CHAPTER 6

To develop a lab-scale continuous system of atmospheric cold plasma (ACP) for juice processing and its performance evaluation with orange juice

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6.1 Introduction

Cold plasma (CP) is a novel technology primarily used for enzyme and microbial inactivation in food products. Plasma, a fourth state of matter, can be produced by passing high voltage current to the air, making air conductive and producing various ions and reactive species. The reactive species include peroxide ions, superoxide ions, free radicals, and ozone (Tappi et al., 2014). In CP, different noble gases can be used; mainly used are He, Ar, etc. When the feed is atmospheric air, it is considered atmospheric cold plasma (ACP) (de Jesús et al., 2022). The orange juice (cv. Wakro) used as the raw material is known for the indigenous variety of Arunachal Pradesh, India. This variety is known for its phytochemical contents and its sweet taste. The raw material is abundantly available in the Northeastern region of India during winter.

From an industrial point of view, in juice processing, a continuous mode of operation helps in the easy flow of the material, leading to less human contact. Therefore, based on the information obtained from batch-type ACP treatment, a continuous-type lab-scale ACP setup was aimed to develop, and its performance evaluation with orange (cv. Wakro) juice.

6.2 Materials and Methods

6.2.1 Raw materials and chemicals

Oranges (cv. Wakro) of Arunachal Pradesh, Northeast India, were acquired from the local vendor of the Tezpur area, Assam, India. Phenolphthalein, 2,2-diphenyl-1-picrylhydrazyl, NaCl, $NaHCO_3$ 2,6-dichloroindophenol salt, methanol, NaOH, Folin-Cocteau reagent (FCR), and distilled water was purchased from HiMedia Laboratories Pvt. Ltd., India, Sisco Research Laboratories Pvt. Ltd., India, and Merck Specialities Pvt. Ltd., India.

6.2.2 Juice preparation

The oranges (cv. Wakro) obtained were cleaned and pressed through a stainless Dynore juicer, then strained with a double-layer muslin cloth. The filtered squeezed

juice was standardized (ºBrix/acid ≈ 30) before continuous ACP treatment (Kumar et al., 2024).

6.2.3 Lab-scale continuous cold plasma setup and juice treatment

The laboratory-scale cold plasma apparatus consists of multiple components: a voltage regulator, dielectric barrier plates, high voltage and ground electrodes, a flow channel, a nylon sheet (dimensions: 20 mm thickness, 300 mm length, 230 mm breadth), and a peristaltic pump (ENPD 100 Victor, Enertech Electronics Pvt Ltd). The nylon sheet containing the flow channel is placed inside the treatment chamber. The gap between the electrodes is fixed at 30 mm. In this setup, orange (cv. Wakro) juice was pumped by a peristaltic pump through a silicon tube to the U-shaped channel (25×15 mm) in the treatment chamber (**Fig. 6.1)**. The juice passed through the channel. At the same time, plasma was generated, and the treated juice was collected at different flow rates and voltages for the experiment. The detailed experimental plan for the effects of continuous ACP treatment on orange (cv. Wakro) juice is presented in **Fig. 6.2**.

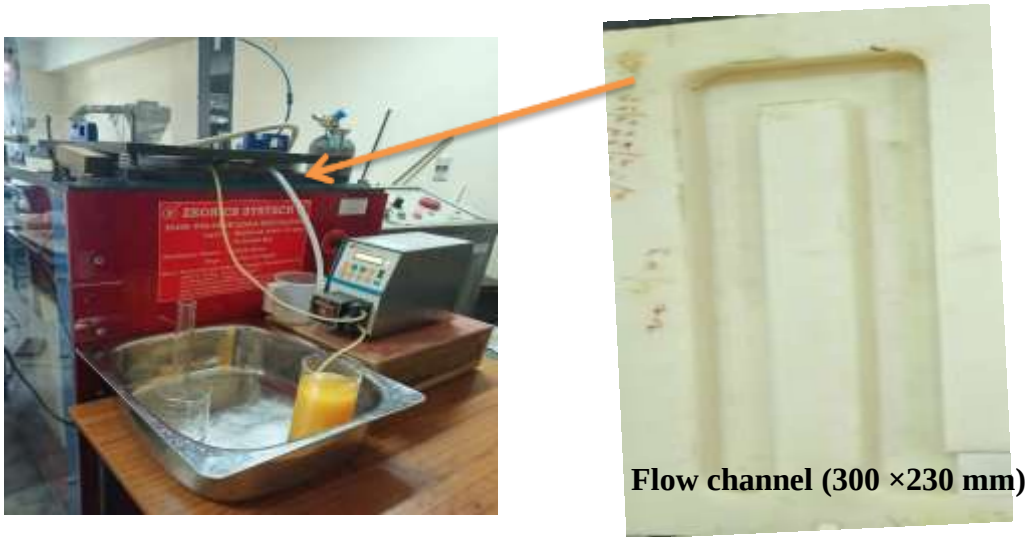

Fig. 6.1 Lab-scale continuous-type cold plasma setup.

6.2.4 pH, total soluble solids, titratable acidity

pH and total soluble solids (TSS) of the orange (cv. Wakro) juice were measured using a digital pH meter and refractometer (Pankaj et al., 2017). The titratable acidity (TA) of the juice sample was measured by titration (Ladaniya, 2010) and the details of the method is mentioned in **Section 5.2.4**. of **Chapter 5**.

6.2.5 Electrical conductivity

The electrical conductivity (EC) of the juice sample was measured by conductivity meter (Pankaj et al., 2017).

