, seizures, coma, and fatality [10]. The level of health risks is alarming in proportion

and unique even after processing products like wine as the residues are transmuted from grapes, and there are higher chances of getting exposed either directly or indirectly [11]. Certain processing techniques have poor biotransformation of pesticides into less harmful metabolites [12].

Cold plasma is a unique nonthermal technology in which the gas molecules are subjected to high voltage to produce reactive species like ultraviolet rays, accelerated electrons, reactive oxygen and nitrogen species (RONS), singlet oxygen, hydroxyl radicals (-OH), nitrogen dioxide ($NO_2\cdot$), and carbonate radicals. These reactive species help in food decontamination (inactivation of pathogens and spoilage microbe), insect disinfestation, food quality improvement, surface modifications (packaging material and food products), toxin removal, and pesticide degradation and also preserve the essential physiochemical properties of food. These reactive species of cold plasma could be highly effective towards the degradation of pesticides, especially against the organophosphate groups [13–15]. Thus, the present study focuses on reducing the target pesticide (chlorpyrifos) in grapes and evaluating product quality changes.

2. Materials and Methods

2.1. Spiking of Pesticide on Grapes. The grapes (variety: "Muscat Hamburg," locally known as paneer grapes) were purchased from the local fruit vendor in Thanjavur, India. The grapes were washed with distilled water surface dried under room conditions. The stalk was kept intact to prevent the release of juice or any contamination. The target pesticide chlorpyrifos (20% EC) was made into three known concentrations of 10, 15, and 20 mg/l (ppm) in distilled water. They were sprayed over the grape's surface using a nozzle sprayer (size 48 mm) for uniform diffusion and allowed for surface drying. Those pesticide-spiked grape samples were the control samples of this study. These control samples were analyzed before the plasma bubbling treatment to determine the initial quality attributes of the grapes.

2.2. Plasma Bubbling (PB) Treatment. Plasma bubbling is the process of generating plasma-activated water (PAW) by bubbling the reactive plasma species in a liquid medium using a dielectric barrier discharge (DBT) system (Figure 1(a)). The atmospheric air is pumped (flow rate of 1 liter/hour) through a DBT plasma tube to produce reactive species at a voltage of 120 V. RONS generated in the plasma system was pushed out as bubbles in the distilled water using a blower [16]. These reactive species in the bubbles interact with the water molecules and produce PAW [17]. In this study, the grapes (100 g each) with three different initial pesticide concentrations (10, 15, and 20 ppm) were immersed in distilled water (weight/volume ratio: 1/2) during plasma bubbling for about three different treatment times (5, 10, and 15 minutes).

2.3. Pesticide Detection and Reduction Percentage

2.3.1. Colorimetric Quantification. The target pesticide level is determined by colorimetric quantification using the UV-visible (model: UV-1800, SHIMADZU) spectrophotometer [17]. Firstly, the samples were immersed in the isopropanol solvent (chlorpyrifos dissolves in alcohol : isopropanol) in the ratio of 1 : 2 for 5 min. Then, the solvent was filtered with Whatman 41-grade filter paper and read at 290 nm. The standard pesticide solutions (0.1 ppm to 25 ppm) were prepared by dissolving the chlorpyrifos in the isopropanol, and the standard curve was obtained at 290 nm. Similarly, the samples were also analyzed using the colorimetric method before and after the plasma bubbling process.

2.3.2. Gas Chromatography and Mass Spectrometry Analysis. Chlorpyrifos degradation was confirmed by gas chromatography and mass spectrometry (GC-MS) [18]. The control sample with the highest pesticide concentration (20 ppm) and the plasma-treated sample with the highest treatment time (15 min) were analyzed and compared. Firstly, the samples were soaked in 99.9% methanol and agitated using a shaker (overnight at 20°C). The methanol extract was then filtered (syringe filter size 0.2 μm) and injected (1 ml) in GC using a split injector (oven temperature: 4°C to 450°C with a constant oven ramp). Monolithic hyperbolic quadrupole was used as the mass filter with a maximum mass of 1050 amu. The inert electronic ionization (EI) was performed with the help of triple-axis HED EM with a scan rate of up to 12,500 amu/sec.

2.4. Analysis of Physicochemical Attributes. The grape samples were analyzed for their changes in physicochemical attributes after plasma bubbling treatment. Throughout the study, all the analyses were carried out in triplicate. Based on the nature of the analysis, whole grapes and juice extract were used.

2.4.1. Color Index. Using hunter color LAB (ColorFlex EZ model: 45/0 LAV, light source: D_{65}, illumination angle: 10°, and calibration disks: black and white tiles), color values (L^* a^* b^*) of the grape juice (extracted juice filtered using muslin cloth) were recorded. Chroma (Eq. (1)) is the degree of dominance of the hue (McGuire 1992). Color difference (ΔE) would help to differentiate the plasma effects (Eq. (2)). However, the standard and optimized method for the grapes called color index for red grapes (CIRG) was used to analyze the samples [19]. Based on the CIRG values (Eq. (3)), the grapes would be classified as follows: green to yellow (CIRG < 2), pink (2 < CIRG < 4), red (4 < CIRG < 5), dark red (5 < CIRG < 6), and blue to black or dark violet (CIRG > 6). The following formulae are used in expressing the CIRG for the grapes:

$$\text{Chroma } (C) = \sqrt{(a^*)^2 + (b^*)^2}, \tag{1}$$

$$\text{Color difference } (\Delta E) = \sqrt{(L^*)^2 + (a^*)^2 + (b^*)^2}, \tag{2}$$

$$\text{CIRG} = \frac{(180 - h\circ)}{(L^* + C)}. \tag{3}$$

2.4.2. Skin Strength. The firmness of the grapes was analyzed using the texture analyzer (Stable Micro Systems texture analyzer; model: TA HD plus, serial number: 5084) having

