

ORIGINAL ARTICLE

Effect of cold plasma on degradation of organophosphorus pesticides used on some agricultural products

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Abstract

This study investigated the potential effect of cold plasma on reducing residues of pesticides diazinon and chlorpyrifos in apples and cucumbers and its effects on property of products. Two separate concentrations of each pesticide with 500 and 1,000 ppm were prepared and the samples were inoculated by dipping them into the solutions. All samples treated with pesticides were exposed to cold plasma in a monopole cold plasma apparatus (DBD) run at 10 and 13 kV voltages. Liquid-liquid extraction (LLE) was used to remove pesticide residues from the samples. Eventually, high-performance liquid chromatography (HPLC) was used to measure the amount of pesticides in the samples. Also, to investigate generated metabolites, extracts were injected into a GC/MS apparatus. In addition, the effects of cold plasma on humidity, tissue hardness, color and the sugar percentage of products were analyzed. The results revealed that treatment of samples with cold plasma considerably reduced pesticide residues without leaving any traces of harmful or toxic substances. Furthermore, it did not have any undesirable effects on the color and texture of the samples. The efficiency of this method increased with higher voltage and longer exposure time. In general, the best results were obtained by the combination of 500 ppm concentration, 10 min exposure and 13 kV voltages. The residues of diazinon were reduced better than the residues of chlorpyrifos. Apples were detoxified much better than cucumbers. Also, cold plasma treatment transformed diazinon and chlorpyrifos pesticides into their less toxic metabolites. The results showed that with increased voltage and longer exposure time, cold plasma caused few changes in moisture and glucose content, texture hardness and color of products. There were no significant difference between treated samples and control in all treatments.

Key words: chlorpyrifos, cold plasma, diazinon, detoxification

Introduction

The use of organophosphorus (OP) pesticides as an alternative to organochlorine compounds all over the world have consequences on both human health and the natural ecosystems. Organophosphorus pesticide residues can be found in some agricultural products and natural waters, due to their extensive use and moderate persistence (Bai *et al.* 2006; Ambrus 2009; Hernandez-Borges *et al.* 2009). In order to avoid adverse effects of OP pesticide residues on the environment, effective methods to remove and degrade pesticide residues from

fresh agricultural products are seriously necessary. Many sterilization methods such as conventional thermal methods, ozonation, UV, ultrasonic and ionizing radiation, photolysis and hydrolysis have been used for the degradation of OP pesticide residues. However, these techniques have low efficiency, potential hazards and are costly (Le Person *et al.* 2007; Oancea and Onescu 2008; Ormad *et al.* 2008).

Recently, the use of cold plasmas operating at ambient air pressure (or other operating gases and gas

mixtures) is a relatively novel technique for the decontamination of fresh foods and food processing surfaces. Misra *et al.* (2011) and Niemira (2012) reviewed the fundamentals of cold plasma and its application to decontamination of foods. There are some reports about the efficiency of cold plasma plasmas to inactivate microorganisms including bacteria, bacterial spores, fungi, and biofilms (Feng *et al.* 2009; Koban *et al.* 2010) and to degrade bio-macromolecules such as proteins and saccharides (Deng *et al.* 2007; Bayliss *et al.* 2009).

Plasma is a glow flow composed of very reactive chemical species such as charged particles, free radicals and some radiation which occurs when a gas is in contact with an electrical discharge. Cold atmospheric plasma, referred to as plasma, is generated under mild conditions of temperature (sometimes ambient conditions) (Fernández and Thompson 2012). The food industry prefers a temperature around 30–60°C because of the low energy required to generate it, and this is the temperature of cold plasma (Misra *et al.* 2011). Plasma can inactivate the microorganisms by heat, charged particles, electric fields, UV photons and some reactive species such as atomic oxygen, metastable oxygen molecules, ozone and hydroxyl radicals, which are commonly found in a gas discharge (Georgescu *et al.* 2010).

Unlike microbiological decontamination limited research has been done on the successful degradation of OP pesticides by cold plasma and the degradation mechanisms are not fully understood. The degradation of dichlorvos and omethoate organophosphorus pesticides sprayed onto maize samples was studied by Bai *et al.* 2009. Recently, Bai *et al.* (2010) also demonstrated the successful degradation of dichlorvos pesticides coated on glass slides. Kim *et al.* (2007) reported the decomposition of paraoxon and parathion with an atmospheric pressure and radio-frequency plasma.

Apple and cucumber are the most commonly used agricultural products in Iran and throughout the world. To control the pests that attack these products, a lot of pesticides such as diazinon and chlorpyrifos are applied by growers in orchards and greenhouses. These two pesticides have undesirable effects on the environment and human health. Therefore, consumers are at risk from residues of these pesticides. In this study we evaluated the potential effects of cold plasma in reducing residues from pesticides such as diazinon and chlorpyrifos in apples and cucumbers.

Materials and Methods

Preparing standard concentrations of pesticides

Pesticides diazinon and chlorpyrifos from Sigma Corporation were used in these experiments. The samples

of standard concentrations of pesticides had 99.9% purity. One mg from each standard was dissolved in 1 cc ethyl acetate separately, and then different concentrations were prepared, namely 0.1, 1, 10, 100 and 1,000 ppm, which were then injected to HPLC.

Cold plasma Monopole Dielectric Barrier Discharge (DBD) device

The device shows the volume and has a screen in its front panel. The output voltage can be changed between 30 to 100% by volume. The voltages in 77 and 100% are 10 and 13 kV, respectively. The cold plasma was formed by creating an electric field with a high voltage power supply and reducing the distance between two electrodes. This device is a plasma generator for work under atmospheric pressure (air mixture). In this experiment the frequency of the device was constant in 13 kHz, and two voltages including 10 and 13 kV were considered as variables and in all experiments, the distance between two electrodes was 7 mm (Fig. 1).