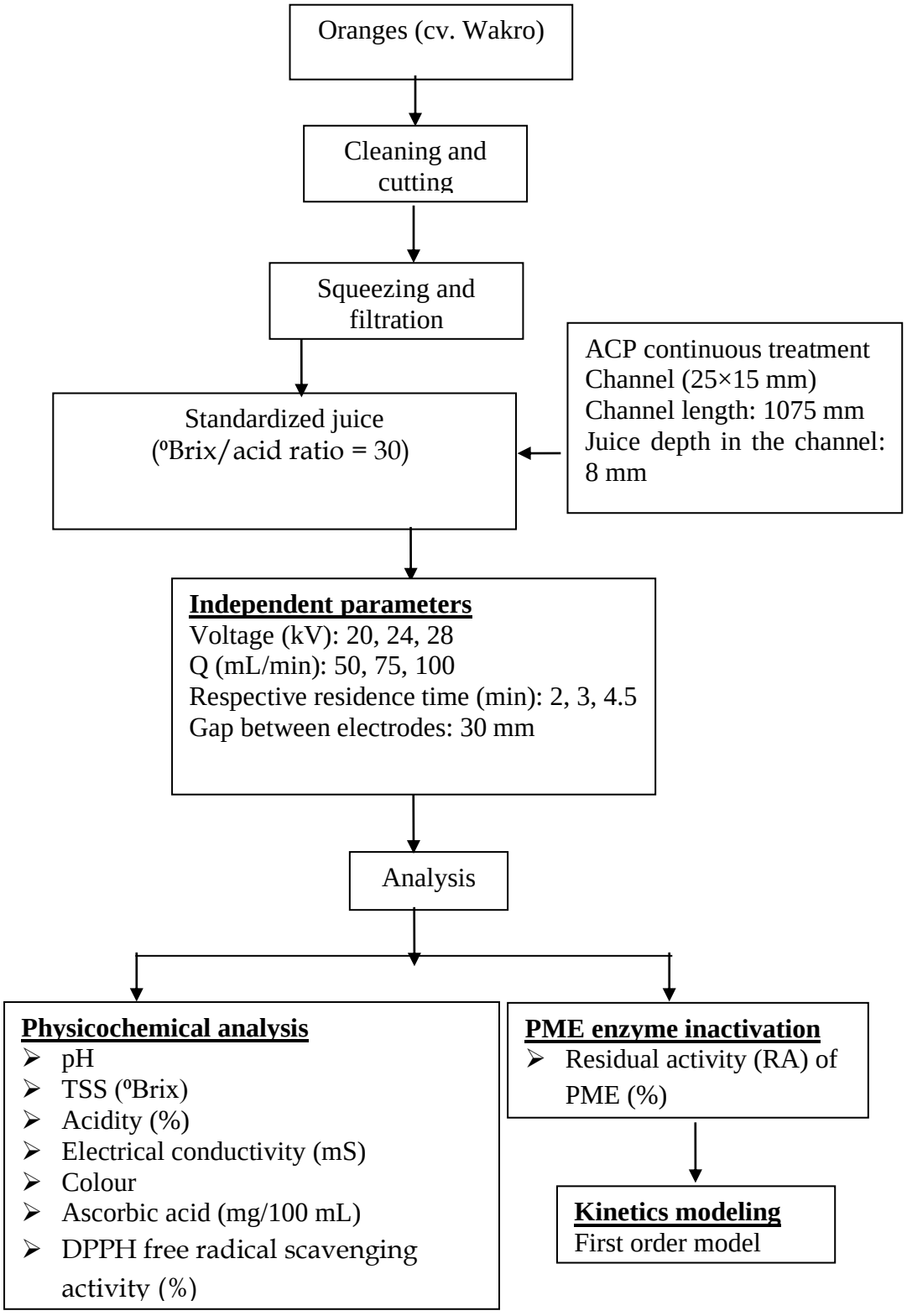

Fig. 6.2 Detailed work plan for objective 4.

6.2.6 Colour

The colour parameters of untreated and continuous ACP-treated juice were measured using the Hunter Colour Lab (Ultrascan VIS, Hunter Color Lab, USA) (Kumar et al., 2023) and the details provided in **Section 5.2.5** of **Chapter 5**.

6.2.7 Residual activity of PME

The initial and residual activity (RA) of PME after ACP treatment in orange (cv. Wakro) juice sample was determined using the method described by Basak and Ramaswamy (1996) and the detail given in **Section 3.3.6** of **Chapter 3**.

6.2.8 Ascorbic acid

The ascorbic acid (AA) concentration in the orange (cv. Wakro) juice was quantified utilizing the titration method developed by Ranganna (2001), the details of the procedure of the method is mentioned in **Section 3.2.5** of **Chapter 3**.

6.2.9 DPPH radical scavenging activity

The DPPH radical scavenging activity of orange (cv. Wakro) juice was measured by using the method outlined by Islam et al. (2019) and given in detail in **Section 3.2.8** of **Chapter 3**.

6.2.10 Ascorbic acid, DPPH radical scavenging activity degradation and RA of PME inactivation kinetics modeling

The first-order model is the commonly used model for characterizing quality degradation and enzyme inactivation kinetics in fruits and vegetable products during processing (Ludikhuyze et al., 1999; Pankaj et al., 2013). The first-order model was given in **Eq. 6.1.**

$$\frac{A_t}{A_0} = \frac{C_t}{C_0} = \frac{D_t}{D_0} = exp(-kt) \quad (6.1)$$

Where 'k' is the inactivation rate constant, min^{-1}; A_0, C_0, and D_0 are the initial values of RA of PME, AA, and DPPH radical scavenging activity, respectively; A_t, C_t, and D_t represents the reduction of RA of PME, AA, and DPPH radical scavenging activity after treatment time 't'.

6.2.11 Statistical analysis

The results of the experiments were expressed as mean ± standard deviation (SD). The significance test at 95% confidence interval was conducted using the Duncan multiple range test in the SPSS program (IBM SPSS Statistics, USA). A significant difference was taken as $p < 0.05$. AA and DPPH radical scavenging activity degradation and PME inactivation kinetics modelling were performed in MATLAB R2015a software. Statistical parameters R^2 Moreover, RMSE was obtained from the MATLAB.