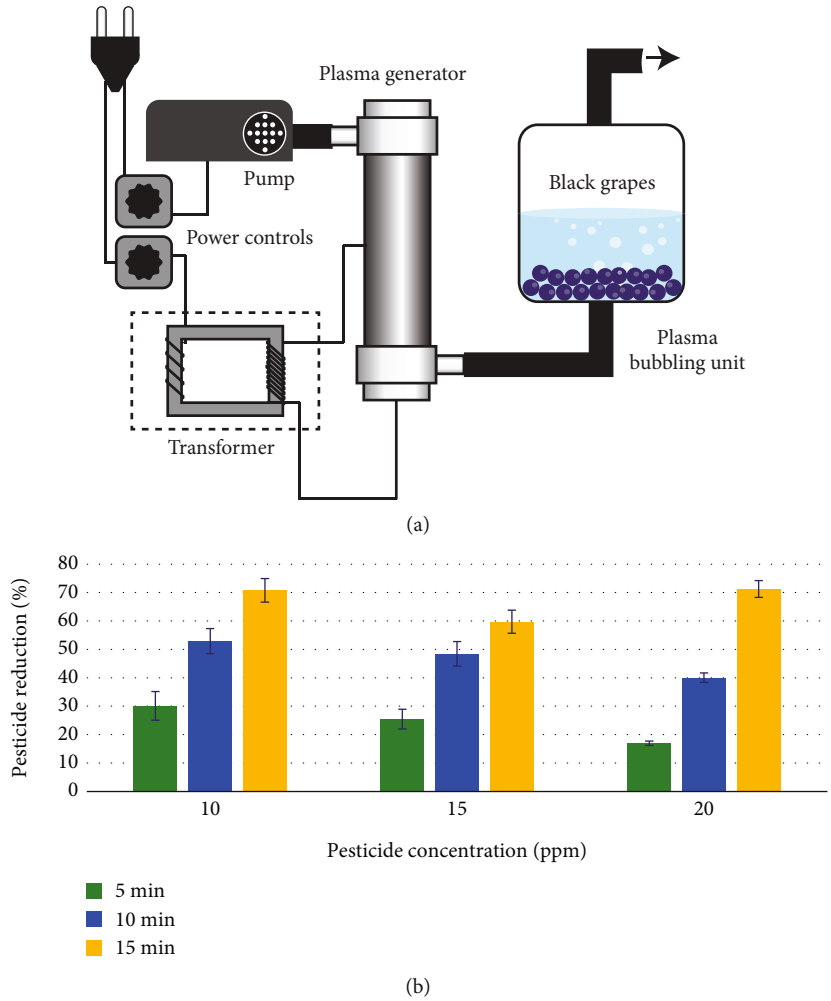

Figure 1: (a) Schematic representation of plasma bubbling system. (b) Changes in residual chlorpyrifos level after plasma bubbling.

a load cell of 30 kg with the needle probe P/2N to puncture the grape. The control and treated grapes were subjected to compression with a distance of 5 mm to incur 10% strain. The firmness of the grape was considered to be the force required to collapse the structure of the grape by puncturing the skin.

2.4.3. pH, Titrable Acidity, Total Soluble Solids, Total Suspended Solids, and Turbidity. The filtered juice was used to determine the pH and total soluble solids (TSS, in °Brix) using a pH meter (model: HANNA, H198107) and digital refractometer (model: HANNA, H196801), respectively. The titrable acidity (TA) was calculated by the modified method described by the AOAC (S.S. Nielsen 2010). In a conical flask, 0.5 ml of grape juice supernatant (centrifuged at 1500 rpm for 10 min) was added to 49.5 ml of distilled water. Then, two drops of phenolphthalein were added as an indicator and titrated against the 0.1 N NaOH. Titration was repeated to get a concordant value. After titration, the TA is expressed in g of tartaric acid/L. Similarly, the total suspended solid (TS, in g/l) and turbidity (in NTU) values were analyzed for grape supernatant using the turbidity meter (model: LABWAN, LW-TM136; calibration standards: 200 NTU, 500 NTU, and 1000 NTU).

2.4.4. Total Phenolic Content. The total phenolic content (TPC) was estimated using the Folin-Ciocalteu method described by Slinkard and Singleton with minor modifications [20]. 0.5 ml of 10% Folin's reagent was added to 0.5 ml of grape juice supernatant. After 6 min, 2 ml of 20% sodium carbonate (Na_2CO_3) was added and made up to 10 ml with distilled water. Then, the mixture was allowed to stand for 90 min in the dark. The purple-colored mixture was read at 760 nm using a UV-visible spectrophotometer (model: UV-1800, SHIMADZU). From the standard curve (gallic acid: 0.1 to 10 mg/ml) absorbance values, the TPC of control and treated grape samples were calculated and expressed in mg of gallic acid equivalent (GAE) per ml of juice.

