Effect of Gliding Arc Plasma on Plant Nutrient Content and Enzyme Activity

M. El Shaer,* M. Mobasher, & A. Abdelghani

PEARLZ (Plasma & Energy Applications Research Laboratory), Faculty of Engineering, Zagazig University, Zagazig, Egypt

*Address all correspondence to: M. El Shaer, Department of Engineering Physics and Mathematics, Faculty of Engineering, Zagazig University, 44599, Egypt; Tel.: +20-100-695-0077; Fax: +20552304987, E-mail: melshaer@link.net

ABSTRACT: In treating agro products by gliding arc plasma (GAP), most efforts are usually directed toward elimination of common pathogens. In this work we consider mainly the effect of GAP on valorization of nutritional values as enzymes and nutrients in fresh fruits. We analyze the effect of plasma on polyphenol oxidase (PPO) enzyme contained in plant cells of Golden Delicious apples. PPO enzyme is considered to be the main cause of apple browning. PPO enzyme content in treated apple slices and its residual activity are found to decrease with plasma treatment time as compared to control untreated samples. We consider also the effect of GAP treatment on nutrients as total polysaccharide content in apple and sugarcane specimens. Gradual increase of total polysaccharides with plasma treatment in those fruits could be related to effects of plasma active species on cell wall disintegration.

KEY WORDS: vegetables and fruits, nutrient, polyphenol oxidase, polysaccharides

I. INTRODUCTION

Plasma applications have been oriented in the last few years toward finding unconventional solutions to some inherent problems in many branches, seeking to enhance quality of life and increase availability of resources in a safe way.1 Processing of agro products is one of the fields where the use of plasma is beginning to contribute to raising standards and solving problems.2 During plasma treatment, one should ensure the safe nature and benefits of plasma processing of vegetables and fruits to insure the good health of consumers. Plasma can be involved in most stages of the food production cycle. Starting with soil treatment, where plasma activated water can have remarkable effects on growth of some crops.3 Plasma treatment of seeds can increase germination and promote plant growth.4 Microbial decontamination is the most popular application of plasma in food processing. Plasma exposure has largely demonstrated its efficacy in the inactivation of a wide range of microorganisms and foodborne pathogens.5 Benefits of plasma treatment have expanded to cover its use in food packaging,6 in which plasma can give some packaging materials interesting properties for food preservation. Many research activities have proved the efficacy of applying nonthermal plasma in food preservation for chemical-free elimination of common pathogens in vegetables and fruits.7 However, less work has been done in analyzing changes in food nutritional constituents and enzyme activities during plasma treatment. There is a need to analyze effects of plasma on plant cell enzymes and food nutrients, which may be altered during
plasma treatment. We consider here the effect of gliding arc plasma (GAP) on enzymes and nutrients in fresh produce fruits. As our model, we used polyphenol oxidase (PPO), which is contained in apple plant cells and may cause apple browning. As nutrients, we consider total polysaccharide content in apple and sugarcane. During plasma treatment by GAP, pH value and temperature of fresh produce specimens are measured.

II. MATERIALS AND METHODS

A. Experimental Setup

GAP used in this work operates in DC mode and consists of two knife-shaped stainless steel electrodes. Nominal values of operation are 3 kV and 2 A. A current limiting resistance of 500 Ω is connected in series with the power supply. The discharge occurs at atmospheric pressure under argon gas flow injected through a nozzle placed above the 3-mm gap at the neck between the two electrodes. During treatment, specimens are placed 13 cm from the extreme tips of the electrodes, in the afterglow region where the temperature is reasonably similar to that in the arc region. Therefore, specimens are not excessively heated during treatment with the highly reactive species, ozone, and UV radiation emitted by the plasma. The setup of the experiment is sketched in Fig. 1.

B. Extraction and Determination of Polyphenol Oxidase Activity in Apples

1. Enzyme Extraction

We used the enzyme polyphenol oxidase (PPO), which is contained in plant cells of apples. The enzyme extraction was carried out at 4°C to prevent loss of enzyme activ-

FIG. 1: Experimental arrangement of gliding arc plasma.
ity. PPO was extracted from apple using Triton X-100 detergent. Ascorbic acid was used as reducing agent of endogenous phenolic compounds. Finally, polyvinylpolypyrrolidone (PVPP) was used to prevent quinone formation. Samples, which were 10 g, were blended with 25 mL 0.1 M phosphate buffer, 0.2 g of ascorbic acid, 3% PVPP, and Triton X-100 for 10 min and left for 2 hr at 4°C in the dark. Samples were centrifuged at 4,800 rev/min for 5 min at 4°C; the supernatants were then collected and filtered with Whatman Grade 4 filter paper. The filtrate was used as the crude enzyme extract.