Preparation of fruits and vegetables

The fresh fruit including apple and cucumber was prepared from organic product centers. Slices of the fruits and vegetables (apple and cucumber: 3 × 3 cm square, 0.5 cm thick) were cut with a knife. The size of the square samples of apple and cucumber was determined by the electrode size of the cold plasma device. All the samples were identical in weight and size.

Experiments

Two concentrations, 500 and 1,000 ppm, from two pesticides were used separately. The samples of apple and cucumber were dipped in the pesticide solutions for 2 min, then air dried and this procedure was repeated. There were two treatments: exposure to cold plasma and the control without cold plasma. Every sample exposed to cold plasma was introduced to the cold plasma Monopole DBD device with two voltages, 10 and 13 kV, for 2, 4, 6, 8 and 10 min. Slices of fruit and vegetables were put in a Petri dish and exposed to the plasma 7 mm from the exit nozzle of the device. Then we used a HPLC device for analysis of pesticide residues in the samples. Also the obtained extracts were injected into a gas chromatography/mass spectrometry (GC/MS) device to determine the generated metabolites.

Extraction and determination of pesticide residues

Liquid-liquid extraction (LLE)

In order to extract the pesticide residues from all of the samples we used LLE. Liquid-liquid extraction, also

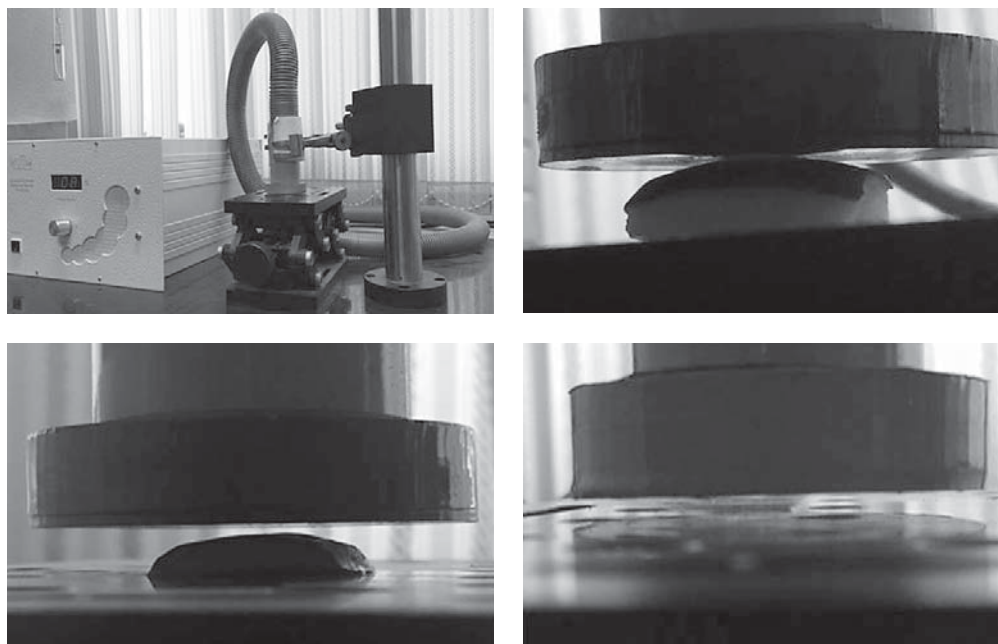


Fig. 1. Cold plasma device with apple and cucumber samples

known as solvent extraction and partitioning, separates compounds based on their relative solubility in two different immiscible liquids, usually water and an organic solvent. It extracts a substance from one liquid into another liquid phase. Separately each sample was pounded in a mortar. In these experiments, every solvent, including ethyl acetate, hexane and acetone, was added to the samples, separately. In the first step, 5 g of homogenized cucumber and apple samples and 5 ml ethyl acetate were blended inside a container and then shaken for 5 min. Then the container was stabilized until two phases were separated. In the above phase ethyl acetate was removed from the sampler. In the second step, 5 ml hexane was added to the solution and shaken for 5 min. After immobilization of the solution, the above phase (hexane) was removed from the sampler. In the last step, 5 ml acetone was added to the solution and the previous steps were repeated. Finally, in order to concentrate the extracted solutions, they were passed from anhydrous sodium sulfate.

Gas chromatography coupled to mass spectrometry (GC/MS)

Gas chromatography coupled to mass spectrometry is widely used in the analysis of pesticides that are highly volatile. In order to identify the potentially generated metabolite we used the GC/MS device model 5973 which included MS and capillary column (non-polar) Hp-5MS (Phenyl dimethyl siloxane) 30 mm in length, 250 μm in inner diameter and 0.25 μm in thickness of immobile phase. The operating conditions were: MSD detector; injection port temperature, 200–220°C; column oven temperature, 80–160°C ramped at 20°C \cdot min⁻¹, then 1.0 min at 160°C, followed by 10°C \cdot min⁻¹

from 160°C to 280°C; helium carrier gas (flow rate of 1.0 ml \cdot min⁻¹); with ionization achieved by electron impact at 70 eV in multiple reaction monitoring (MRM) mode and the mass range was 50–500 amu.

Measurement of physical and chemical properties

A direct drying method was applied to investigate the moisture content of the vegetable and fruit slices after plasma treatment. Fruit and vegetables slices were placed in dried weighing bottles and kept at 105°C for at least 7 h in a drying oven. They were then allowed to cool down to room temperature in desiccators for half an hour and weighed again. The difference in this final weight and the initial weight before drying is the moisture weight. This procedure was repeated in three consecutive cycles until the final and precise weight was obtained and as much moisture as possible was eliminated. The total moisture content was then defined as the difference between the initial weight prior to the first drying cycle and the final weight after the last drying cycle.

Changes in color parameters due to plasma exposure was another property that influence the marking of fruits and vegetables. In order to quantify the color differences of fruit and vegetable slices, the slices were examined with a spectrophotometer, model Xrite Sp-64 with D65 source of light. The device, based on the absorption of light in samples, measures parameters such as L (color transparency), a (green-red) and b (blue-yellow). The data were analyzed using color of control software. Finally the results were reported as the color difference between treated samples and control.