2.4.5. Total Flavonoid Content (TFC). The modified aluminium colorimetric method determined the total flavonoid content [21]. 1 ml of grape juice supernatant was mixed with 0.3 ml of 5% sodium nitrite ($NaNO_2$). After 6 min, 0.6 ml of 10% aluminium chloride ($AlCl_3$) was added to the mixture

and allowed to react for 5 min. Again, 2 ml of 1 M NaOH was added to the mix and vortexed well. The resultant bright yellow colored solution was read after 15 min at 510 nm colorimetric method. The standard curve was prepared for the quercetin concentration range of 0.1 to 10 mg/ml and correlated with the absorbance of the sample. The TFC of the grape juice is expressed in mg of quercetin equivalents (QE) per ml of juice.

2.4.6. Antioxidant Scavenging Activity. The antioxidant scavenging activity was determined by inhibiting the DPPH solution with minor modifications described in the Blois method [22]. 100 μl of grape juice supernatant was made up to 3 ml with ethanol and mixed with 2 ml of 0.5 M DPPH. After incubating the content in a dark condition for 20 minutes, the mixture was read at 517 nm using a UV-visible spectrophotometer. The radical scavenging by the DPPH was calculated by the following equation:

$$\text{DPPH (\%inhibition)} = \left(1 - \frac{\text{Abs.of the sample}}{\text{Abs.of the control}}\right) \times 100. \quad (4)$$

2.4.7. Vitamin C Content. The ascorbic acid content was estimated using the 2,6-dichlorophenolindophenol (DCPIP) titration method described by Zubeckis with some minor changes [23]. 5 ml of working standard (100 μg/ml) of ascorbic acid and 10 ml of 4% oxalic acid were pipetted out into a conical flask and titrated against the DCPIP dye solution (V1) until the appearance of pale pink color. Initially, 0.1 ml supernatant was made into 100 ml with 4% oxalic acid. The diluted sample of 5 ml and 10 ml of 4% oxalic acid was titrated against the dye (V2). From equation (5), the ascorbic acid content (mg/ml) was estimated for the grapes.

$$\text{Ascorbic acid content} = \frac{0.5\ \text{mg} \times \text{V2} \times \text{make up volume}}{\text{V1} \times 5\ \text{ml} \times \text{volume of sample taken}}. \quad (5)$$

2.5. Statistical Analysis. For this study, statistical analysis was carried out using SPSS statistical software (SPSS Inc., Chicago, USA, version 20.0). Significant changes in the chlorpyrifos reduction percentage and physiochemical attribute parameters were analyzed using multivariate ANOVA with the Tukey honesty test at a 95% confidence level. All the data were obtained and collected in triplicate for this study.

3. Results and Discussion

3.1. Effect of PB Treatment in Chlorpyrifos Reduction on Grapes

3.1.1. Interpreting the Colorimetric Quantification. A significant reduction in the chlorpyrifos levels was observed (Figure 1(b)) in all the pesticide-spiked samples (10 ppm, 15 ppm, and 20 ppm) after plasma bubbling. Among the three treatment times (5 min, 10 min, and 15 min), 15 min bubbling was found to be more effective as it reduced about 70.79%, 59.54%, and 71.27% of the chlorpyrifos from the samples spiked with 10 ppm, 15 ppm, and 20 ppm pesticide, respectively. Meanwhile, 10 min treatment removed only 52.9%, 48.41%, and 40.03% of the chlorpyrifos in these samples. However, the lowest effectiveness was observed in 5 min treatment as their effectiveness was only 30.11%, 25.49%, and 16.99% in 10 ppm, 15 ppm, and 20 ppm pesticide-spiked samples, respectively. This proved that the application PAW prepared using cold plasma technology could reduce the pesticide level in black grapes. Similar results were observed in chlorpyrifos-spiked fruits such as mangoes (74% reduction) [24], tomatoes (89.18%) [18], grapes (79%), and strawberries (69%) [25] after plasma treatment. Apart from that, plasma treatment was also effective against the pesticide in the food grains. For example, chlorpyrifos content in soybean and corn can be reduced to 50% [7] and 86.2% [26], respectively, after plasma treatment. In PB treatment, the degradation of the chlorpyrifos has been achieved by the cleavage of the bonds between P-S and S-C through the oxidative action of RONS, ions, free electrons, and atoms that were generated by the atmospheric air in the water [27]. This is because the molecules with the same bond dissociation energy and ionization energy will get damaged due to the energy of electrons varying from 0 to 10 eV, which produces many free radicals with high oxidative potential, which may result in pesticide degradation. The increase in reactive species generation upon high voltages enhanced these oxidative reactions. Hence, the increased plasma voltage caused more pesticide degradation [28].