6.3. Results and Discussion

6.3.1 Effect of continuous ACP treatment on the quality parameters

6.3.1.1 pH, total soluble solids, and titratable acidity

In every analysis, the treated juices have been compared with the control. There was no significant variance ($p > 0.05$) in the case of all three variables. The value of pH ranged from 3.48 ± 0.01 to 3.50 ± 0.01. As the flow rate was kept constant and the voltage was changed, there was no change in the pH, which shows that the pH was unaffected by the flow rate. In the case of TSS, the value ranges from 11.93 ± 0.12 to 12.20 ± 0.10 (**Table 6.1**). While comparing the 20 kV treatment with different flow rates, initially, the TSS remained unchanged; at 100 mL/min, there was a slight decrease even though it was insignificant.

Table 6.1 Effect of continuous ACP treatment on physiochemical characteristics (pH, TSS, and TA) of orange (cv. Wakro) juice.

Voltage (kV)	Flow rate (mL/min)	Parameters		
		pH	Total soluble solids (°Brix)	Titratable acidity (%)
Control		3.49 ± 0.01[a]	12.20 ± 0.10[a]	0.41 ± 0.04[a]
20	50	3.49 ± 0.02[a]	12.07 ± 0.25[a]	0.41 ± 0.04[a]
	75	3.50 ± 0.01[a]	12.07 ± 0.12[a]	0.45 ± 0.00[a]
	100	3.48 ± 0.01[a]	11.93 ± 0.12[a]	0.45 ± 0.06[a]
24	50	3.48 ± 0.01[a]	11.93 ± 0.12[a]	0.41 ± 0.04[a]
	75	3.49 ± 0.02[a]	12.00 ± 0.20[a]	0.43 ± 0.04[a]
	100	3.48 ± 0.02[a]	12.10 ± 0.17[a]	0.39 ± 0.00[a]
28	50	3.47 ± 0.01[a]	11.93 ± 0.12[a]	0.43 ± 0.04[a]
	75	3.49 ± 0.02[a]	12.00 ± 0.00[a]	0.45 ± 0.00[a]
	100	3.48 ± 0.01[a]	12.00 ± 0.20[a]	0.43 ± 0.03[a]

Note: Superscript 'a' represents insignificant difference ($p > 0.05$)

In the case of 24 kV, the TSS was slightly increased as the flow rates increased. In the case of 28kV, there was also a slight increase as the flow rate increased from 50 mL/min to 75 mL/min, after which it remained unchanged. TSS in juice was observed to have an insignificant increase or decrease when the data were analyzed with the control (**Table 6.1**). In TA, the lower value was 0.39 ± 0.00 for the treatment combination of 24 kV with 100 mL/min, and the higher value was shown by the treatment combinations of 20 kV with 75 mL/min, 20 kV with 100 mL/min, and 28 kV with 75 mL/min. While comparing with control, there was no significant change. In the case of 20 kV, there was a slight increase followed by a stationary stage.

All the above-mentioned results showed no significant changes in the pH, TSS, or TA of orange juices when treated with continuous ACP. As per the research done by Xiang et al. (2018), there was an increase in the acidity of apple juices. Even though acidity showed an increase, the change was insignificant when a comparison of those values was made with the control juice. A study was done by Pankaj et al. (2017) in white grapes have shown no significant change in the pH of the juice, but in the case of TA, there was a significant change, which is not in accordance with this study.

6.3.1.2 Electrical conductivity

The EC of orange (cv. Wakro) juice was 8.57 ± 0.03 mS. At 50 mL/min, the maximum EC was 8.88 ± 0.02 mS while treated with 28 kV. On comparing the samples with different flow rates, the maximum EC was shown by the treatment combination of 28 kV with 100 mL/min (**Fig. 6.3**). This can be explained as a higher voltage; there will be maximum free radical species and gaseous ions, which would have been produced due to air ionization. The increased concentration of these reactive species would have been absorbed during the juices' flow, thereby increasing the conductivity of the treated juices. Comparing the samples with the control, there was a significant increase in EC, showing that at higher voltage and higher flow rate, they are positively correlated with the EC.

The research carried out by Pankaj et al. (2017) on white grapes, which has shown a significant difference in EC. These results are not similar to those carried out in orange juice with the continuous system, as explained earlier. According to the above-mentioned study in white grapes, the reason they put forward suggests that the

hydroxyl radicals generated from the plasma discharge will have higher solubilizing power. Due to its higher solubilizing power, an increase in EC was observed.

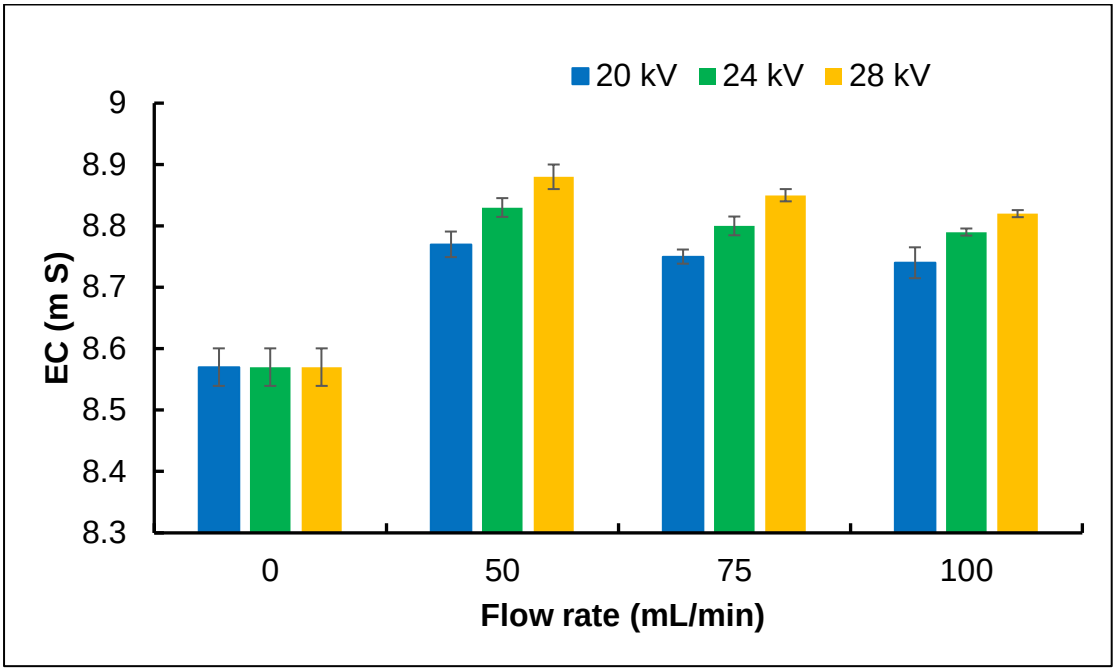

Fig. 6.3. Effect of continuous ACP on electrical conductivity (EC).

