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Effect of gliding arc on plant cell enzyme activity

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Abstract: Gliding arc plasma operating at atmospheric pressure under argon gas is used for treatment of vegetables and fruits. Plasma by its ability to generate active species is found to influence enzyme activity in apple cell tissue. The activity of polyphenoloxidase, which is the enzyme responsible of favourizing apple browning, has been reduced by exposing apple slices to gliding arc plasma at moderate temperature without any additives.

Keywords: gliding arc, atmospheric pressure plasma, enzyme, polyphenoloxidase

1. Introduction

Work has been extensively performed in describing the effect of plasma against microorganisms [1,2]. Many research activities have proved the efficacy of applying nonthermal plasma in food preservation for elimination of common pathogens in vegetables and fruits [3,4]. However less work have been done in analysing changes in food nutritional constituents and enzymes activities during plasma treatment [5,6,7]. There is a need to analyse effects of plasma on food nutrients and plant cell enzymes that can be altered during plasma treatment either positively or negatively. We consider here the effect of gliding arc plasma on fresh apples. During postharvest period, apples can be deteriorated by the release of an enzyme called polyphenoloxidase (PPO) contained in plant cells. In the presence of oxygen, this enzyme cause the formation of a brown pigment in apple tissue called melanin [8]. Reducing the activity of this enzyme by plasma treatment could be profitable concerning the preservation of apple nutritional values during storing and industrial food processing.

2. Materials and methods

2.1 Experimental setup

Gliding arc plasma discharge have been used in many applications related to food preservation [9]. Gliding arc is considered as the transition between thermal and nonthermal plasma, this combine the ability of producing energetic reactive species together with mild treatment of bio-products without excessive heating. The gliding arc used in this work consists of two knife-shaped stainless steel electrodes as shown in Fig. 1. The arc is operated at DC mode. The nominal values of operation are 2 kV and 1 Amps. A limiting resistance of 500 Ω is connected in series with the applied voltage. The discharge occurs at atmospheric pressure under the flow of argon gas injected through a nozzle placed above the 3-mm gap at the neck between the two electrodes. The distance between electrodes extreme tips and specimen position is set fixed at 13 cm. During plasma treatment, temperatures are measured at specimen position by an infrared thermometer and in the electrodes region by an infrared thermal sensor FLIR A5sc.

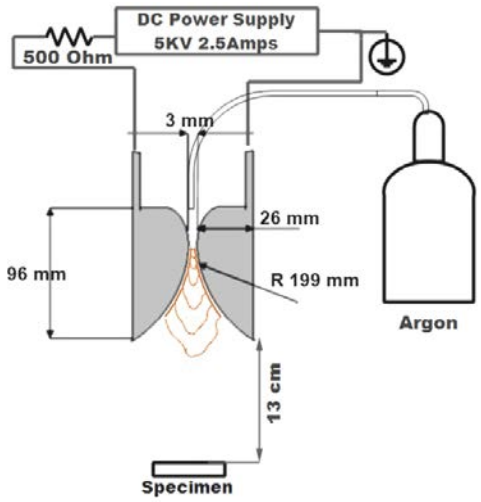

Fig.1. Gliding arc discharge

2.2 Enzyme extraction

One gram of apple pulp ground in liquid nitrogen was homogenized with 25 ml of 0.05 M phosphate buffer (pH 7) and left for 2 hours at 4 °C in the dark. The homogenates were then centrifuged at 4800 rev/min for 5 minutes, then supernatants were used for assay. The protein content was determined in preparation used for PPO assay by the colorimetric method described by Bradford [10]. The values were obtained by graphic interpolation on a calibration standard curve with bovine serum albumin (BSA) at 595 nm.

2.3 Determination of PPO activity

PPO activity is defined as the change of 0.001 in the absorbance value per minute under the conditions of assay and expressed per µ gram of fresh mass of fruit sample taken for extraction (U/µg protein/min). PPO activity was determined at 25 °C by measuring the rate of increase in the absorbance at 420 nm, using the spectrophotometer. The activity was assayed after incubation for 2 minutes in 3 ml of reaction mixture, consisting of 2.7 ml of M catechol (1,2 dihydroxybenzene) in 0.2 M sodium

phosphate buffer (pH 5.5) plus 0.3 ml of the prepared enzyme.

3. Results

Golden delicious apples are used in this study. Apple slices are exposed to gliding arc argon plasma at atmospheric pressure for different time intervals. PPO enzyme contents and activities in control and plasma treated samples are extracted and analysed using the above described methods. Enzyme contents in treated apple slices are found to decrease with plasma treatment time compared to control one, as shown in Fig. 2.

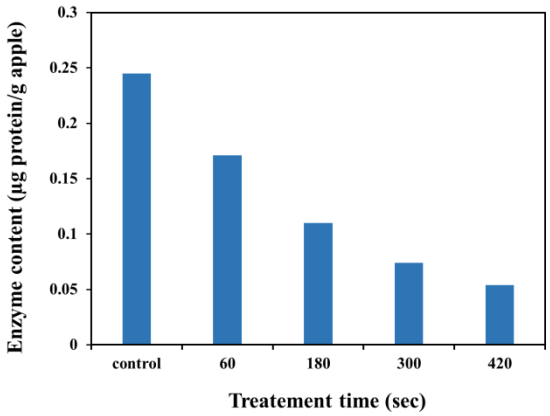

Fig. 2. PPO enzyme activity versus treatment time

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) x 100.