3.1.2. Validation of Pesticide Reduction through GC-MS Assay. The obtained GC-MS results for the 20 ppm chlorpyrifos-spiked control (untreated) and 15 min plasma-treated samples are shown in Figures 2(a) and 2(b). The peak for the target pesticide chlorpyrifos was obtained at the retention time of 19.3962 min with a molecular weight of 348.926 g. The component area for the control was 235586.8 with a match factor of 93.3, and the area covered was 0.506%. After the treatment, the component area was reduced to 81848.2 with a match factor of 79.4, and the area covered was 0.2328%. The results indicated a 65.25% reduction in the chlorpyrifos level of grapes, which positively confirms the chlorpyrifos degradation and the colorimetric quantification data. However, the deviation in the colorimetric quantification data might be due to the variations in the process of pesticide degradation and its oxidative byproduct (both grape and chlorpyrifos) nature. After the interaction of oxidative species with the chlorpyrifos, it could have been converted into secondary metabolites (degraded chlorpyrifos metabolite like trichloropyridinol). But no other peaks were observed near the chlorpyrifos peak in the GC-MS assay, which indicates that there were no traces of those secondary metabolites or traces of broken phosphorus and chlorine as in previous studies [7, 18]. In conclusion, the interpretation suggests that the weak binding effect of these secondary metabolites to the surface of grapes, owing to a hydrophobic barrier, may result in their removal through water washing or bonding with other free radicals present in the plasma-activated water (PAW). This scenario provides multiple pathways for degradation [25].

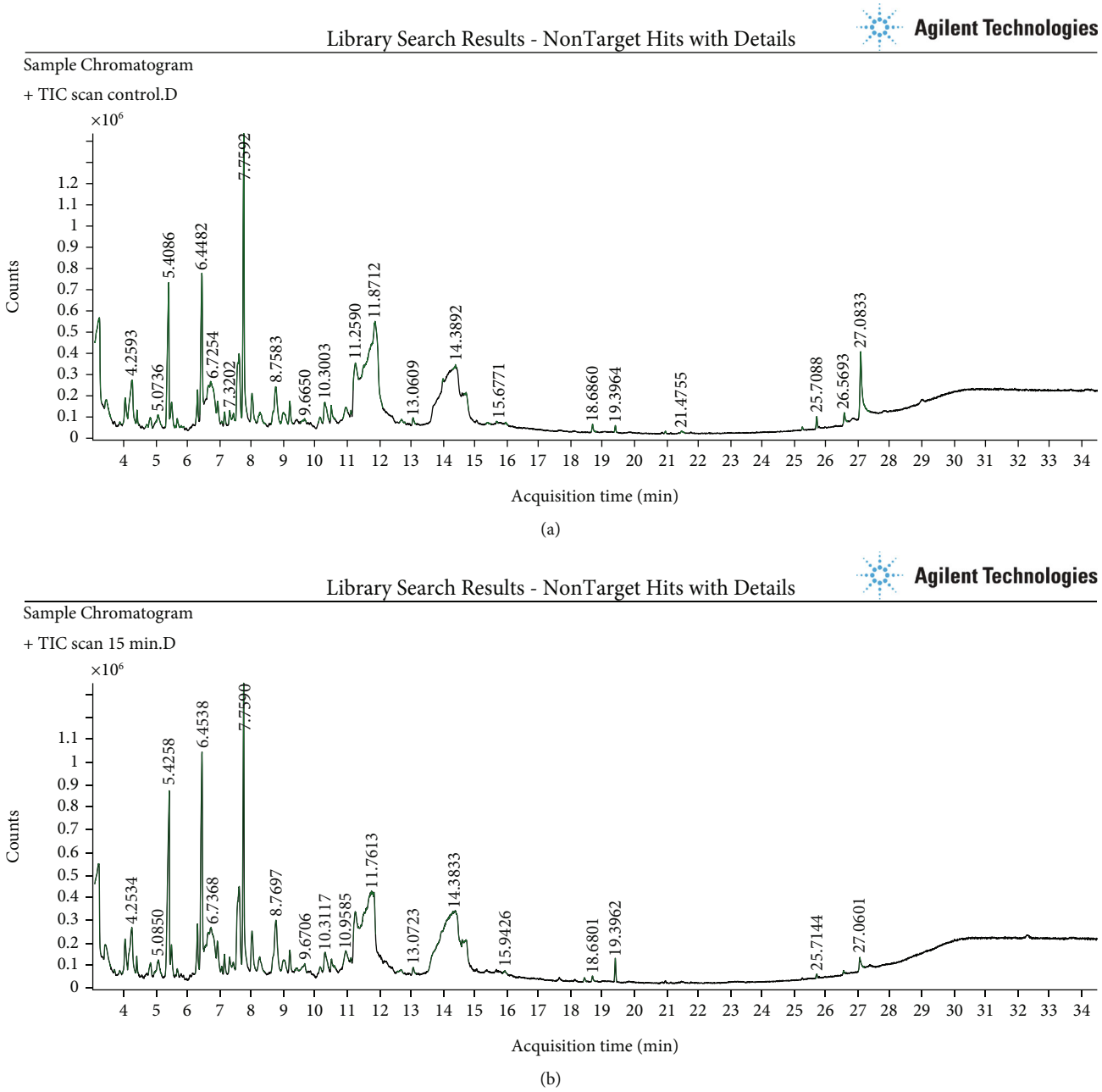

Figure 2: GC-MS spectra of (a) the control (20 ppm) and (b) 15 min plasma-bubbled grape samples.