6.3.1.3 Colour

The colour of fruit juices is a critical quality indicator parameter that significantly impacts consumer preferences and their decision to accept or reject the product (Guerrouj et al., 2016). The initial values of color parameters *L**, *a**, and *b** were 36.35 ± 0.10, 2.19 ± 0.07, 13.01 ± 0.11, respectively. The *L** and *a** were not significantly varied during continuous ACP treatment ($p > 0.05$). However, the *b** value increased with the decrease (100–50 mL/min) in the flow rates at each constant voltage. The *b** value ranged from 13.01 ± 0.12 to 13.53 ± 0.08, 13.84 ± 0.04 to 14.35 ± 0.05, 14.43 ± 0.03 to 14.63 ± 0.03 at 20, 24, and 28 kV, respectively. The minimal variations in *b** and ΔE observed could be attributed to pigment oxidation and the interaction with plasma species generated during continuous ACP treatment (Wang et al., 2012). However, ΔE is generally considered a significant factor in determining juice quality. The ΔE in juice implies how the colour of the treated sample varies from that of the control sample. The changes in ΔE obtained during the analysis are shown in **Fig 6.4.** From the data obtained from the analysis, it can be drawn that maximum colour change has occurred at the flow rate of 50 mL/min in all the voltages. On

comparing the voltage ranges with the color difference, it was observed that as the voltage increases, the Δ*E* increases. Flow rate had a positive correlation with an Δ*E*, showing that if there is a higher flow rate, there will be a lesser Δ*E* variation. The change of Δ*E* may be because the increased contact with the charged species was browning in juices, causing color changes.

In the experiments conducted by Ramazzina et al. (2015**)** on CP treatment of kiwi fruits, *L** decreased and was insignificant during storage compared to the control sample. As per the above-mentioned paper, CP treatment did not impact the color of the samples. So, the low flow rate can affect the ΔE in the juice. The colour changes may be due to the reaction with reactive species and pigment oxidation (Islam et al., 2024).

Another study by Liao et al. (2018) on apple juice showed that the *L** value of the juice decreased, and the treated samples became darker. The same trend was also seen in the case of many other fruit juices (Almeida et al., 2015; Kovačević et al., 2016; Pankaj et al., 2017). In one study done with white grapes, the changes in *L** values were insignificant, but the values of *a** and *b** were significant (Pankaj et al., 2017).

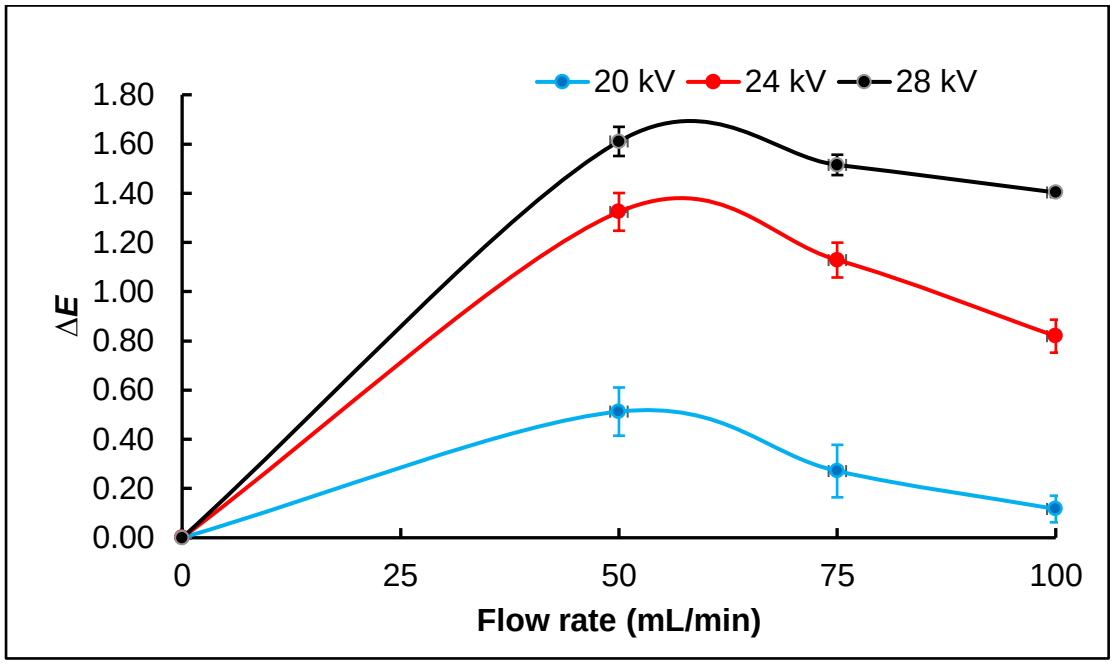

Fig. 6.4 Effect of continuous ACP on the total color difference (Δ*E*) in juice.

