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Kinetics of inactivation of peroxidase and polyphenol oxidase in tender coconut water by dielectric barrier discharge plasma

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Running title: Effect of cold plasma on tender coconut water

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ABSTRACT

The spoilage enzymes in tender coconut water were inactivated by atmospheric cold plasma technology. The kinetics of peroxidase (POD) and polyphenol oxidase (PPO) inactivation was studied at voltage levels varying between 18 and 28 kV dielectric barrier discharge plasma treatments (DBD) at atmospheric air. The time of treatment and applied voltage were significant factors for inactivation of the enzymes. POD was more resistant than PPO. The experimentally observed data were fitted to establish different kinetics models and model parameters were evaluated. The sigmoidal logistic was the best fitting model to explain the kinetics of browning enzymes inactivation based on high RMSE values. The time required for half maximal activity values for POD was 0.84, 1.67 and 2.53 min at 18 kV, 23 kV and 28 kV, respectively which was higher than PPO with half maximal activity values of 0.67, 1.18 and 1.35 min, respectively. This indicates that POD is more resistant to cold plasma than PPO and its inactivation in tender coconut water by cold plasma can be considered as a crucial

quality parameter. DBD generated cold plasma can therefore be used to process fruits and vegetables juices where in enzymes activity is one of the quality deterioration parameters.

Keywords: Cold plasma, peroxidase, polyphenol oxidase, tender coconut water, inactivation model

1. Introduction

Tender coconut *(Cocos nucifera L.)* water is a widely consumed refreshing beverage. Its unique flavour and presence of all essential nutrients makes it very popular among consumers (Mahnot et al., 2014). The beverage is useful for health and medicinal purposes due to its significant antioxidant, anti-ageing, anti-carcinogenic, anti-thrombotic effects and balanced electrolytes (Jean et al., 2009).

Among the various intrinsic enzymes, POD and PPO being more stable are more responsible for spoilage through biochemical reactions and are taken as indicators of effectiveness of thermal processing (Matsui et al., 2007; Robinson, 1991). These enzymes are known to adversely change the sensory, nutritional and textural properties (Matsui et al., 2007; Misra et al., 2016). PPO is responsible for browning and discoloration (McEvily et al., 1992), whilst POD catalyses peroxidation reaction wherein the generated end products give off-flavour in food products (Pankaj et al., 2013).

Heat treatment is conventionally used for inactivation of the enzymes (Anthon & Barrett, 2002); however adversely affects sensory and nutritional qualities of food products. Researchers are therefore, interested in novel nonthermal technologies for enzyme inactivation like high pressure processing (Bermejo-Prada at al., 2014), gamma irradiation (Jha et al., 2013), pulsed electric fields (Samaranayake & Sastry, 2016), ultraviolet light (Augusto et al., 2015) and sonication (Cao et al., 2018). Many of these non thermal technologies are complex, expensive and difficult for commercial scaling up (Pankaj et al., 2013).

Cold plasma is a nonconventional technology found to be effective for decontaminating foods. Surowsky et al. (2014) inactivated *Citrobacter freundii* in apple juice using cold plasma and found that Weibull model was not good for low amount of *Citrobacter freundii*. Cold plasma was found to stabilise wheat germ by inactivating lipase and lipoxygenase which is desirable for extending the shelf life (Tolouie et al., 2018). Pankaj et al. (2013) reported the effects of cold plasma from DBD to inactivate tomato POD and also studied the kinetics of tomato POD inactivation at different voltages (30, 40 and 50 kV) for different time intervals (15 s to 5 min). The authors found logistic model to properly describe tomato POD inactivation. Up to 45% reduction in PPO activity as compared to the control in cold plasma treated fresh cut samples (Tappi et al., 2014) and up to 17% reduction in POD in cold plasma treated fresh cut melon (Tappi et al., 2016) have been reported. Vukic et al. (2017) studied the color changes of orange and carrot juice blend due to atmospheric air DBD plasma treatment and they reported that the DBD reduces cloud loss and can cause minor degradation of color attributes. Segat et al. (2016) reported that dielectric barrier discharge-atmospheric cold plasma treatment at high voltages (40, 50 and 60 kV) for durations ranging between 15 s and 5 min had significantly inactivated alkaline phosphatase enzyme and the enzyme inactivation kinetics was best described by Weibull method.

The effects as well as the kinetics of PPO and POD activity in tender coconut water using DBD at cold atmospheric pressure and modelling of the inactivation have not been reported. This study was undertaken (i) to investigate the parameters involved in the inactivation of POD and PPO in tender coconut water, (ii) to model kinetics of enzymes activity, and (iii) to compare the kinetics of activity of POD and PPO.