3.2. Effect of PB on the Physiochemical Properties

3.2.1. Color Index of the Grapes. The grapes did not undergo any characteristic color change regardless of different treatment times (Table 1). However, there was a significant color difference between the control and treated samples (ΔE ranges from 0.00 to 4.21). Similar results were observed in the Guo et al. [29] study with minimal changes in overall color as PAW preserved the pigment responsible for the grapes' color. Meanwhile, the significantly unaffected chroma values (Table 1) also indicated the retention of high-intensity dark color in grape skin. The maximum variations in the chroma values were 2.94 to 2.35, 3.64 to 2.96, and 5.65 to 4.29 for 10 ppm, 15 ppm, and 20 ppm grape samples, respectively. In addition, the samples of all three treatment times (5, 10, and 15 min) remained at the CIRG index value of six (CIRG > 6, Table 1), which denotes that the grapes remained in the dark-colored (violet to black) spectrum of the CIRG index [19]. Therefore, it is evident that the color of plasma-bubbled grapes was preserved even after 15 min of exposure time. At the same time several phenolic compositions like anthocyanins, which are primarily responsible for the dark color quality in black grapes are preserved.

3.2.2. Effect on the Textural Attributes. Skin strength recorded (Table 1) to determine the firmness of grapes showed no significant difference in skin strength after the treatment. Though the mean values of puncture force varied

Table 1: Color, textural, TSS, and pH changes in plasma-bubbled grapes.

Parameter	Treatment	10 ppm	15 ppm	20 ppm
ΔE	Control	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
	5 min	2.24 ± 1.08^{ab}	1.55 ± 0.6^{b}	1.95 ± 0.6^{ab}
	10 min	3.72 ± 0.27^{b}	2.26 ± 0.82^{b}	3.4 ± 2.25^{b}
	15 min	2.70 ± 2.29^{b}	2.00 ± 0.78^{b}	4.21 ± 1.88^{b}
Chroma	Control	2.94 ± 1.79^{a}	3.24 ± 0.88^{a}	5.65 ± 2.15^{a}
	5 min	3.15 ± 1.82^{a}	2.96 ± 0.67^{a}	5.32 ± 1.49^{a}
	10 min	3.57 ± 1.03^{a}	3.64 ± 1.2^{a}	4.29 ± 1.41^{a}
	15 min	2.35 ± 1.01^{a}	3.1 ± 0.81^{a}	5.2 ± 1.08^{a}
CIRG	Control	13.47 ± 1.91^{a}	12.87 ± 1.72^{a}	8.61 ± 1.55^{a}
	5 min	11.91 ± 1.35^{a}	14.05 ± 3.57^{a}	9.89 ± 2.32^{a}
	10 min	10.53 ± 1.37^{a}	12.2 ± 1.31^{a}	9.79 ± 1.78^{a}
	15 min	12.54 ± 1.94^{a}	12.99 ± 2.93^{a}	11.18 ± 2.56^{a}
Skin strength in kg-force	Control	90.1 ± 22.7^{a}	106.31 ± 11.06^{a}	91.75 ± 8.46^{a}
	5 min	83.69 ± 30.17^{a}	91.86 ± 29.71^{a}	100.87 ± 16.29^{a}
	10 min	96.3 ± 27.3^{a}	87.04 ± 12.77^{a}	83.71 ± 10.17^{a}
	15 min	74.85 ± 6.92^{a}	103.75 ± 26.11^{a}	77.27 ± 26.94^{a}
TSS (°Brix)	Control	15.18 ± 0.8^{a}	15.78 ± 0.8^{a}	14.23 ± 1.96^{a}
	5 min	14.35 ± 0.65^{a}	13.48 ± 1^{b}	12.28 ± 1.96^{a}
	10 min	13.98 ± 0.95^{a}	13.55 ± 0.33^{b}	12.33 ± 1.67^{a}
	15 min	14.03 ± 0.58^{a}	13.7 ± 0.73^{b}	11.95 ± 1.86^{a}
pH	Control	3.43 ± 0.05^{a}	3.45 ± 0.06^{a}	3.43 ± 0.05^{a}
	5 min	3.48 ± 0.05^{a}	3.35 ± 0.06^{a}	3.28 ± 0.22^{a}
	10 min	3.38 ± 0.05^{a}	3.38 ± 0.05^{a}	3.28 ± 0.15^{a}
	15 min	3.43 ± 0.05^{a}	3.38 ± 0.05^{a}	3.35 ± 0.06^{a}

Different alphabet superscripts in the same column indicate the significant difference between the values at different treatment conditions for a given parameter ($p \leq 0.5$).

from 90.1 g to 74.85 g, 106.31 g to 87.04 g, and 100.87 g to 77.27 g for 10 ppm, 15 ppm, and 20 ppm samples, respectively, the standard deviation between the triplicate values was higher enough to avoid the significant difference between these treatment times. Therefore, the appeared differences (Table 1) in the skin strength at different treatment times are due to the natural variations in the grape's skin strength and not due to the treatment effect. Moreover, grape skin is made up with phenols tightly bounded to polysaccharides through hydrogen bonds, and the interactions are hydrophobic in nature. For a high degree of skin's firmness, the level of the calcium availability in the cell wall membrane structure should be high, and hypothetically, it could not be reduced through the PB treatment at this voltage, flow rate, and time exposure. Since "accelerated electrons" are absent during the bubbling process, the possibilities for skin surface etching are minimal. Similar results were observed in chlorpyrifos-spiked tomatoes when treated in PAW using bubbling technology [17]. Thus, plasma bubbling did not degrade the structural polysaccharides in the grapes' cell wall. Hence, the samples retained their structural integrity even after the treatment. However, a nonlinear decrease in the skin strength was observed during the treatments owing to the natural skin strength variations and extended soaking time.