6.3.1.4 PME inactivation

The results of the study showed that continuous ACP treatment at minimum flow (50 mL/min) rate and maximum voltage (28 kV) resulted in minimum RA of PME (29.64 ± 1.47%), indicating higher PME inactivation (70.36%) (**Fig. 6.5**). As shown in **Fig. 6.5**, at lower flow rate, RA of PME significantly decreased with the increase in CP voltage. The study by Andreou et al. (2023) states that Helium gas plasma could inactivate the activity of PME by 80% in orange juice. So, the gas used also significantly affected the inactivation of PME in juices. In the case of orange juice, the fed gas had a higher impact on PME reduction. According to Xu et al. (2017), when the feed gas was MA65, which contains a large number of oxygen species, it resulted in the inactivation of PME approximately by 81%. The mechanism involved for the reduction of RA of PME during ACP treatment was previously discussed in Chapter **3** and section **3.3.2.2.**

As per the research carried out by Tiwari et al. (2009) depicted that by using ultrasonication in orange juice, PME can be reduced. In the case of orange juice, the inactivation of PME was 62%. PME inactivation using ultrasonication is less effective than CP treatment.

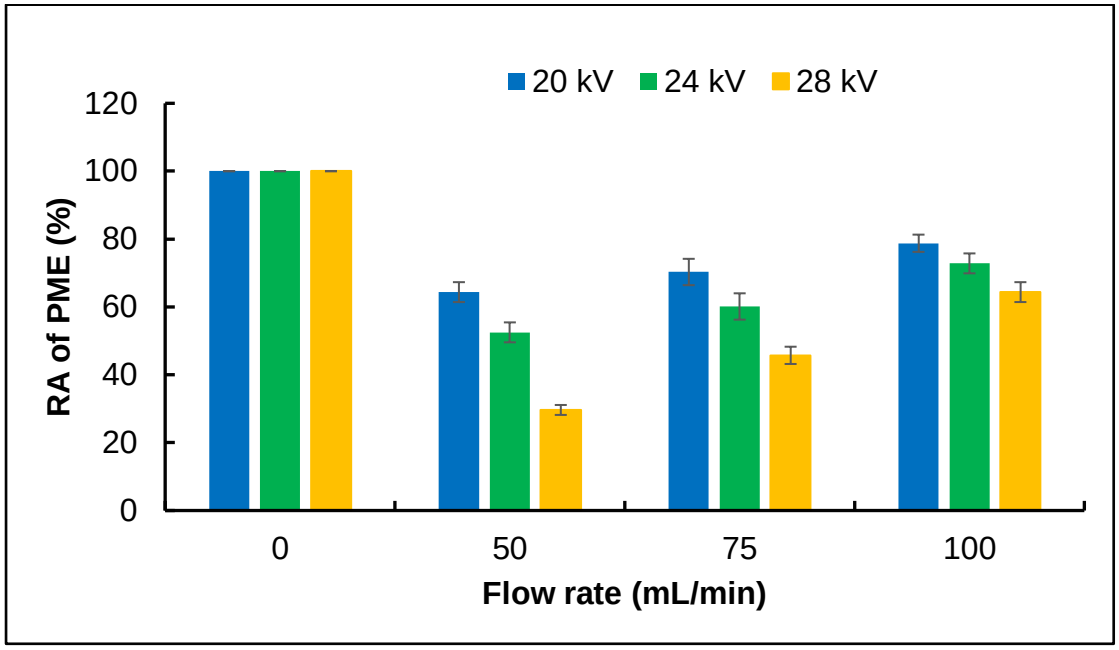

Fig. 6.5 Effect of continuous ACP on residual activity (RA) of PME in juice.

6.3.1.5 Ascorbic acid

As shown in **Fig. 6.6**, the continuous ACP treatment significantly affected the AA content in orange (cv. Wakro) juice. The initial AA content of orange (cv. Wakro) juice was 36.35 ± 1.34 mg/100 mL. The maximum reduction (23.20 ± 1.16 mg/100 mL) was found to be at low flowrate (50 mL/min) and higher voltage (28 kV) (**Fig. 6.6**). The main reason for this was that when there was a high retention time in the channel, as the flow rate was kept less, the juice was supposed to have more time with the radicals; thereby, maximum AA reduction would have occurred.

AA degradation is hypothesized to occur through either the direct attack of ozone, known as the Criegee mechanism, or indirectly by a reaction due to singlet oxygen and exited molecular oxygen (a free radical mechanism) (Kumar et al., 2023). Some studies also indicated that ACP treatment can reduce the AA content in juice, which aligns with the findings of this study (Xu et al., 2017; Hosseini et al., 2021). However, the reduction in AA could be mitigated by adjusting or changing the feed gas (Xu et al., 2017).

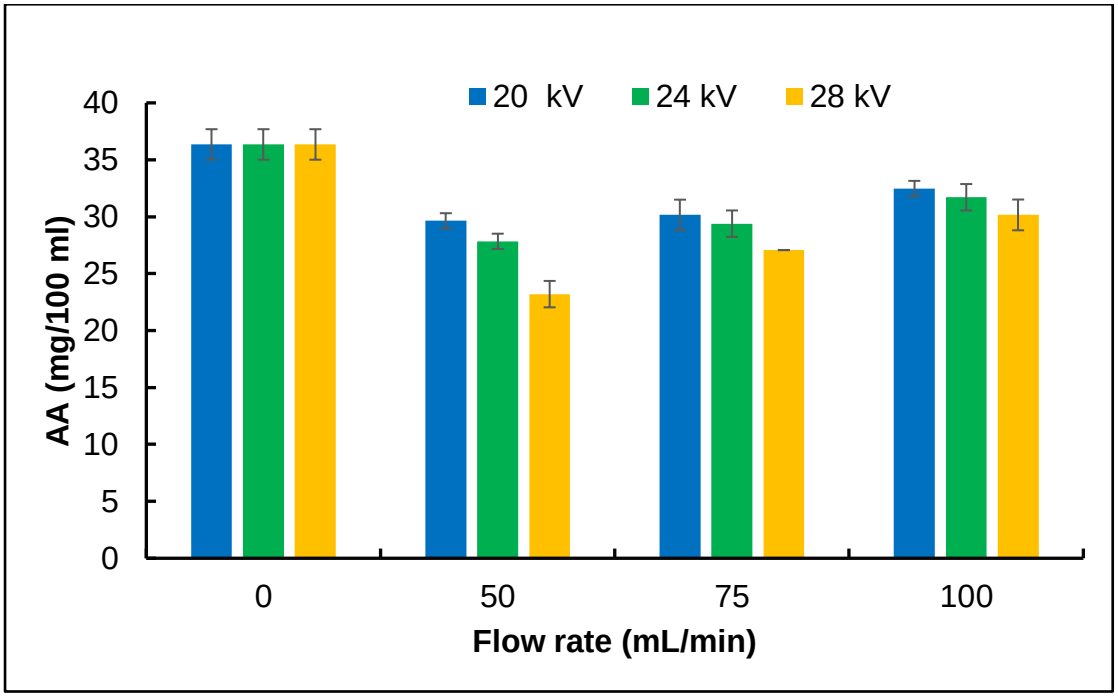

Fig. 6.6 Effect of continuous ACP on ascorbic acid (AA).