Another property that is important to the marking of fruits and vegetables is texture hardness. We used a ct3 texture analyzer made in the USA to investigate texture hardness of products that were exposed to cold plasma. We also measured the effect of cold plasma on Brix or solid material dissolved in apple fruits. For this purpose we used an ABBE refractometer. First, the refractometer was calibrated by using a drop of distilled water. Then a drop of the extract of samples was exposed to the device and in front of the light, the number of Brix solid material dissolved in apple fruits was recorded. This number mainly shows the amount of glucose produced within the fruits.

Analysis

The one-way ANOVA was done in factorial design in a completely randomized design with SPSS14 to analyze the effect of cold plasma on the degradation of

organophosphorus pesticide and the means were compared by Duncan's multiple range test.

Results

Effect of cold plasma on detoxification

Our results showed that the effects of the main factors such as type of fruit or vegetable, type of pesticide, concentrations, procedure and time on percentage of residue were statistically significant. The effect of type of fruit or vegetable was significant at level 5%, while the effects of other factors were significant with probability of 99 ($p < 0.001$). The high value of coefficient of determination ($R_2 = 0.994$) meant that the model was chosen well. This suggested that factors in the model cover more than 99% of all changes which affect parameters involved in the percentage of

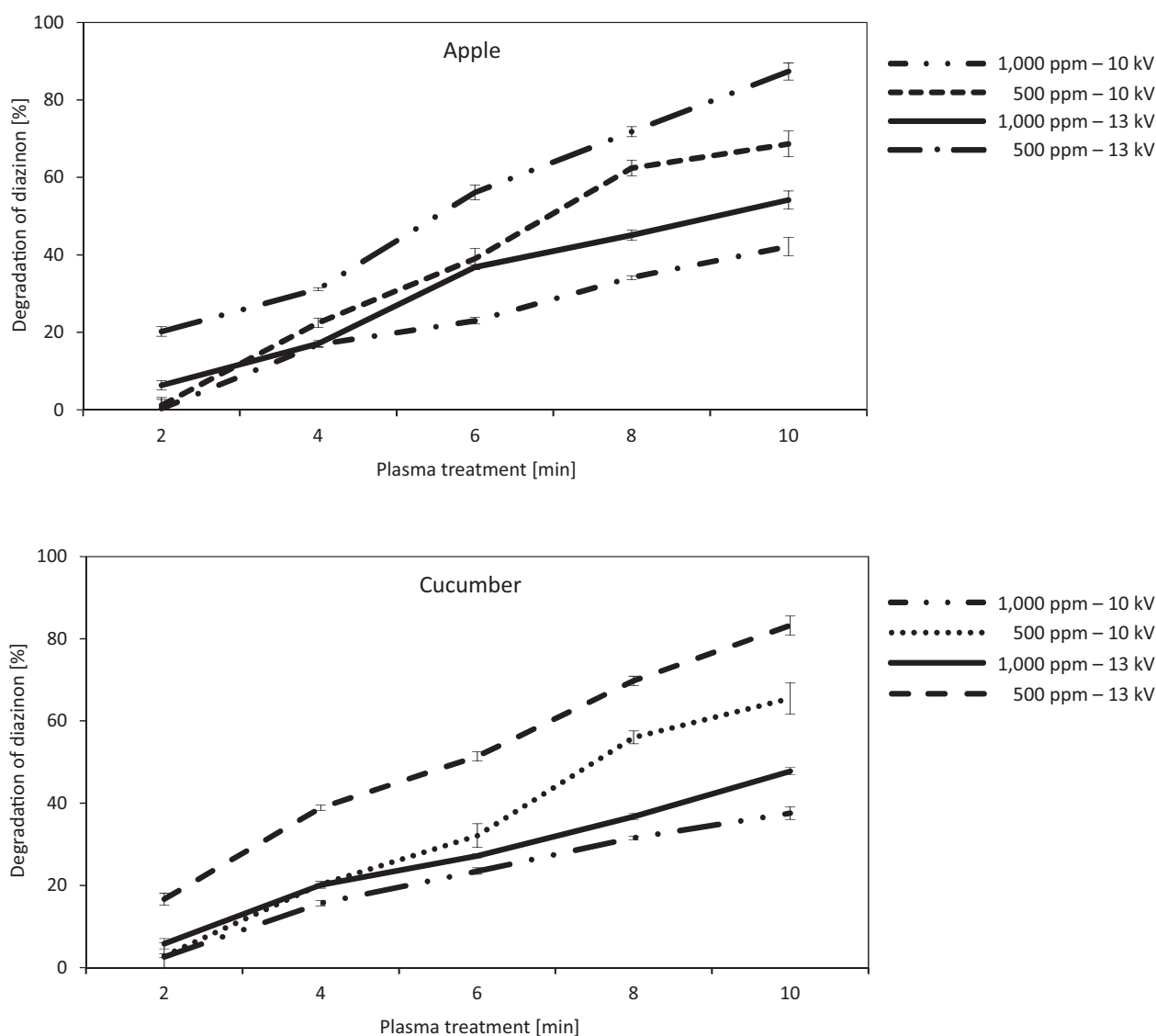


Fig. 2. Effect of the duration of cold plasma treatment on the degradation of diazinon at different voltages in apple and cucumber

detoxification of pesticide residues and a very small percentage of the variations are caused by random factors (errors).

Percentage of detoxification of diazinon in different products

According to Figure 2, with longer cold plasma treatment, from 2 to 10 min, the mean percentage of detoxification of diazinon in both concentrations and also both products increased. We found the highest percentage of detoxification with cold plasma treatment in apple for 10 min for 500 ppm at 13 kV and 10 kV. This reduced the residue of diazinon to 87.38% and 63.67%, respectively. These values for 1,000 ppm at 13 kV and 10 kV were 16.54% and 18.42%, respectively. Also, the percentage of detoxification of the residue of diazinon in cucumber for 500 ppm at 13 kV and 10 kV was 82.24% and 65.49%, respectively and for

1,000 ppm at 13 kV and 10 kV was 79.47% and 56.37%, respectively.