2. Assay for Polyphenol Oxidase Activity

PPO enzymatic activity was evaluated at 25°C by measuring the rate of increase in the absorbance at 420 nm, using the UV/VIS spectrophotometer (Shimadzu Corp, Tokyo, Japan). The activity was assayed after incubation for 2 min in 3 mL of reaction mixture, consisting of 2.7 mL of 0.6 M catechol (1,2-dihydroxybenzene) in 0.2 M sodium phosphate buffer (pH 5.5) plus 0.3 mL of the prepared enzyme. The reference cuvette contained only the substrate solution. The unit (U) for enzymatic activity was defined as a change of 0.001 in the absorbance value per minute under the conditions of the assay, and it was expressed per μg of fresh mass of fruit sample taken for extraction (U/μg protein/min). All determinations were performed in triplicate.

3. Protein Determination

Protein concentrations of the extracts were measured by the Bradford method, measuring optical density (OD) at 595 nm, with bovine serum albumin (BSA) as a standard.

C. Determination of Polysaccharides in Apples and Sugarcane

For the determination of polysaccharides, first 500 mg were taken from each sample and subjected to extraction with 25 ml of 80% ethanol at 80°C for 20 min. The supernatants were discarded to remove the soluble sugars and other soluble compounds. This last step was repeated six times. The residual alcohol-insoluble residue (AIR) was washed with distilled water and dried at 60°C for 24 hr. Approximately 10 mg of AIR was dissolved in 100 mL of distilled water, and 1 mL of 5% phenol was added, followed by 5 mL of concentrated H₂SO₄ to 1 mL of the sample solution, then the absorbance was measured after 10 min at 485 nm against the blank solution. Then the results were compared with standard solution of glucose. The standard curve preparation is made by placing 10 mg of pure glucose in a 100-mL volumetric flask and diluted up to the mark with deionized water; 1 mL of 5% phenol solution was added to 1 mL of this sugar solution, followed by 5 mL of concentrated H₂SO₄. After 10 min, the absorbance was measured at 485 nm against a blank. The comparison between the standard curve of the glucose and the curve obtained from the absorption of the dissolved AIR at 10 min against a blank gives the polysaccharide content in each sample.
III. RESULTS

Golden Delicious apple slices a few millimeters thick are exposed to GAP argon plasma at atmospheric pressure for different time intervals. PPO enzyme contents and activities in control and plasma-treated samples were extracted and analyzed using the methods just described. PPO content in treated apple slices were found to decrease with plasma treatment time compared to control one, as shown in Fig. 2.

![Graph showing PPO enzyme contents versus plasma treatment time for Golden Delicious apples.](Plasma Medicine)

Residual PPO activity percentage changes of apple specimens due to plasma treatment are calculated using the following simple relation: % residual PPO activity = (PPO activity during plasma treatment ÷ activity without plasma) × 100. Residual PPO activity percentages, (Fig. 3), are found to decrease with treatment time. The slope of PPO activity reduction is fast in the first 180 sec and then becomes much slower to reach an activity reduction of around 80% after 420 seconds of plasma treatment time as compared to untreated control specimen. This result is found at nearly constant pH value of 4 during plasma treatment of the apple samples. The value of the temperature at the treatment position in the afterglow region is of reasonable value for biomaterials treatment compared to the value in the arc region. Results of infrared sensor giving temperature distributions around the electrodes in the arc region and expansion zone are given elsewhere. The maximum temperatures were found to occur in the central region of the gliding arc plasma column at the thermal zone. The minimum temperatures were found at the outer contour of the gliding arc plasma column where the nonthermal zone is supposed to occur. The apple temperature, measured in the afterglow region at specimen
position was found to increase reasonably from an environmental temperature of 17°C to around 30°C after 420 sec of plasma treatment. Total polysaccharide content during plasma treatment in Golden Delicious apples (Fig. 4) and sugarcane (Fig. 5) show gradual increase with plasma treatment time as compared to control untreated samples.

In Fig. 6, sugarcane samples 10 cm long and 1 cm thick were examined with scanning electron microscop (SEM). Figure 6a shows a control specimen. Figures 6b to 6e show the effect of GAP for time intervals of 1, 3, 5, and 7 min of plasma exposure. In

![Graph showing residual PPO activity versus plasma treatment time for Golden Delicious apples.](image1)

**FIG. 3:** Residual PPO activity versus plasma treatment time for Golden Delicious apples.

![Graph showing total polysaccharide content in Golden Delicious apples during plasma treatment.](image2)

**FIG. 4:** Total polysaccharide content in Golden Delicious apples during plasma treatment.
Fig. 6a, the control sample shows regular shape of cells in the form of boxes. In Fig. 6b, after 1 min treatment, cracks begin to appear. After 3 and 4 min in Fig.s 6c and 6d, cell membranes become increasingly irregular. In Fig. 6e, cell membranes completely collapse.