2. Materials and methods

2.1. Materials

Fresh tender coconut (*Cocos nucifera L.)* was harvested from plants grown in a nearby area of Tezpur University, Assam, India. Tender coconut water had a pH of 5.20 ± 0.2, (pH meter model PB 11, Sartorius), and soluble solids content of 3.95 ± 0.25 ^{o}Brix (refractometer model Erma, Tokyo, Japan).

2.2. Preparation of sample

The water from the coconut was collected following the method of Mahnot et al, (2014) with slight modification. Two days before processing, formaldehyde and potassium chromate were used to clean the working area to prevent contamination of coconut water. The nuts were thoroughly rinsed with tap water and sanitized by dipping in 300 mg/l sodium hypochlorite solution. The processing equipment were sterilized in an autoclave before use.

2.3. Cold plasma treatment

In the experimental setup as shown in Fig. 1, the DBD plasma system comprised of two square copper plate electrodes, with an area of 225 cm^2 and thickness of 5 mm and was covered with glass dielectric plate (2 mm thickness). A high voltage power supply (0-50 kV, 50 Hz, Zenoics Systech, India) gave power to the upper electrode and the lower electrode was grounded. An uncovered Petri plate (90 mm diameter) containing coconut water (15 ml per plate) was placed between the two dielectric plates with a gap of 15 mm. Stable and uniform air plasma discharge was obtained across the discharge gap at atmospheric pressure (1 bar) and at different applied voltages (Break down voltage for the current set-up was above 12 kV) of 18 kV, 23 kV and 28 kV. All treatment processes were carried out at relative humidity (RH) of 58% and temperature of 27°C using a humidity-temperature probe (Testo 176T2, UK).

2.4. Enzyme activity and inactivation kinetics

POD and PPO activities were measured using the modified spectrophotometric method described by Purkayastha et al. (2012). Inactivation kinetics of POD and PPO reported by Pankaj et al. (2013) was followed.

2.5. Statistical analysis

The data obtained were statistically analysed by ANOVA test in SPSS 24.0 (SPPS Inc., Chicago, IL, USA). The model parameters for all equations were estimated by non-linear least squares regression using Microsoft Excel Solver (Microsoft office, USA). The goodness of fit was determined from adjusted coefficient of determination, R^2 (adj) and the adequacy of the model fittings was indicated by root mean squared error (RMSE). Higher R^2_{adj} value and lower RMSE value indicated that the model was best fitted.

3. Results and Discussion

3.1. Effect of treatment time and voltage on activity of POD and PPO

Significant reduction in enzyme activity after treatment with DBD plasma was observed (P<0.05). A rise in temperature by 5°C only was recorded by infrared thermometer (Maplin Electronics, UK) in all the experiments. It was observed that temperature required for inactivation of enzymes was not reached. Both treatment times and voltage significantly reduced the activity of POD and PPO ($P \leq 0.05$). All three applied voltages showed significant difference in residual activity ($P \leq 0.05$). Also, voltage was found to significantly ($P \leq 0.05$) interact with time.

3.2. First order kinetics model

Kp, the inactivation rate constant for first order kinetic model of POD and PPO was calculated from the slope of the lines. The values of Kp and R^2 for the kinetic model at the different voltage levels studied are given in Table 1. Even though the R^2 values were quite high (0.93 to 0.99) for both enzymes, satisfactory RMSE values were not obtained, being in the range of 2.7 to 8.61 for POD and 2.81 to 8.59 for PPO. Therefore, the residual activity of

both PPO and POD was not satisfactorily described by the first-order model as can be seen from Figs. 2a and 2b for POD and PPO, respectively. This may be due to the complex structure of the enzyme and difference in the mechanism of disruption of a single bond or structure (Pankaj et al., 2013). The proposed first-order kinetics model in the inactivation of enzymes appeared to be exceedingly simple. However, this model showed that inactivation kinetics of PPO was slightly higher as compared to POD.

3.3. Weibull distribution model

Experimental data were taken to determine scale parameter (α) and shape parameter (Y) of the Weibull model. The values of α and Y along with R^2 values for POD and PPO are summarised in Table 1. Weibull model could strongly predict residual activity for both POD and PPO after DBD plasma treatments as seen from the high R^2 value ($R^2 \geq 0.99$) obtained for the different voltage levels taken for study.