Percentage of detoxification of chlorpyrifos in different products

According to Figure 3, with an increase in the time of cold plasma treatment from 2 to 10 min, the mean percentage of detoxification for diazinon in both concentrations and also both products increased. According to our results, the best results for the percentage of detoxification by using cold plasma in apple, for 10 min for 500 ppm at 13 kV and 10 kV reduced the remaining of diazinon to 86.96% and 32.55%, respectively. These values for 1,000 ppm at 13 kV and 10 kV were 7.53% and 44.33%, respectively. Also, the percentage of detoxification of the residue of diazinon in cucumber for 500 ppm at 13 kV and 10 kV was 33.74% and 8.54%, respectively and for 1,000 ppm at 13 kV and 10 kV was 58.33% and 33.27%, respectively.

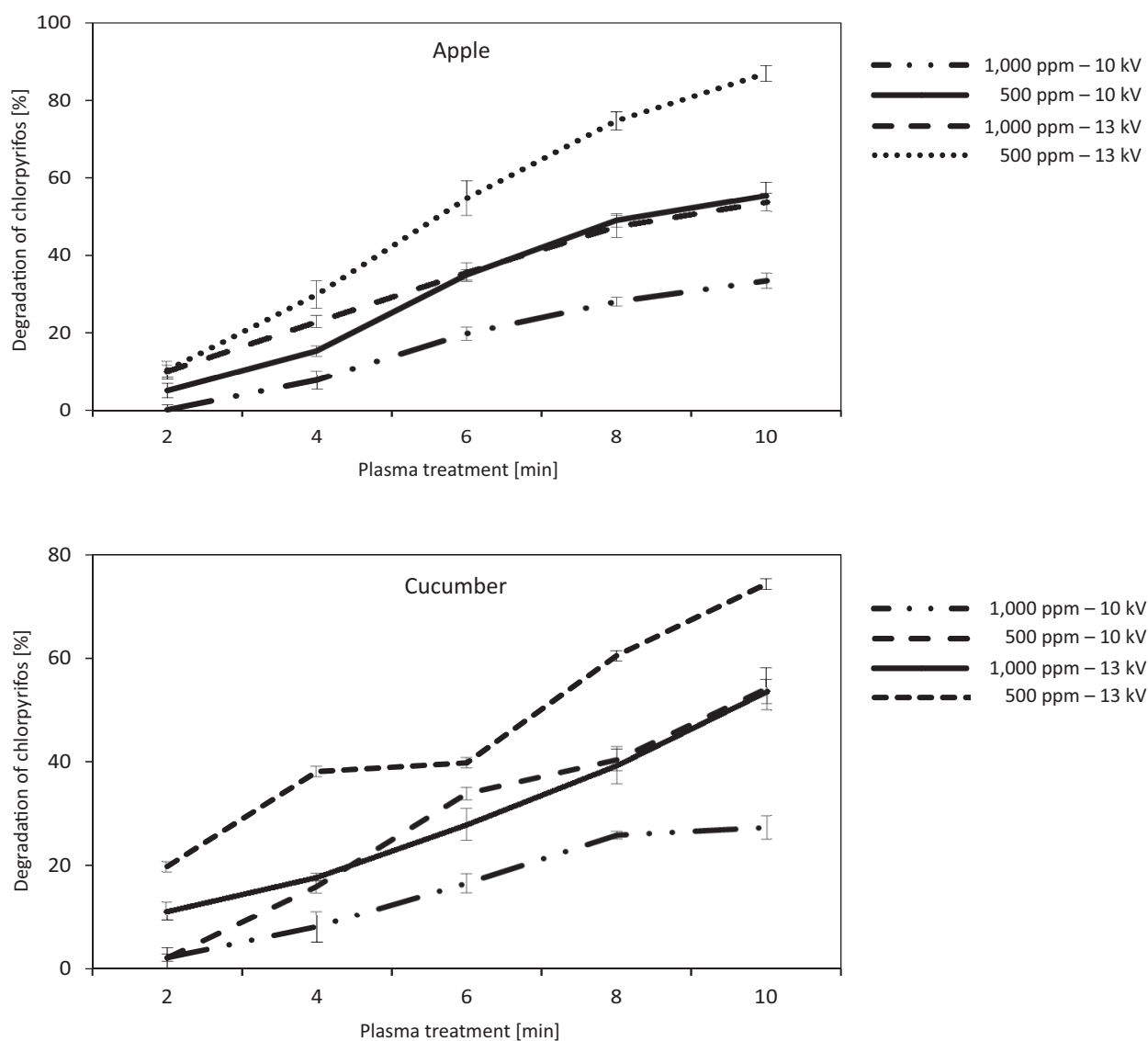


Fig. 3. Effect of the duration of cold plasma treatment on the degradation of chlorpyrifos at different voltages in apple and cucumber

Effect of cold plasma on moisture content of different products

With increasing the time of using cold plasma from 2 to 10 min, the mean moisture content of both products treated with cold plasma at 10 and 13 kV decreased slightly (2–3%) compared to control. The greatest decrease (2.6% in apple and 3.03% in cucumber) was found for the use of cold plasma at 13 kV. Therefore, as a result, cold plasma treatment had no significant effect on the moisture content of apple and cucumber ($p > 0.05$). In general, the mean of moisture content of cucumber in comparison with apple was reduced by about 0.43 (Fig. 4).

Effect of cold plasma on glucose content in apple

We did not observe any changes in glucose content of apple with longer use of cold plasma. As a result, cold plasma treatment did not affect the glucose content of apple significantly ($p > 0.05$) (Fig. 5).