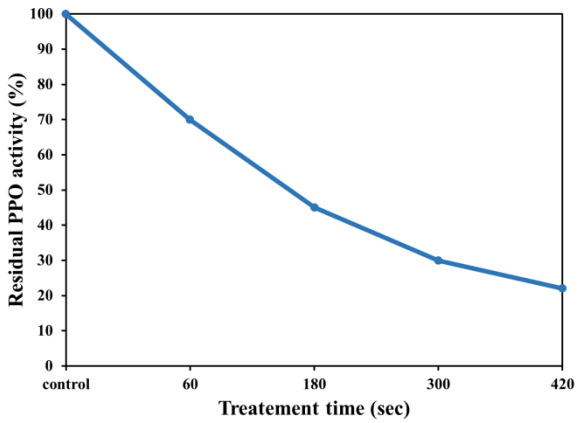

Fig. 3. Residual PPO activity versus treatment time

Residual PPO activity, as shown in Fig. 3, is found to decrease with treatment time. The slope of PPO activity reduction is faster in the first 180 seconds and become much slower after that to reach an activity reduction of around 80% at 420 seconds of plasma treatment time as compared to control untreated specimen. This result is found at nearly constant apple pH value of 4 during plasma treatment. A similar behaviour of PPO activities with plasma was found by B. Surowsky et al [5].

In Fig. 4, the apple temperature is shown to increase reasonably from an environment temperature of 17 °C to reach around 30 °C after 420 seconds of plasma treatment. Results of infrared sensor giving temperature distributions around electrodes and arc regions are given elsewhere [11]. The maximum temperatures were found to occur in the central region of the gliding arc plasma column at thermal zone. While, the minimum temperatures were found at the outer contour of the gliding arc plasma column where the nonthermal zone is supposed to occur.

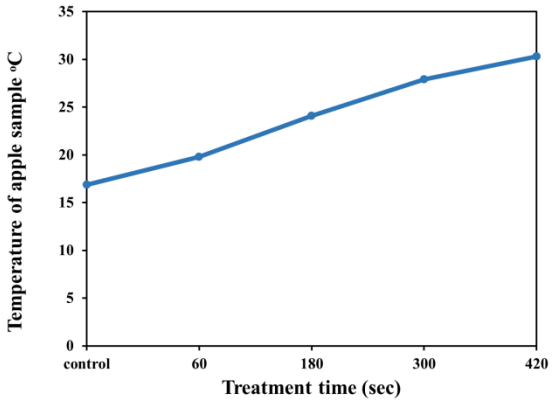

Fig. 4. Temperature increase during plasma treatment

4. Discussion

Some legumes and fruits when peeled or cut release PPO enzyme. In the presence of oxygen of ambient air, this enzyme catalyses plant phenolic compound to form brown pigment substance called melanin [12].

Several methods can be used to prevent legumes and fruits from browning. This can be achieved by simply dipping in water bath for preventing oxygen to react or by heating which inactivate PPO. In food industry, substances may be added as sulfites to prevent melanin formation or ascorbic acid (vitamin C), which acts as antioxidant and prevents oxygen from reacting in the presence of PPO. Citric acid and acetic acid lower the pH of plant tissue, this will decrease the enzyme activity and will retard or even suppress the action of PPO.

Nonthermal plasma occurring at atmospheric pressure will also have beneficial effects on lowering PPO which may hinder apple browning. Gliding arc discharge occurring at atmospheric pressure in air under argon gas flow generates many reactive species as reactive oxygen species (ROS), and reactive nitrogen species (RNS) as well as Ozone (O_3), atomic oxygen (O) and hydroxyl radical (OH). PPO enzyme, being a form of protein, is subject to ROS, atomic oxygen and OH radicals during plasma treatment. This can lead to oxidation of amino acid residue side chains, formation of protein-protein cross-linkages, and oxidation of the protein backbone

resulting in protein fragmentation [13]. This may explain the lowering of PPO enzyme by plasma.

This result is confirmed by comparing the color change of two apple slices, one as control and the other treated by plasma for 7 minutes. The two slits are left in ambient conditions. The photo in Fig. 5 have been taken after three hours. We notice more pronounced browning for the untreated sample compared to the plasma treated one.

Fig. 5. Photo of apple slices. Left control untreated specimen and right plasma treated specimen for 7 minutes, both left 3 hours in ambient conditions.

5. Conclusion

More efforts should be done to control enzymatic browning in vegetables and fruits. Plasma has proved its ability to reduce polyphenoloxidase, the enzyme responsible of apple browning. Stability of plasma treated apple samples after long time storing should be proved. Plasma may bring some benefits considering food nutrition facts, but one should assess that no unwanted by-products will be generated. Extensive work should be done to provide evidences to food industry and consumers about safe and profitable natures of plasma treatment of vegetables and fruits.

6. Acknowledgments

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7. References

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