The scale parameter for POD ranged from 1.18 to 2.22 and for PPO it ranged from 0.76 to 1.95. Voltage levels inversely influenced the activity of the enzymes as lower applied voltage level showed higher scale parameter. The shape parameter for both POD and PPO ranged from 0.79 to 1.43 and 0.73 to 1.35, respectively. Figs. 3a and 3b show the fitted curves of the Weibull model for POD and PPO, respectively at different voltage levels. The figures revealed tailing effect at 23 kV treatment voltage that could not be accounted for by Weibull fit. The higher RMSE value at 23 kV treatment voltage as given in Table 1(4.55 and 6.29 for POD and PPO, respectively) also suggested the inadequacy of Weibull model. Even though $R^2 \geq 0.99$, the RMSE values were higher especially for 23 kV indicating that Weibull distribution model was not sufficient.

3.4. Logistic Model

The curves for both POD and PPO inactivation that were fitted using logistic model are shown in Figs. 4a and 4b, respectively. From the R^2 values, it can be said that this model

147 explained ≥ 99 % of the variability in residual activity of the enzymes. Tailing effect for POD
148 and PPO for 18 kV treatments was explained by the A^a_{min} values (6.39 ± 0.56 for POD and
149 8.06±0.99 for PPO). The t^a_{50} values for both POD and POD indicated the rapid inactivation
150 for both POD and PPO at 28 kV compared to 23 kV and 18 kV treatments. The R^2 values
151 were very high (≥ 0.99). RMSE values for both enzymes were very low ranging from 0.6 to
152 0.99 for POD and 0.2 to 1.02 for PPO. Thus, it can be inferred that the POD inactivation was
153 sigmoidal, which was adequately supported by the logistic type model. Table 1 also shows
154 that at same voltage and time treatments, the t^a_{50} values for POD were 0.84, 1.67 and 2.53
155 min which were higher than PPO showing values of 0.67, 1.18 and 1.35 min at 18 kV, 23 kV
156 and 28 kV respectively. The values indicate that POD is more stable than PPO in terms of
157 cold plasma treatment. Surowsky et al. (2013) also reported that cold plasma is effective in
158 reducing the activity of both PPO and POD in a model food system. There was 90%
159 reduction in PPO activity after cold plasma treatment for 180 s and 85% reduction of POD
160 activity after 240 s of treatment. There is no reported study on effects of cold plasma from
161 DBD on the activity of enzymes in tender coconut, mainly POD and PPO.

162 Inactivation by cold plasma can be attributed to the reaction between plasma
163 generated reactive species and chemically reactive side-chain of the amino acids in the
164 enzyme (Pankaj et al. 2013; Surowsky et al., 2013; Takai et al., 2012;). Superoxide anion
165 radicals, hydroxyl radicals, hydrogen peroxide radicals and oxide of nitrogen are the well
166 known reactive species and the reactive amino acid side chains are cysteine, aromatic rings of
167 phenylalanine, tyrosine, and tryptophan and their reaction causes modifications in the
168 secondary structure of the proteins (Attri & Venkatesu, 2017; Pankaj et al. 2013; Surowsky et
169 al., 2013; Takai et al., 2012). Segat et al. (2016) also observed that the inactivation of alkaline
170 phosphatase enzyme was attributed to the loss of α- helical and β-sheet secondary structures

of the proteins. The decomposition of bonds in protein (C–H, C–N and N–H) may also cause enzyme inactivation (Hayashi et al. 2009).

4. Conclusion

This study for the first time reports the effect of atmospheric cold plasma from DBD on POD and PPO activity in tender coconut water. POD and PPO inactivation increased with voltage and time of plasma treatment and POD was more resistant than PPO. Logistic model best described the inactivation of both enzymes. Further, voltage and time were noted to be important parameters that influenced the rate of inactivation and determined the shape of enzyme inactivation curve. These results indicated that cold plasma has the ability to inactivate enzymes in addition to microbial inactivation that has been widely reported. Further work can be done to study the mechanism of enzyme inactivation by cold plasma.

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DECLARATION OF CONFLICTING INTERESTS

The authors have no conflict of interest to declare.

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Figure legends

Fig. 1. Schematic of the experimental setup for DBD plasma system.

Fig 2. First order model curve fitting at different voltage levels for residual activity of (a) POD and (b) PPO.

Fig 3. Weibull model curve fitting at different voltage levels for residual activity of (a) POD and (b) PPO.

Fig 4. Logistic model curve fitting at different voltage levels for residual activity of (a) POD and (b) PPO.