Effect of cold plasma on texture hardness of different products

The texture hardness of apple and cucumber with an increase in the time that cold plasma was used from 2 to 10 min at 10 and 13 kV had no significant difference (1–1.5 unit) compared to the control. By using cold plasma at 13 kV, we observed the greatest decrease in texture hardness (1.75 unit in apple and 1.18 unit in cucumber) that was not significantly different from the control. According to our results, cold plasma treatment had no significant effect on texture hardness of apple and cucumber ($p > 0.05$) (Fig. 6).

Effect of cold plasma on the color of different products

Our results showed that cold plasma treatment had no significant effect on the color of apple and cucumber. Properties such as brightness, redness and greenness were not affected by using cold plasma on apple and cucumber ($p > 0.05$) (Fig. 7).

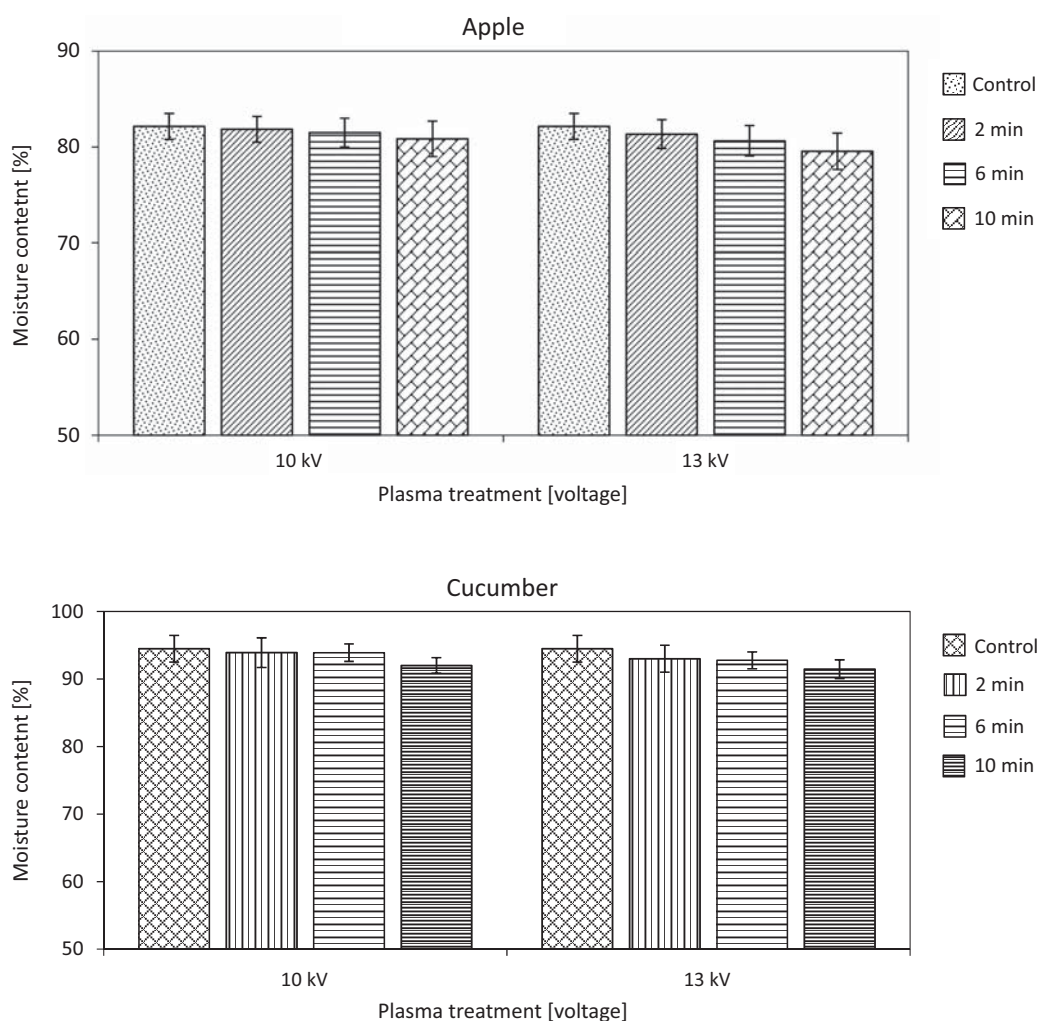


Fig. 4. Effect of cold plasma treatment on moisture content in apple and cucumber at different voltages

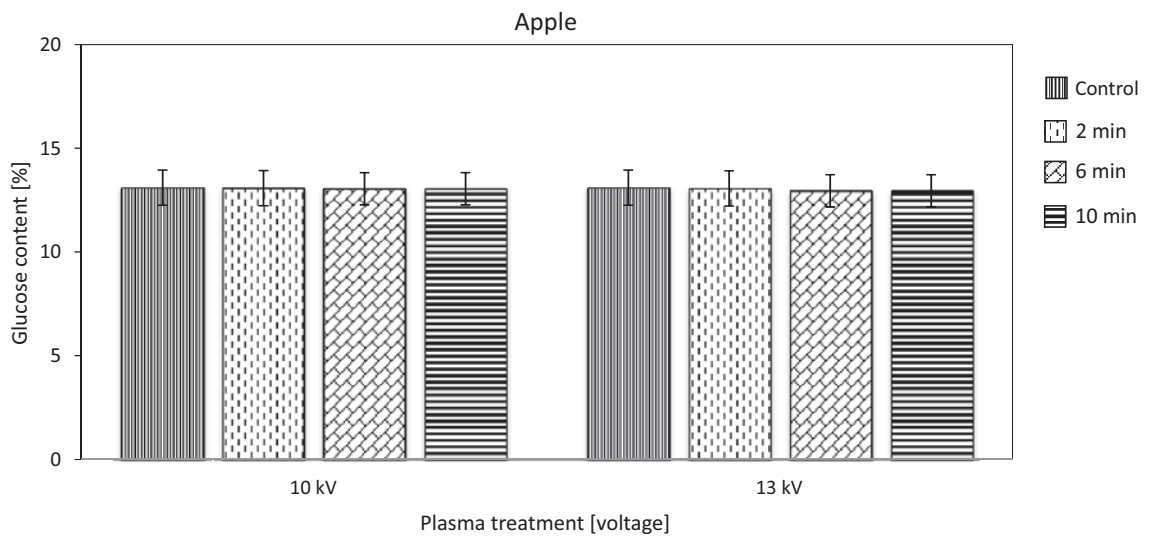


Fig. 5. Effect of cold plasma treatment on glucose content in apple at different voltages