3.2.3. Changes in the TSS and pH. Plasma bubbling did not induce any significant changes in the TSS value of 10 ppm (ranges from $15.18 \pm 0.80^{\circ}$ to $13.98 \pm 0.95^{\circ}$) and 20 ppm (ranges from $14.23 \pm 1.96^{\circ}$ to $11.95 \pm 1.86^{\circ}$) grape samples. However, a nonlinear reduction trend was observed in those samples with respect to the treatment time increase. On the other hand, a significant reduction was observed in the TSS value of 15 ppm chlorpyrifos-spiked grape samples ($14.23 \pm 1.96^{\circ}$ to $11.95 \pm 1.86^{\circ}$) in all the treatment times (Table 1). The endosmosis could be the reason for the reducing trend in the TSS values of plasma-bubbled samples where the water acted as hypotonic medium in which grapes were treated (water penetrating the grapes can dilute the TSS content of the samples). This penetration could be happened due to either the skin's permeability was high or naturally susceptible easily due to the poor structural arrangement of the cells in the skin. On the other hand, no significant changes were observed in the pH values of plasma-bubbled samples. There were instances of the water molecule penetration into grapes due to osmosis. But it did not affect the

overall acidic pH range of the grapes which might be balanced or retained by the overall ion distribution in it. The overall variation in the pH was only 3.45 ± 0.06 to 3.28 ± 0.22. Evidently, [30, 31] also did not observe any significant changes in the pH and TSS of PAW-treated grapes. Since plasma reactive species react only at the surface level of grapes, they did not alter the pH values of bubbled grapes.

3.2.4. Changes in the TS and Turbidity. As shown in Table 2, bubbling treatment did not induce any significant changes to the TS values of grape juice. However, the nonlinear reducing trend observed in TS (10 ppm: 1.95 to 1.93 g/l, 15 ppm: 1.89 to 1.74 g/l, and 20 ppm: 1.95 to 1.80 g/l) could be due to the migration of water molecules into grape samples. Meanwhile, nonlinear uncharacterized turbidity changes were recorded for the juice extracted from plasma-bubbled whole grapes. However, for all three samples (10 ppm: 253.9 to 247.15 NTU, 15 ppm: 246.63 to 224.1 NTU, and 20 ppm: 251.78 to 236.73 NTU), a considerable (not significant) reduction in the turbidity was recorded after 15 min of bubbling. Removal of surface dirt due to the continuous collapsing of the pressurized bubbles leads to cavitation phenomenon and impregnation of water molecules (endosmosis) into the grapes which would have been the reasons for the reduced TS and turbidity of plasma-bubbled grapes.

3.2.5. Effects in the Titrable Acidity. The TA content (tartaric acid) of plasma-bubbled grapes reduced linearly (Table 2) with respect to the treatment time in all the pesticide-spiked samples (10 ppm, 15 ppm, and 20 ppm). The initial acidity values of 10.88 ± 3.09, 11.25 ± 1.94, and 10.5 ± 2.74 g acid/l were reduced to 9.75 ± 1.94, 9.75 ± 1.5, and 7.5 ± 0.0 g acid/l in 10 ppm, 15 ppm, and 20 ppm pesticide-spiked grape samples, respectively, after 15 min of treatment. Similar results were observed in [30] study, where 30 min of PAW treatment at 25°C reduced the initial grape TA value from 0.674 to 0.656 g acid/100 g fresh weight. The drop in the level of TA could be the activation of redox reactions (enzymatic oxidation) taken inside the pulp due to the continuous temperature difference between the water molecules and molecules inside the bubble to the grape surface. However, the observed changes were not significantly different from the control sample's TA value.

3.2.6. Effect of PB on the TPC of the Grapes. The plasma bubbling of whole grapes induced an insignificant positive response to the TPC values of extracted juice. Mainly after 10 min of bubbling, the maximum TPC values were recorded in all three pesticide-spiked samples (10 ppm: 7.14 ± 1.28 to 7.54 ± 0.93 mg GAE/ml, 15 ppm: 7.11 ± 0.69 to 7.34 ± 0.56 mg GAE/ml, and 20 ppm: 5.86 ± 1.71 to 6.1 ± 2.06 mg GAE/ml) as shown in Table 3. However, extended bubbling caused a reducing trend in the TPC values of all three samples as the grapes were impregnated with PAW. Although the phenolic content is rich in freshly crushed grape juice, it is readily oxidized by the polyphenol oxidase and laccase through both rapid enzymatic oxidations. Further, the slow nonenzymatic oxidation also takes place based on the limitation of glutathione. The observed insignificant reducing trend could be happened due to the glutathione depletion and precipitation of the polyphenolic compounds in the juice as oxidation promoted.

Activation of enzymes responsible for the phenolic compound synthesis could be the reason for the increase in the TPC of plasma-bubbled grapes [32]. Studies also revealed that the increase in TPC might be related to the activation of phenylalanine ammonia-lyase enzyme in the fruits [33]. When cell wall polysaccharides are exposed to a strongly oxidizing environment, it might result in their depolymerization and dissolution, which makes it easier to extract or degrade conjugated phenolic chemicals [25]. When the grapes were crushed, the phenols confined in the vacuoles of the cells especially in the skin which would be spiked overall TPC easily. The extraction method also affects the free and bound phenolic in the grape skin, pulp, and seed [34]. Moreover, Bao et al. [35] observed the capability of cold plasma on enhancing the phenolic compound extraction in the grape pomace. Thus, bubbling releases the bound phenolic components from the grape's peel and pomace, raising the TPC content.