6.3.1.6 DPPH radical scavenging activity

The initial DPPH radical scavenging activity of orange (cv. Wakro) juice was 69.74 ± 0.69%. The maximum DPPH radical scavenging activity degradation was 54.68 ± 0.36% at 50 mL/min and 28 kV, indicating voltage was significantly affected (p < 0.05). The lower flow rate indicates the maximum residence time of juice in the flow channel during ACP treatment. As the flow rate decreases, DPPH radical scavenging activity reduction increases at varying voltages (20–28 kV). The percentage inhibition of DPPH radical scavenging activity of samples and comparing it with control had helped to know about the prospects in the antioxidant pattern of the orange juice after high voltage ACP treatment. **Fig. 6.7** shows the effect of continuous ACP on DPPH radical scavenging activity. As shown in **Fig. 6.7**, at 50 mL/min, DPPH radical scavenging activity showed higher than flow rates of 75 mL/min and 100 mL/min. The reduction of DPPH radical scavenging activity may be attributed to the plasma reactive species (Kumar et al., 2023).

In the case of the experiments conducted by Hou et al. (2019), there was a significant variation in the DPPH radical scavenging activity of plasma-treated samples with the control sample. The changes in the DPPH radical scavenging activity correlated with the changes observed in AA content. AA is an antioxidant, and its loss would have caused a decrease in the DPPH radical scavenging activity. Along with AA, other antioxidants would also have degraded.

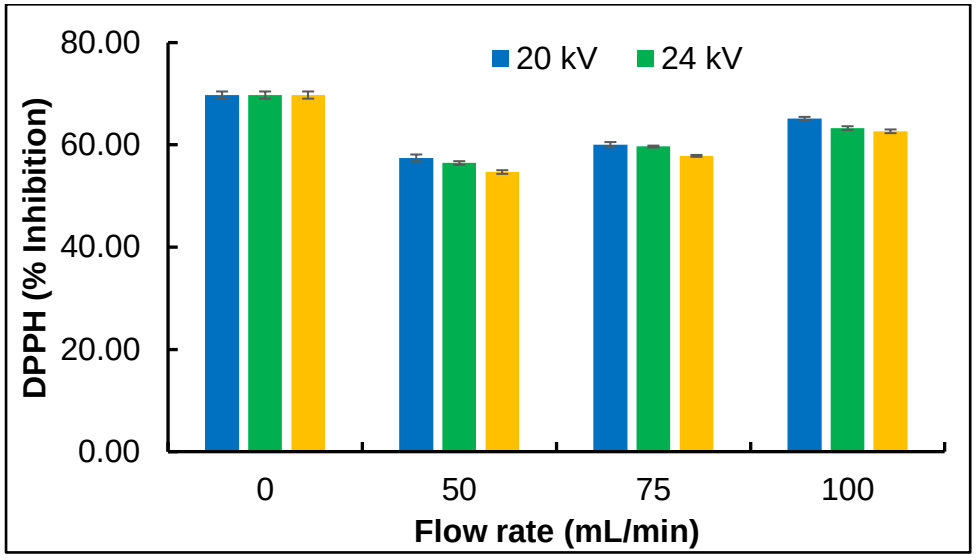

Fig 6.7 Effect of continuous ACP on DPPH radical scavenging activity.

6.3.2 Kinetics modeling

6.3.2.1 Residual activity of PME

The effect of the input voltage and treatment duration on the PME enzyme's RA was explained using the first-order kinetic model equation. The first-order model's statistical parameters (R^2 and RMSE) and inactivation rate constant and are listed in **Table 6.2**. A higher R^2 value (> 0.97) and lower RMSE (< 0.03) indicate higher accuracy in fitting the model. **Fig. 6.8** illustrates the curves for PME inactivation, and the behavior was linear. The inactivation rate constant, k, was calculated from the slope of the lines for different samples at each input voltage, and its value was in the range of 0.108–0.253 min^{-1}. The rate constant (k-value) increases with the input voltage, indicating that voltage significantly impacts PME inactivation in orange juice. The similar observations were in line with the results of Andreou et al. (2023). The author reported that the inactivation rate constant ranged from 0.033 to 0.192 min^{-1} when the voltage was 4–7 kV. The high R^2 values indicate that this kinetic model successfully described the PME inactivation of orange juices by cold plasma.

6.3.2.2 Ascorbic acid degradation

Most of the work was done on ascorbic acid degradation during the storage duration of cold plasma-treated samples (Chutia and Mahanta, 2021; Nasri et al., 2023). So, this study was done to analyze the kinetics of degradation of AA during cold plasma treatment at different voltages and times. The first model was used to explain the behavior of AA during cold plasma treatment at different input voltage and treatment times. The degradation rate was 0.0518, 0.064, and 0.098 min^{-1} at 20, 24, and 28 kV, respectively, as mentioned in **Table 6.2.** This is because cold plasma treatment generates more reactive oxygen species (ROS) and high-voltage ozone, which can contribute to the quick breakdown of ascorbic acid. Nasri et al. (2023) have reported that the impact of low voltage (i.e., 8 kV) on the AA of the carrot juice sample was insignificant. Degradation behavior during CP treatment followed a linear trend, as shown in **Fig. 6.9.** Statistical parameters like R^2 (> 0.98) show that the first-order model fitted well for 24 and 28 kV as compared to 20 kV (R^2 < 0.94) treatment conditions. Similarly, RMSE values were lower (< 0.03) for all three conditions. It was also observed that degradation of AA was more for more prolonged exposure in

the treatment chamber, and it occurs at a faster rate at a higher voltage. Hou et al. (2019) have also reported similar trends and shown that more AA degradation occurs with increasing treatment.