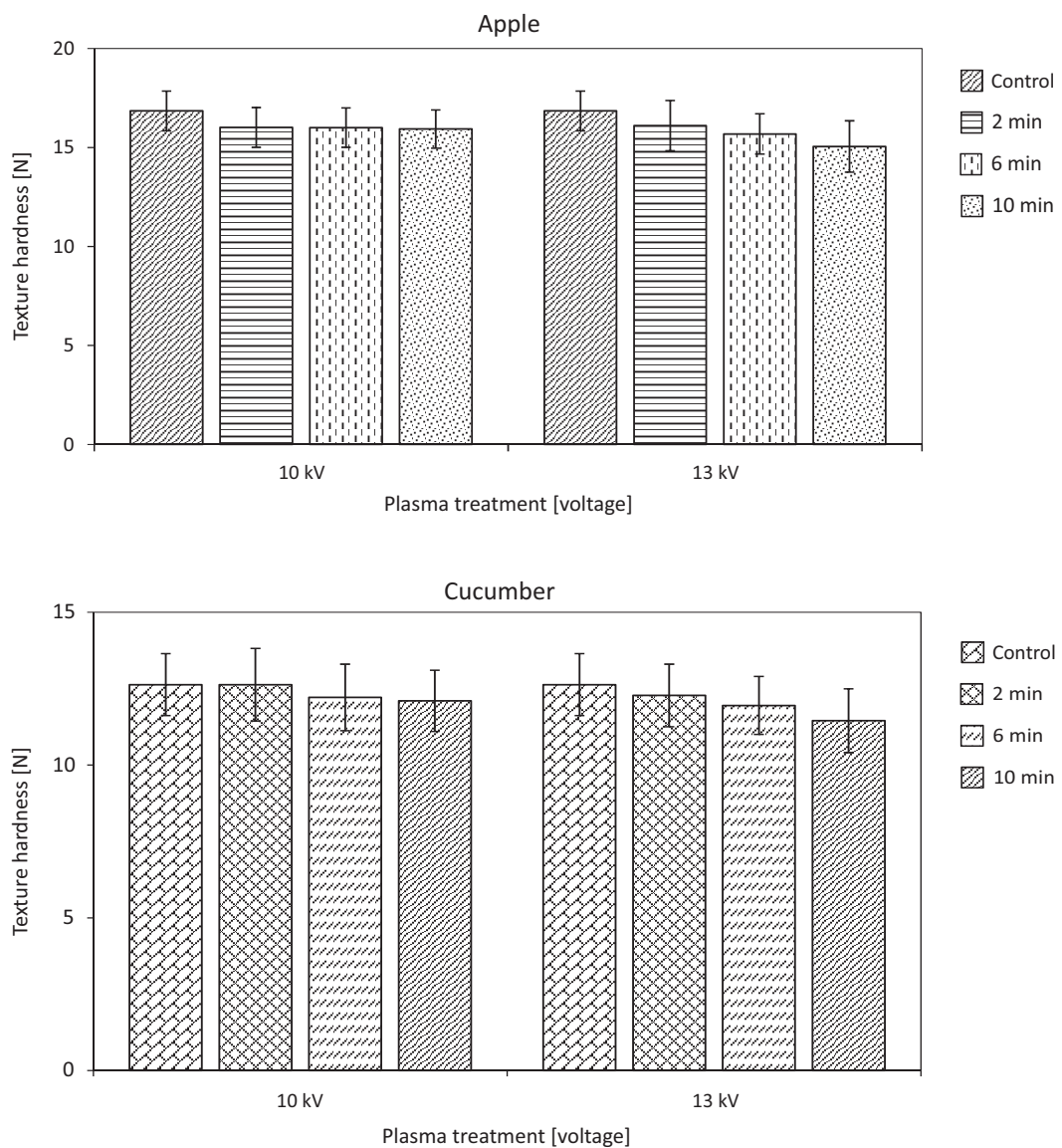


Fig. 6. Effect of cold plasma treatment on texture hardness in apple and cucumber at different voltages