**FIG. 5:** Total polysaccharide content in sugarcane during plasma treatment.

**FIG. 6:** SEM photos for sugarcane specimens treated for different plasma exposure times: (a) control; (b) 1 min; (c) 3 min; (d) 5 min; (e) 7 min.
The concentration of ozone ($O_3$) versus plasma treatment time has been measured in air at the location of the sample with an Eco Sensors A-22 Portable Ozone Meter, which has a range of 0.03 to 20 ppm. The ozone concentration versus plasma treatment time, plotted in Fig. 7, shows a gradual smooth increase.

GAP produces chemical active species. Figure 8 shows spectral intensity of some lines during operation under the flow of argon gas in air measured using a fiber optics spectrometer (AVANTES AvaSpec-ULS2048 StarLine).

FIG. 7: Ozone concentration versus plasma treatment time.

FIG. 8: Measured optical emission spectra for GAP working with argon at atmospheric pressure.
IV. DISCUSSION

During the postharvest period, apples can deteriorate because of the release of polyphenol oxidase (PPO), an enzyme contained in plant cells. In the presence of oxygen, this enzyme induces the formation of melanin in the form of brown pigment in apple tissues. Reducing the activity of this enzyme by plasma treatment could help preserve the nutritional value of apples during storing and processing in industrial food applications. Several methods can be used to inactivate PPO to prevent legumes and fruits from browning. First, simply dipping them in a water bath prevents oxygen from reacting with the tissue. The apples may also be heated. In the food industry, substances such as sulfites may be added to prevent melanin formation, or ascorbic acid (vitamin C) may be added to act as an antioxidant and prevent oxygen from reacting in presence of PPO. Citric acid and acetic acid will lower the pH of plant tissue, leading to a decrease in enzyme activity, which retards or even suppresses the action of PPO on plants.\textsuperscript{13} GAP discharge occurring at atmospheric pressure in air under argon gas flow generates nonthermal plasma, which will have beneficial effects on lowering PPO. During plasma treatment, PPO, which is a proteinaceous enzyme, is subjected to the actions of reactive species produced in plasma as reactive oxygen species (ROS), atomic oxygen, and hydroxyl radicals. This can lead to oxidation of amino acid residues on side chains, formation of protein-protein cross-links, and oxidation of the protein backbone resulting in protein fragmentation and lowering of PPO enzyme activity by plasma.\textsuperscript{14} Similar behavior of PPO activity with plasma was found by Surowsky et al.\textsuperscript{15} and Tappi et al.\textsuperscript{16} This result has been confirmed by comparing the color change of two apple slices, one untreated and the other treated for 7 min with plasma. The photo in Fig. 9 has been taken after letting the two samples sit for 3 hr in ambient conditions. We notice more pronounced browning for the untreated sample compared to the one treated with plasma.

![Fig. 9: Photo of apple slices. Left, control untreated specimen; right, plasma treated specimen for 7 min; both were left 3 hr in ambient conditions.](image-url)
Effects of plasma on sugarcane cells are shown in Figs. 6a to 6e. Plasma affects cell size and shape, as observed on SEM photos for different treatment times. As plasma treatment time increases, cellular shapes become more irregular and even ruptured after longer exposure time, shown in Fig. 6e. This may explain the increase in polysaccharides in the treated samples shown in Fig. 5.

In order to understand the effect of plasma on plant cells, an analogy can be made with phenomena accompanying the application on plants of the well-studied pulsed electric field (PEF). The phenomenon of electroporation occurring due to PEF on the cell membranes causes the charges to accumulate on the outer and inner cell membranes. This may alter cell membrane properties, leading to an increase in permeability of cell walls and subsequent increase of nutrients due to diffusion of inner plant cell components from intracellular to extracellular medium. The effect of PEF on the disintegration of sugarcane cells reported by Eshtiaghi et al. is similar to the results obtained with GAP processing.

V. CONCLUSION

More efforts should be focused on controlling enzymatic browning in vegetables and fruits. Plasma has been proven to reduce the effects of PPO, the enzyme responsible for apple browning. Stability of plasma-treated apple samples after long-term storage should be examined. Plasma may bring some benefits, considering food nutrition facts, but additional experiments are required to determine whether unwanted by-products are generated. Extensive work should be done to provide evidence to the food industry and consumers about the safe and profitable nature of plasma treatment of vegetables and fruits.

ACKNOWLEDGMENTS

Apple enzyme activities were measured in central labs of the faculty of agriculture, Ain Shams University, Cairo. Electron microscopy measurements are done at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Authors wish to acknowledge the contribution of the Research Development and Innovation Programme of the Egyptian Ministry of Scientific Research funded by the European Union, grant RDI2/S2/038.

REFERENCES