6.3.2.3 DPPH radical scavenging activity degradation

The first-order model was used to know the degradation behavior of DPPH radical scavenging activity during ACP. Model parameters show that the degradation rate (k) increases with increasing input voltage, and its values were 0.044, 0.049, and 0.056 min^{-1} at 20, 24, and 28 kV, respectively, as listed in **Table 6.2**. Just like AA, the first order showed the best fit for 24 and 28 kV treatment conditions with a high R^2 value (> 0.98) and low RMSE (< 0.02) compared to the 20 kV treatment condition. Further, **Fig. 6.10** shows that the degradation behavior of DPPH is linear during continuous CP treatment. The DPPH free radical scavenging capacity of orange juice decreased with processing time for all three input voltages during ACP. A similar trend was shown by Pankaj et al. (2017) during the ACP treatment of white grape juice. The author reported that DPPH free radical scavenging was 88.16, 87.63, 82.74, 79.21, and 77.50 % at 0, 1, 2, 3, and 4 min when treated at 80 kV. It was also observed that there is a significant reduction in DPPH activity with processing time, i.e., the longer the exposure, the more degradation of DPPH will occur. The behavior of DPPH degradation is similar to the behavior observed for AA, which may be the dominant factor determining the antioxidant activity at different times.

Table 6.2 The goodness-of-fit and model parameters of the first-order model fitted RA of PME, ascorbic acid (AA), and DPPH radical scavenging activity degradation.

Parameter		**Voltage**		
Dependent	**Model constant**	**20 kV**	**24 kV**	**28 kV**
RA of PME	k (min^{-1})	0.1076	0.1547	0.2531
	R^2	0.9787	0.9886	0.9914
	RMSE	0.02277	0.02227	0.02797
AA	k (min^{-1})	0.05176	0.06402	0.09822
	R^2	0.9385	0.9809	0.9988
	RMSE	0.02085	0.01412	0.005335
DPPH	k (min^{-1})	0.04378	0.04866	0.0564
	R^2	0.9682	0.9945	0.9882
	RMSE	0.01399	0.006069	0.0102

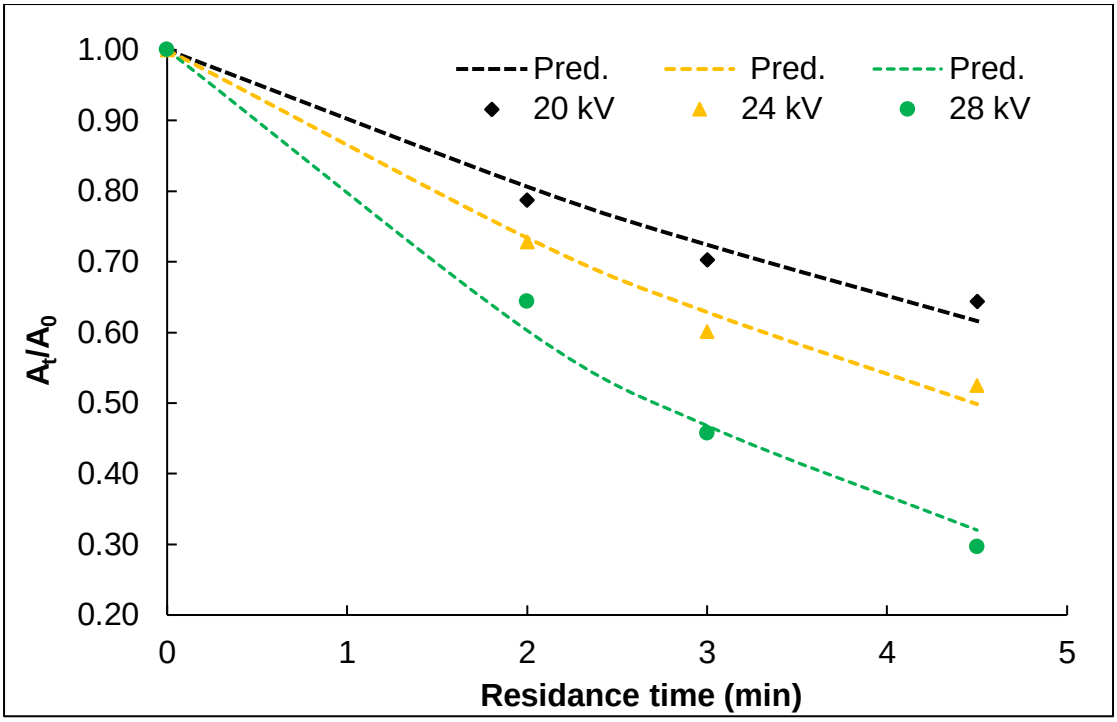

Fig. 6.8 First-order model kinetics of residual activity of PME in orange (cv. Wakro) juice during continuous ACP treatment.