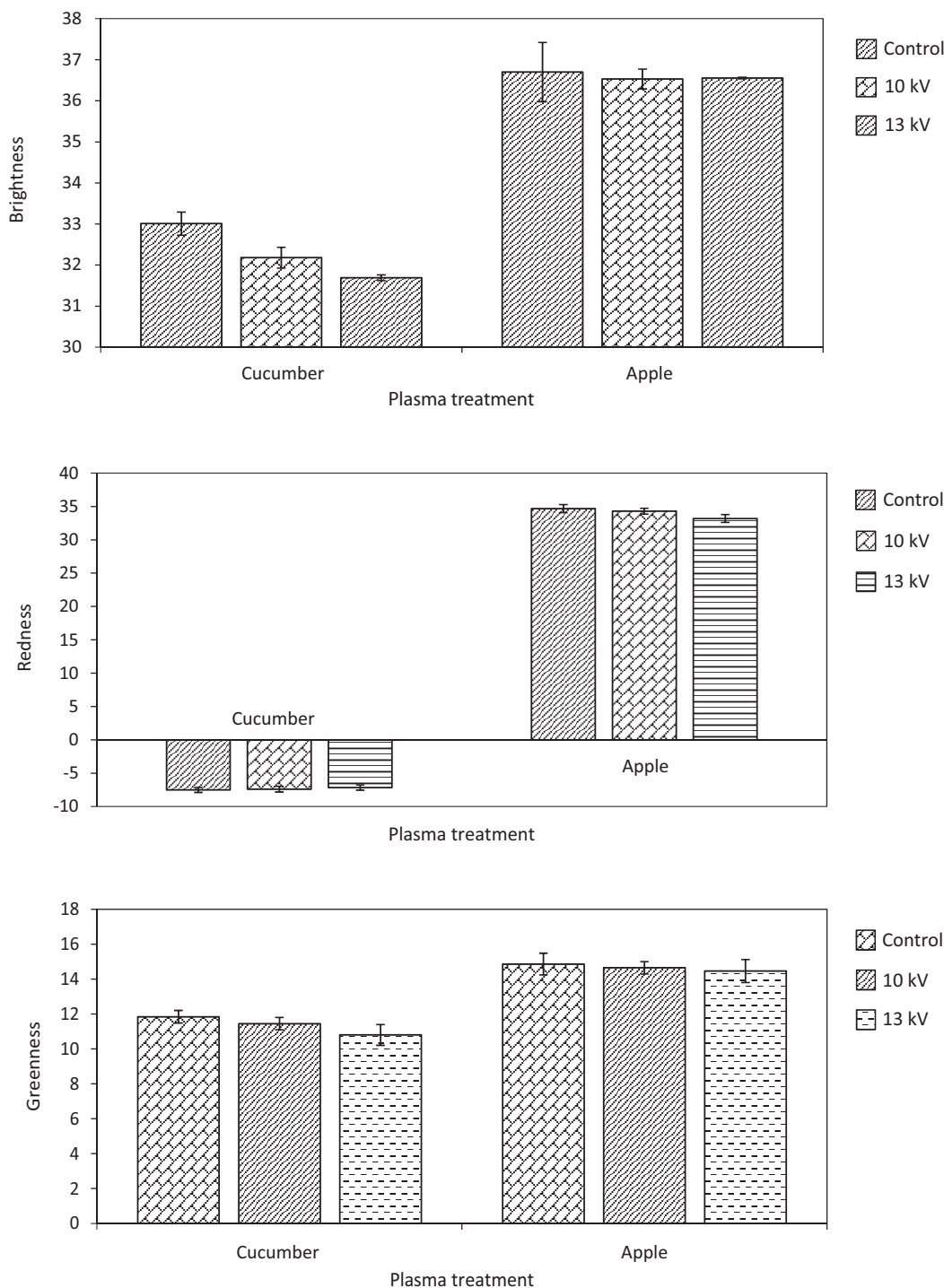


Fig. 7. Effect of cold plasma treatment on color in apple and cucumber at different voltages

The results of GC/MS device

In this research due to the insignificant amount of known metabolites, we only did a qualitative identification of metabolites with respect to the molecular weight of pesticides and their metabolites known by using GC/MS. Their names are listed below. We identified three known metabolites of diazinon [2-isopropyl-4-methoxy-pyrimidine, diazoxon and 2-Isopropyl-6-methyl-4-pyrimidinol (IMP)] and one

metabolite of chlorpyrifos, 3,5,6-trichloro-2-pyridinol (TCP) by GC/MS (Fig. 8).

Discussion

The results showed that the efficiency of different factors on pesticide detoxification varied, so that the duration of plasma treatment had an important role on