3.2.7. Effect of PB on the TFC Content of the Grapes. Similar to TPC, the TFC of plasma-bubbled grapes also increased (not significant, $p \leq 0.5$). However, the maximum TFC was recorded within 5 min of treatment in all the samples, and a further increase in the treatment time reduced the TFC. In the initial TFC content of 10 ppm (2.7 ± 0.42 mg QE/ml), 15 ppm (3.0 ± 0.53 mg QE/ml), and 20 ppm (2.73 ± 0.44 mg QE/ml), pesticide-spiked grape samples increased to 3.51 ± 0.42, 3.93 ± 0.07, and 3.55 ± 0.49 mg QE/ml, respectively, within 5 min of plasma bubbling. Most commonly available flavonoids in grapes are flavonols (quercetin), catechins, and anthocyanins, and during crushing, the flavonoids are easily extractable compared to the other phenolic components due to their lower energy binding [36]. Hence, the shortest treatment time of 5 min extracted more flavonoids from grapes than the control samples. They could exist in both free and polymer with a sugar, other flavonoids, and nonflavonoids. Regardless they are synthesized in the endoplasmic reticulum and stored in the central vacuole of that cell. However, overexposure increased the water content of grapes due to the water impregnation (endosmosis) where the combined forms (like flavonoid glycosides) are prone to soluble in water. Therefore, the TFC content per milliliter of grape juice is reduced after prolonged plasma bubbling.

3.2.8. Effect of PAW on the Free Radical Scavenging in the Grapes. Phenolic compounds and ascorbic acid are the main components responsible for fruits' free radical scavenging/antioxidant activity. These substances can neutralize free radicals that harm the body and lower the risk of several diseases and conditions caused by oxidative stress [37]. In the present study, plasma bubbling reduced the free radical scavenging activity of grape juice extracted from plasma-bubbled grapes. The overall reduction in the radical scavenging percentage of plasma-bubbled grapes is shown in Figure 3. In brief, the antioxidant scavenging activity of 10 ppm, 15 ppm,

Table 2: Effect of PB on the TS, turbidity, and TA of the grapes.

Treatment time	TS (g/l)			Turbidity (NTU)			Titrable acidity (g acid/l)		
	10 ppm	15 ppm	20 ppm	10 ppm	15 ppm	20 ppm	10 ppm	15 ppm	20 ppm
Control	1.95 ± 0.16^{a}	1.89 ± 0.09^{ab}	1.95 ± 0.04^{a}	253.9 ± 18.58^{a}	246.63 ± 11.42^{ab}	251.78 ± 5.14^{a}	10.88 ± 3.09^{a}	11.25 ± 1.94^{a}	10.5 ± 2.74^{a}
5 min	1.96 ± 0.11^{a}	2.02 ± 0.08^{b}	1.84 ± 0.07^{a}	255.63 ± 14.5^{a}	261.48 ± 11.61^{b}	240.33 ± 8.1^{a}	9.75 ± 1.94^{a}	10.5 ± 1.22^{a}	9.38 ± 0.75^{a}
10 min	1.99 ± 0.12^{a}	1.82 ± 0.09^{ab}	1.93 ± 0.24^{a}	259.13 ± 11.13^{a}	236.03 ± 12.97^{ab}	254.63 ± 28.6^{a}	9.75 ± 1.94^{a}	10.5 ± 1.22^{a}	7.88 ± 0.75^{a}
15 min	1.93 ± 0.05^{a}	1.74 ± 0.2^{a}	1.8 ± 0.03^{a}	247.15 ± 14.3^{a}	224.1 ± 24.85^{a}	236.73 ± 6.23^{a}	9.75 ± 1.94^{a}	9.75 ± 1.5^{a}	7.5 ± 0^{a}

Different alphabet superscripts in the same column indicate the significant difference between the values at different treatment conditions for a given parameter ($p \leq 0.5$).

Table 3: Effects of PAW in the TPC, TFC, and ascorbic acid of the grapes.

Treatment time	TPC (mg GAE/ml)			TFC (mg QE/ml)			Ascorbic acid (mg/ml)		
	10 ppm	15 ppm	20 ppm	10 ppm	15 ppm	20 ppm	10 ppm	15 ppm	20 ppm
Control	7.14 ± 1.28^{a}	7.11 ± 0.69^{a}	5.86 ± 1.71^{a}	2.7 ± 0.42^{a}	3 ± 0.53^{ab}	2.73 ± 0.44^{a}	19.97 ± 2.69^{a}	17.64 ± 2.91^{a}	19.19 ± 11.53^{a}
5 min	7.6 ± 0.99^{a}	7.25 ± 0.65^{a}	6.02 ± 1.58^{a}	3.51 ± 0.42^{a}	3.93 ± 0.07^{a}	3.55 ± 0.49^{a}	12.35 ± 0.5^{b}	11.3 ± 0.96^{b}	17.12 ± 11.89^{a}
10 min	7.54 ± 0.93^{a}	7.34 ± 0.56^{a}	6.1 ± 2.06^{a}	2.9 ± 0.35^{a}	3.02 ± 0.43^{ab}	3.46 ± 0.45^{a}	9.15 ± 2.02^{b}	9.21 ± 2.41^{b}	11.3 ± 0.96^{a}
15 min	7.27 ± 0.65^{a}	6.45 ± 0.61^{a}	5.36 ± 1.75^{a}	2.77 ± 0.9^{a}	2.61 ± 0.57^{b}	3.39 ± 0.28^{a}	10.2 ± 0.61^{b}	12.86 ± 3.96^{ab}	14.21 ± 6.11^{a}

Different alphabet superscripts in the same column indicate the significant difference between the values at different treatment conditions for a given parameter ($p \leq 0.5$).