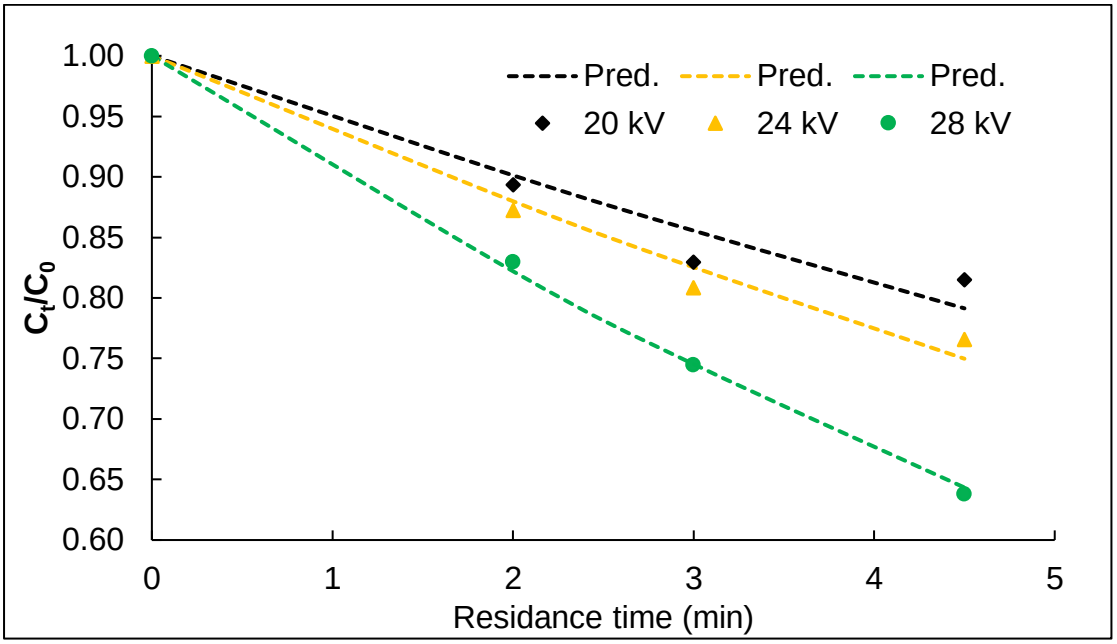

Fig. 6.9 First-order model kinetics of ascorbic acid degradation in orange (cv. Wakro) juice during continuous ACP treatment.

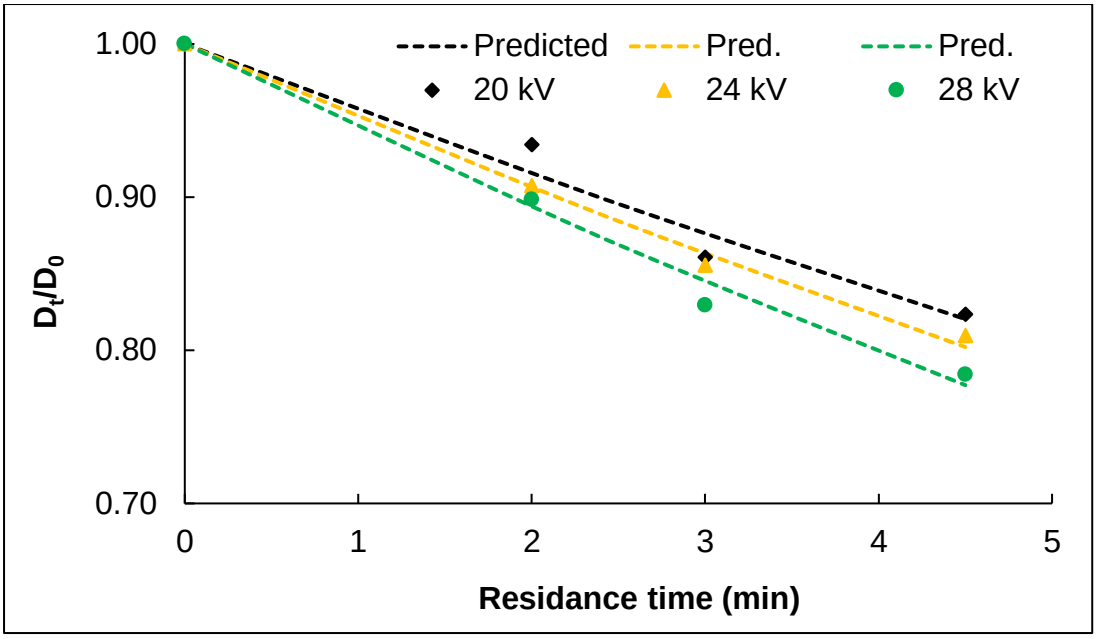

Fig. 6.10 First-order model kinetics of DPPH radical scavenging activity degradation in orange (cv. Wakro) juice during continuous ACP treatment.

The scalability and feasibility of this technology must be carefully assessed to determine its practical application on an industrial scale. At the industrial level, CP reactors need to be designed for higher throughput, ensuring that the residence time, voltage, and juice flow rates can be adjusted to accommodate mass production. On the other hand, the trade-off between enzyme and microbial inactivation and nutrient loss, such as reduced vitamin C, requires optimization. The economic feasibility of ACP treatment is an important factor that can be evaluated through a cost-benefit analysis and the ongoing operational costs. Regulatory approval is essential, with extensive testing to meet food safety standards. Consumer acceptance is also a critical challenge. Consumers must be aware of the benefits and safety of ACP technology.

6.4 Conclusion

The present work developed a flow channel for a continuous-type lab-scale ACP setup and its performance evaluation with orange (cv. Wakro) juice by applying voltage from 20–28 kV and a flow rate from 50-100 mL/min. The continuous ACP treatment at varying voltages (20–28 kV) and flow rates (50–100 mL/min) had minimal impact on key physicochemical properties such as pH, TSS, and TA, with no significant changes observed. However, EC increased with higher voltage and flow

rates due to enhanced production of reactive species during ACP treatment. The treatment was most effective in inactivating PME, with a maximum inactivation of 70.36% at 28 kV and 50 mL/min. AA content and DPPH radical scavenging activity were significantly reduced with higher voltage and lower flow rates, indicating the degradation of antioxidants during the process.

Kinetic modeling using a first-order model was successfully applied to describe the degradation of PME, AA, and DPPH activity, with high R^2 (> 0.93) and low RMSE (< 0.03), confirming the reliability of the model in representing the degradation behaviors. Overall, the continuous ACP setup showed promising results for juice treatment, especially for PME enzyme inactivation. Further optimization of treatment parameters could enhance the process for industrial-scale applications.

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