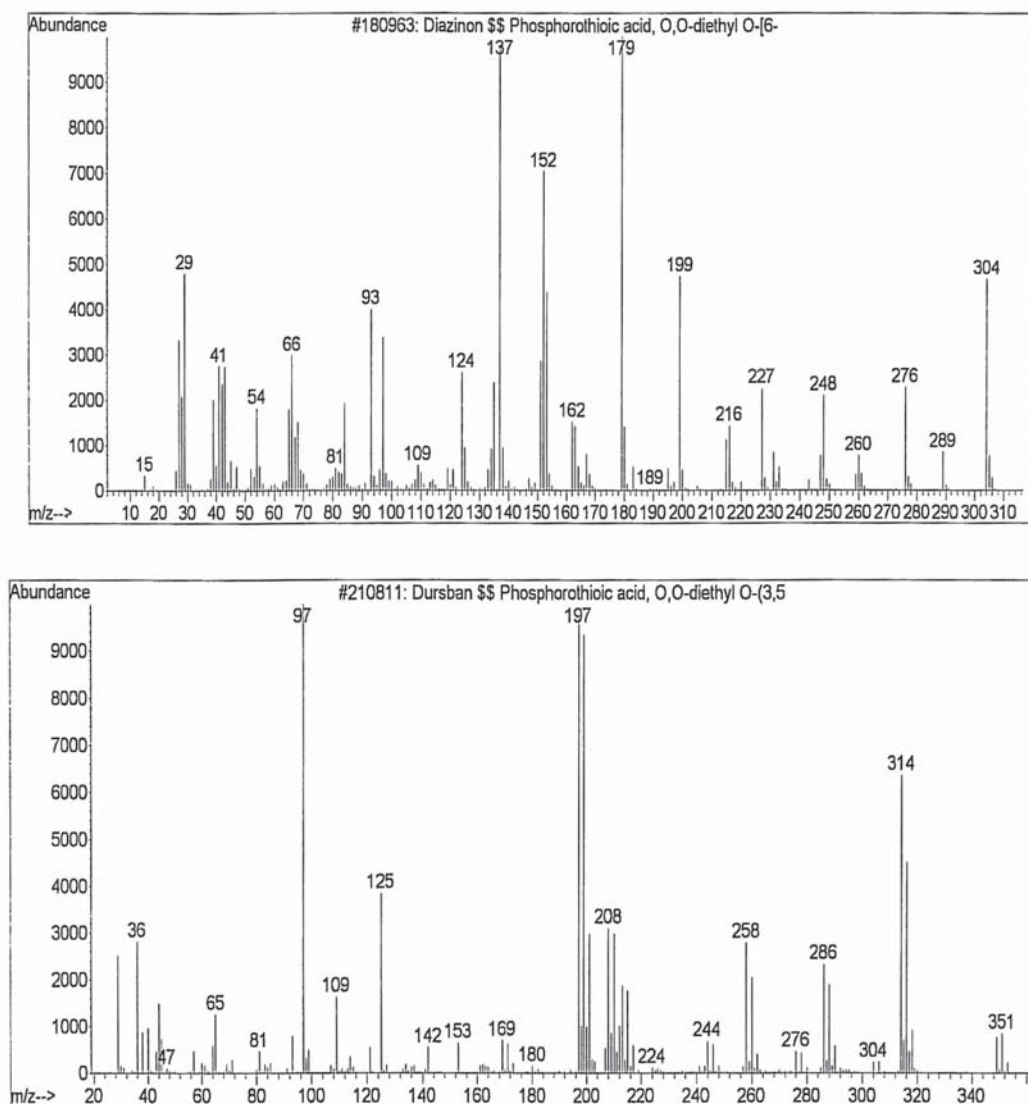


Fig. 8. Mass spectrum of diazinon and chlorpyrifos and their metabolites

detoxification. We found that as the duration of plasma treatment increased, the pesticide detoxification also increased. We observed that when the voltage of cold plasma was increased, the percentage of detoxification increased and 13 kV was more effective than 10 kV.

The pesticides used in this research were chosen from different groups. Diazinon and chlorpyrifos are non-halogenated and halogenated, respectively. Both pesticides are not systemic and are slightly soluble in water. Our results showed that the percentages of detoxification varied according to the type of pesticide, noticeably because of differences in the chemical and physical structures of pesticides such as molecular structure, solubility in water, polarity, penetration in plant tissues and stay away from the detoxification factors. We suggested that the percentage of detoxification of diazinon was more than that of chlorpyrifos. Our findings were similar to the results of other researchers such as Li *et al.* (2013), Misra *et al.* (2014) and Reddy *et al.* (2014). Li *et al.* (2013) showed that

nitenpyram (NTP) pesticide could effectively be removed from aqueous solutions by low-temperature plasma. The results of Misra *et al.* (2014) revealed that nonthermal plasma treatment using a dielectric barrier discharge successfully degraded pesticide residues such as azoxystrobin, cyprodinil, fludioxonil and pyriproxyfen on strawberries. The studies of Reddy *et al.* (2014) showed the potential of a non-thermal plasma generated in a DBD configuration for the removal of endosulfan. The percentage of degradation during the experiment at 60 min of plasma treatment in different concentrations of endosulfan and different voltages was between 83.6 to 85.2%.

According to our results, with increasing preliminary concentrations of pesticides, the percentage of detoxification with cold plasma decreased. At high concentrations of pesticides, the production of hydroxyl radicals that cause strong oxidation and decomposition rates was reduced. On the other hand with low concentrations of pesticides more molecules of toxins

exposed to cold plasma active oxidation agents, and result in an increase in the percentage of detoxification (Zhang *et al.* 2010).

We suggest that the detoxification in apple was 6% higher than cucumber. Different agricultural products have varying uptake and penetration of pesticides. In general it could be said that the pesticide accumulates in cucurbits such as cucumber, tomato, etc. more than other agricultural products. Also, because the apple has skin that is thinner than cucumber, the cold plasma active oxidation agents can penetrate more easily and more oxidation can happen than in the cucumber.

Investigation of the effect of ozone on residues of fenitrothion in lettuce, strawberry and cherry tomatoes showed that ozone could reduce the residue of the pesticide in lettuce more effectively than in the others. Cherry tomatoes have a pericarp harder than lettuce and strawberry that the dissolved ozone and free hydroxyls cannot penetrate and become inactive in contact with the skin of cherry tomatoes (Tamaki and Ikeura 2012).

The metabolites that we identified in this research were more polar and less toxic than the main pesticides. The identified metabolites, as a result of strong oxidizing agents in cold plasma, during the oxidation reaction such as hydroxylation and desulfuration (reaction) were turned into oxidized and hydroxylated compounds (Abu-Qare and Abou-Donia 2001; Koulombos *et al.* 2003; Li *et al.* 2015).

Our results about moisture and glucose content, the color of fruits and vegetables and texture hardness were similar to other studies. A slight decrease in the moisture content of samples treated with cold plasma resulted from air flow generated in the plasma at 30°C, the temperature at which partial evaporation of water from the surface of products occurs. A decrease in the moisture content of agricultural products also can decrease texture hardness. On the other hand, the decrease in brightness, redness and greenness of the studied products can be explained by the fact that the hydroxyl radicals formed in the plasma do not attack the molecules selectively, therefore they can influence pigments or anthocyanins. This subject depends on the length of treatment time with cold plasma. The decrease in skin brightness of products may be caused by decreased moisture content. A slight decrease in the glucose content of apple showed that the compounds within products only change on the surface of fruit, because the plasma does not penetrate deep (Wang *et al.* 2012; Bermúdez-Aguirre *et al.* 2013; Misra *et al.* 2014).

Conclusions

The results revealed that treatment of samples with cold plasma considerably reduced pesticides without any harmful, toxic or other undesirable effects on the

color and texture of the sample. Using plasma systems is a new way for detoxification. This method has many advantages, including the ability to detoxify at relatively low temperatures and in a short time. Another advantage is that it is non-toxic and safe for humans. The plasma used for detoxification was cold plasma, because the heavy particles (ions and neutral particles) have a lower temperature than the electrons in cold plasma. Therefore, the electrons can break the molecules and ionize the atoms and molecules of the gases without the need of heavy particles with high energy. The advantage of detoxification under atmospheric pressure is that they allow active species to be formed in abundance. Cold plasma treatment is a non-thermal technology that is used for heat-sensitive foods. Although plasma irradiation on fruits and vegetables can create desirable properties, at the same time long-term exposure may lead to a decrease in the shelf life. So, regarding the application of cold plasma on agricultural products we have to find the optimum time.

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