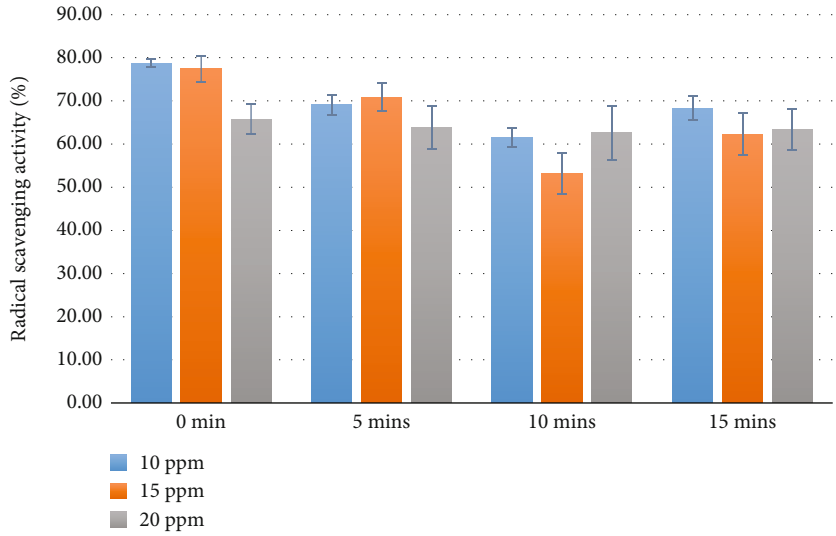

Figure 3: Changes in the free radical scavenging activity of control and plasma-bubbled grapes.

and 20 ppm pesticide-spiked samples reduced from 78.74% to 63.98%, 77.43% to 53.3%, and 65.81 to 63.40%, respectively, after 15 min of treatment. Figure 3 shows that the increase in treatment time results in the reduction of antioxidant activity. In addition, this study discovered that a high concentration of TPC was not always associated with a high level of antioxidant activity. However, from our study, we could conclude that regardless of pesticide concentration, the PB has the same effect on the antioxidant capacity if time increases which are contradicted by the study [35].

3.2.9. Effects of Bubbling on the Ascorbic Content. In the present study, plasma bubbling of grapes reduced the ascorbic acid content significantly in 10 ppm and 15 ppm sample pesticide containing grape samples (Table 3). Though the ascorbic acid content in the 20 ppm sample also reduced (not significant) from 19.19 ± 11.53 to 14.21 ± 6.11 mg/ml, it was significantly different from the initial values. Among the three different treatment times, the lowest ascorbic acid content was recorded after 10 min of bubbling, where 10 ppm, 15 ppm, and 20 ppm pesticide-spiked grape samples reached the lowest ascorbic content values of 9.15 ± 2.02 mg/ml, 9.21 ± 2.41 mg/ml, and 11.3 ± 0.96 mg/ml from their initial values of 19.97 ± 2.69 mg/ml, 17.64 ± 2.91 mg/ml, and 19.19 ± 11.53 mg/ml, respectively. Contrast results were observed in Xiang et al.'s [30] study, where the treatment did not cause any significant reduction in the ascorbic acid content. This could have happened through the preference of conventional extraction method. While the grapes were being crushed, the concentration of vitamins would fall naturally; thus, ascorbic acid is being rapidly oxidized. And it cleaved into oxalic acid and threonic acids which favours the coupled oxidation of ascorbic acid and catechins for the production of diphenols (this whole process promotes polymerization and precipitation).

4. Conclusion

The present study investigated the ability of plasma bubbling to reduce the chlorpyrifos pesticide residue from grapes and evaluated its subsequent impacts on the grape's physicochemical attributes. The residual chlorpyrifos content reduced significantly up to 65.25% after the plasma bubbling period of 15 min, further without any traces of the broken metabolites in the plasma-bubbled grapes. However, the treatment reduced the TFC, turbidity, antioxidant scavenging activity, and vitamin C content of grapes. Meanwhile, only slight changes were observed in color, texture, pH, titrable acidity, TSS, and TPC of the grapes. The significant changes were observed due to the predominant action of extraction method, enzymatic oxidation, osmotic regulation, kinetic polymerization, and minor precipitation in the extracted juice of the plasma-bubbled grapes. The present study reveals that nonthermal cold plasma has the potential to degrade the organophosphorus pesticide without many adverse effects on the characteristics of the commodities throughout the growth period. Research can be carried out on how different hydrophobic agrochemicals, often employed in various food commodities, are degraded by cold plasma bubbling and the post effects during different storage conditions. Kinetic pesticide degradation research may be carried out to improve and optimize the plasma processing conditions for providing effective pesticide reduction.

Data Availability

All the data is presented in the manuscript itself.

Conflicts of Interest

The authors have declared no conflicts of interest for this article.

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