Table1

Results on the parameters of the models fitted to inactivation kinetics of PPO and POD enzymes

Model	Parameters	POD			PPO		
		18 kV	23 kV	28 kV	18 kV	23 kV	28 kV
First Order	K_p (Min)	0.49±0.06	0.57±0.05	0.73± 0.07	0.59± 0.08	0.73±0.06	1.01±.05
	R^2	0.93	0.95	0.99	0.93	0.96	0.99
	RMSE	8.61	6.79	2.7	7.72	8.59	2.81
Weibull	α (min)[a]	2.22±0.06	1.84±.05	1.18 ± 0.03	1.95±0.05	1.46±0.02	0.76±0.03
	Y[a]	1.43±0.2	1.17±0.16	0.79±0.008	1.35±0.13	1.19±0.09	0.73±0.06
	R^2	0.99	0.99	0.99	0.99	0.99	0.99
	RMSE	2.87	4.55	1.95	2.99	6.29	1.38
Logistic	A^a_{min}	6.39±0.56	11.02±.99	6.82±0.89	8.06±0.99	5.67±0.89	2.63±0.51
	t^a_{50}	2.53±0.02	1.67±0.03	0.84±0.05	1.35±0.023	1.18±0.016	0.67±0.09
	P[a]	1.59±0.09	2.85±0.16	2.07± 0.3	2.78±0.56	3.24±0.15	2.02±0.21
	R^2	0.99	0.99	0.99	0.99	0.99	0.99
	RMSE	0.08	0.01	0.95	1.02	0.08	0.02

R^2 = regression coefficient; a = value ± standard error

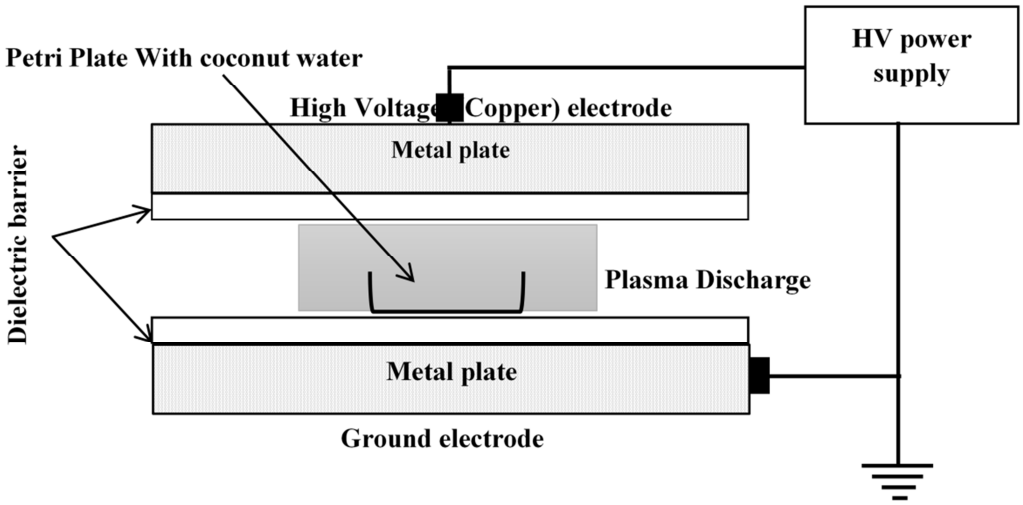
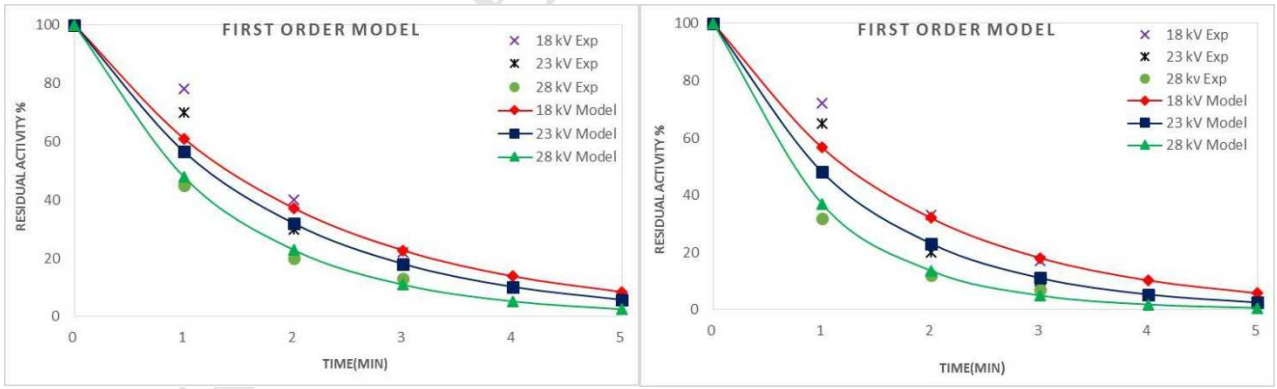
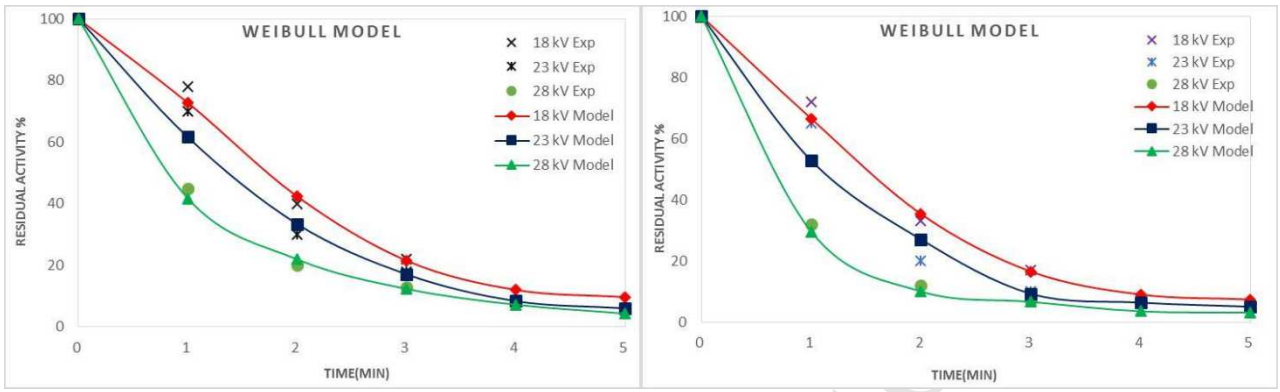

Petri Plate With coconut water
HV power supply
High Voltage (Copper) electrode
Metal plate
Dielectric barrier
Plasma Discharge
Metal plate
Ground electrode

Fig. 1.


FIRST ORDER MODEL
RESIDUAL ACTIVITY %
TIME(MIN)
18 kV Exp
23 kV Exp
28 kV Exp
18 kV Model
23 kV Model
28 kV Model
FIRST ORDER MODEL
RESIDUAL ACTIVITY %
TIME(MIN)
18 kV Exp
23 kV Exp
28 kV Exp
18 kV Model
23 kV Model
28 kV Model

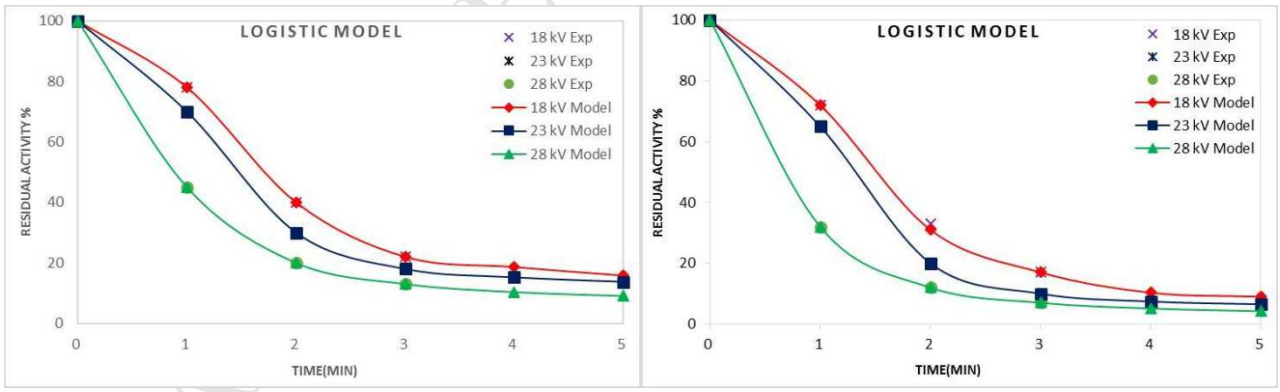
(a) (b)

Fig 2.



(a) (b)

Fig 3.



(a) (b)

Fig 4.

- DBD cold plasma used to inactivate POD and PPO in tender coconut water
- Treatment time and applied voltage showed significant inactivation
- POD was more resistant than PPO to DBD cold plasma
- Sigmoidal shaped Logistic model adequately described the enzymes inhibition
- DBD cold plasma can be used as a quality indicator for enzymatically spoiled foods